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Title

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries

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http://mycotox.cirad.fr

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The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries

Abstract

The MYCOTOX project (http://mycotox.cirad.fr) (ref ICA4-CT-2002-10043) entitled "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries" started at the beginning of 2003. It involved partners from France, UK, Argentina, Brazil, Chile and Uruguay. The overall objective of the project was to improve the competitiveness of domestically and internationally traded cereals by controlling the occurrence of mycotoxins in maize and wheat products used as human food and animal feed. The project was based on a multidisciplinary approach, including analytical, technological, socio-economic components in order to ensure, jointly with all stakeholders, quality and safety throughout maize and wheat whole chains.

The project's activities included "lab" activities (within Work Packages 1&2&3) and "field" ones (within Work Packages 4&5&6). The different activities performed are listed below, as well as the methodologies used, the major results obtained, the problems encountered and the steps taken to ensure application of the results.

The chromatographic methods classically used for mycotoxin determination were standardised and harmonised among the partners, through inter-laboratory work and proficiency rounds. The same reference materials (either purchased or prepared internally) were analysed by the participating laboratories, and the results subjected to statistical analysis in order to evaluate the performance of each laboratory. When needed, corrective actions and adjustments were made to analytical procedures to bring performance up to standard. This work allowed a network to be set-up among the partners with the aim of strengthening the regional analytical support to trade and regulatory bodies. Exchanges are now effective among partners beyond the project's end. This was a challenge and a highlight of the project.

Alternative techniques (toxicity bioassays, near infrared reflectance spectroscopy and the Toximet-T system) were tested as tools for semi-quantitative measurement or screening of contaminated bulks. The results confirmed the potential of these techniques for this purpose, however, further research is needed. The MYCOTOX project acted in this sense as starter of investigation.

The human exposure to ochratoxin A was assessed in Argentina and Chile, through the OTA analysis in blood samples collected from volunteers, and the statistical exploitation of questionnaires on the food diet and consumption habits of the blood donors, as well as the screening of the fungal OTA-producing flora of some cereals. The generated data was pioneer in the South Cone region, it will help decision-makers for health policies.

Cereal processing steps (cleaning, sieving, grading, milling, de-hulling) were evaluated in terms of their impact on the mycotoxin distribution in the resultant fractions. This resulted in technical recommendations and potential control measures and corrective actions to be put in place by the cereal industry, for reducing the mycotoxin content and/or re-orienting the use of resultant fractions.

The priority combination (commodity/mycotoxin) was chosen at the start of project and the Commodity Flow Diagram (CFD) was then elaborated for a selected case-study in

each country. The project succeeded in establishing, in each country, a multidisciplinary team (agronomist, socioeconomist, HACCP specialist, analyst) which applied the HACCP (Hazard Analysis and Critical Control Points) method and surveillance studies for identifying where mycotoxin hazard entered or increased in the whole cereal chain. Control measures were tested and validated. The generated technical and socio-economic data were fully integrated into HACCP plans and the associated pre-requisite Good Agricultural Practices, to elaborate the final architecture of an efficient Food Quality Management System, adapted to each local context and helpful for the cereal stakeholders and decision makers.

The project has used specific case-studies to pioneer the application of a number of methodologies and approaches to the control of mycotoxins in cereal chains in the Southern Cone. The application of HACCP (Hazard Analysis and Critical Control Points), which was initially focusing on technical aspects at lab level, to the whole agri-food chain including stakeholders, and the full integration of socio-economic and technical inputs, has been successfully demonstrated and can therefore be recommended for use throughout the Southern Cone for the control of mycotoxins (and by extension to other types of contaminants) in all cereals and cereal products (and by extension to other types of commodities).

To ensure application of the results achieved, the project consortium had a very dynamic dissemination strategy, organising various meetings and workshops with the cereal stakeholders, representatives of the academic sector and official bodies. This was essential according to the expressed needs in the Southern Cone region for considering mycotoxin prevention and control in an holistic way, and for external interventions driven by authorities and regulatory bodies to ensure the application of Quality Assurance and Management Systems along the cereal chains in the region.

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Summary

The MYCOTOX project (http://mycotox.cirad.fr) (ref ICA4-CT-2002-10043) entitled "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries" started at the beginning of 2003. It involved partners from France, UK, Argentina, Brazil, Chile and Uruguay. The overall objective of the project was to improve the competitiveness of domestically and internationally traded cereals by controlling the occurrence of mycotoxins in maize and wheat products used as human food and animal feed. The project was based on a multidisciplinary approach, including analytical, technological and socio-economic components in order to ensure, jointly with all stakeholders, quality and safety throughout maize and wheat whole chains.

The activities and outputs of the project are summarised below:

Work Package 1. Development and Standardisation of Effective Analytical Tools for Mycotoxin Determination in Cereals and By-Products

***Objective of WP 1*:** To develop, validate and make available between all partners accurate, precise, fast, reliable, sensitive, and simple analytical tools, for the detection or determination of mycotoxins, for use during the risk analysis, validation, verification and monitoring components of the HACCP approach to mycotoxin control in maize and wheat.

Activity 1. The **inter-laboratory work and proficiency rounds** performed among the laboratories involved in the project allowed:

i) Standardisation and harmonization of the analytical procedures (sampling, sample preparation and analysis) used in the four Southern Cone countries for mycotoxin determination. The same reference materials were analysed, using the chromatographic techniques currently available at the participating laboratories, and the results were subjected to statistical analysis in order to evaluate the performance of each laboratory. When needed, corrective actions and adjustments were made to analytical procedures to bring performance up to standard.

ii) Preparation of internal reference materials (mycotoxin-free and naturally or spiked contaminated cereals) that were used among the partners. These reference materials might be used by extension within the Latin American continent. This can then answer the need expressed many times by scientists in different conferences on mycotoxins for available reference materials in the region, but less expensive than those marketed at the international level such as the FAPAS materials. *This was one challenge of the project*.

iii) Setting-up a regional Southern Cone analytical network with harmonised procedures for aflatoxin, zearalenone, ochratoxin A and fumonisin determination in wheat and maize. This network aimed at strengthening the regional analytical support to trade and regulatory bodies. *This was another challenge taken up by the project*.

Activity 2. Alternative techniques were tested as tools for semi-quantitative mycotoxin determination and/or screening of contaminated batches. These techniques were:

i) The **toxicity bioassays** based on the natural ability of the *Vibrio fischeri* bacteria to emit light, and the potential decrease of light emission in presence of mycotoxins. *This study pioneered the application of this technique to mycotoxins*, as most existing literature addressed chemical pollutants in water or wastewater. Our results showed that the use of toxicity bioassays through *V. fischeri* luminescence had potential as a semi-quantitative screening tool for rapid discrimination of mycotoxin-contaminated grain bulks. Further research is needed for fine-tuning; *the MYCOTOX project acted in this sense as a starter of investigation*.

ii) **Near Infrared Reflectance Spectroscopy (NIRS)** was investigated as a tool for screening grain bulks and predicting mycotoxin content in cereals. The potential of this technique was confirmed, especially as indirect assessment of the changes induced by the DON mycotoxin in the product rather than a direct measurement of the mycotoxin itself. This should orientate the future research. Here again, *the MYCOTOX project acted as a starter of investigation*.

iii) The **Toximet-T system** was evaluated as a simple, inexpensive, robust, user-friendly and efficient procedure for accurately detecting and measuring mycotoxins in food. A prototype instrument was successfully designed and manufactured which automatically determined the concentration of mycotoxins, immobilised on a polymer cartridge, by measuring the fluorescence signal generated by the toxin under UV light irradiation. *An international patent was published in November 2006 and two UK patent applications were respectively filed in February and April 2007*. Additional funds to those of the MYCOTOX project were sought for to make possible these achievements. *The Toximet Ltd. Company (*http://www.toximet.com*) was created for further R&D and commercial exploitation of the Toximet-T technology*.

Work Package 2. Risk Assessment of Human Exposure to Ochratoxin A

***Objective of WP 2*:** To evaluate the risk of human exposure to ochratoxin A (OTA) in Latin America South Cone region.

Activity 3. The **risk of human exposure to ochratoxin A** (OTA) was evaluated in Argentina and Chile, through OTA analysis of blood samples collected from volunteers in both countries, and exploitation of dietary surveys on the food habits and consumption of the blood donors. In Chile, cereals were the most likely source of OTA. In Argentina, the dietary inquiry showed that bakery products were the most consumed daily and might be a source of exposure to OTA. However, Argentinean wines did not show high levels of OTA contamination and were not pointed out as source of exposure to this mycotoxin. Additionally, potential sources of contamination with OTA were screened by identification of the fungal flora in some cereals and comparison to the OTA content in those cereals and derived products. This correlation between fungal flora and mycotoxin production was a very interesting line of investigation which was not initially foreseen in the technical contract annex but which emerged as a priority during the project's

running. These studies were initially limited to Argentina, but were later extended to include Uruguay and Chile. *This pioneering work on the occurrence of ochratoxin A in the Southern Cone region generated original data and contributed to many scientific exchanges and training among the partners*. *The outputs provided baseline studies and recommendations for helping the health-related decision-makers to overcome the risk of human exposure to ochratoxin A*.

Work Package 3. Evaluation of Milling Procedures as Potential Critical Control Points (CCPs)

> ***Objective of WP 3*****:** To evaluate distribution and variability of deoxynivalenol (DON) in wheat and fumonisins in maize in the fractions obtained through wet and dry milling processes in the Southern Cone.

Activity 4. The **technical steps of grain processing were evaluated as potential control measures to be applied at Critical Control Points** in the cereal chains. The impact of cleaning, sieving, grading, milling, de-hulling of grains (wheat and/or maize) were studied in terms of distribution of mycotoxins (deoxynivalenol DON for wheat and fumonisin for maize) in the resultant fractions. The fumonisin reduction through corn wet milling was validated. Starch and gluten were shown to have very low fumonisin content. This finding was very interesting so it could orient towards corrective actions to be put in place by the corn industry for highly contaminated maize. The maize sieving before storage was also investigated and the validated results are under transfer to the corn industry. The impact of frying on deoxynivalenol reduction in the traditionally consumed wheat-made pies (called “empanadas”) in Argentina was studied. The impact of de-hulling, grading by size and conditioning with water on the DON in wheat was also studied. The grain size was shown as a critical parameter for reducing the wheat contamination with DON. *The results allowed technical recommendations to be proposed for potential control measures and corrective actions to be put in place by the cereal industry, in order to reduce the mycotoxin content and/or to economically re-orient the use of resultant fractions*. Some work is still running beyond the end of the project. *The MYCOTOX project acted in this sense as starter of investigation*.

Work Package 4. Hazard Analysis of Mycotoxins

> ***Objectives of WP 4*****:** i) To identify cereal commodities-mycotoxin combinations that present an unacceptable risk to human health or present a constraint to trade and ii) To construct and verify the corresponding Commodity Flow Diagrams (CFDs).

Activity 5. The **priority combination (commodity/mycotoxin) (referred to as selected commodity system) was chosen** at the start of project as a selected case-study and pilot site in each country. This choice was made on the basis of an initial Hazard Analysis, using published mycotoxin occurrence data gathered from the 4 Southern Cone countries. The wheat/deoxynivalenol combination was selected in Argentina, Chile and Uruguay whilst the corn dedicated for poultry feeding was selected as the commodity in Brazil, with a wide range of mycotoxins of interest (aflatoxins,

ochratoxin A, fumonisin and zearalenone).

Activity 6. As the Hazard Analysis and Critical Control Points (HACCP) was the method on which the project's approach was based, **multidisciplinary HACCP teams were assembled** in Brazil, Uruguay, Argentina and Chile, including agronomists, socioeconomists, HACCP specialists, analysts, and representatives of the key players of the selected cereal chain (private sector, seed and grain producers). Confidentiality agreements were signed with the key players in order to legitimise their involvement in the project and define the mutual commitment of the consortium and the cereal stakeholders to join efforts and share field activities and outputs. *This was another challenge of the project, as it was a pioneering approach to extend the HACCP application, initially focusing on technical aspects at lab level, to the whole agri-food chain involving all of the key players and including socio-economic features*.

Activity 7. The **Commodity Flow Diagram (CFD) was then elaborated** for each selected case-study in each country. The collected data allowed a detailed overview on the agricultural practices, the raw material supply, the processing and transport channels and actors, the quality control procedures, the socioeconomic context (trade issues such as volumes, prices, fluxes, seasonal fluctuations, supply chain organisation). The CFDs were regularly reviewed, adjusted and updated throughout the project duration.

Activity 8. A **surveillance programme was designed and implemented** for each selected commodity system in each country. This aimed to identify at which step in the CFD the mycotoxin hazard entered or increased to unacceptable levels throughout the whole selected cereal chain. A sampling plan (procedures, number of samples, sample preparation before lab analysis) was prepared, shared and harmonised among the consortium members before implementation. The **surveillance studies implemented** allowed, for each commodity system in each country, identification of the potential Critical Control Points (CCPs), i.e. the steps in the CFD where contamination by moulds and/or mycotoxin production might occur or increase to an unsafe level, and hence where control measures were required. *In Argentina, Chile and Uruguay*, surveillance studies found levels of DON in wheat to be very low in the selected case-study catchment areas over the whole study period. This illustrates the variable nature of mycotoxin contamination and the MYCOTOX project sought to identify the factors responsible for this. *In Chile*, because of the absence of DON in the South, the HACCP team extended the hazard study and established for the first time DON contamination in wheat produced in North Chile. Hazard analysis had also identified high contamination with the fungus *Alternaria* even if no detectable levels of *Alternaria* toxins were found. *In Brazil*, the surveillance studies were performed at the poultry feed mill and the poultry farm. Here it was found that maize in-coming to the feed mill was not significantly contaminated with aflatoxin, and levels did not increase during subsequent steps of the CFD. However, the in-coming maize was found to be contaminated with fumonisin at moderate levels of 3 to 4 µg/g (ppm), and these levels also remained stable.

The samples collected in-field (at different harvests) were analysed by the partner laboratories in each country, which ensured a *strong collaboration between the "field" Work Packages 4&5 and the "lab" Work Packages 1&2&3 in the same country, and*

strengthened the role of the analytical network set-up within the Work Package 1 (see activity 1 above).

Work Package 5. Identification and Validation of Mycotoxin Control Measures

Objectives of WP 5**:** i) To develop and validate control measures to be applied in the CFD, ii) To evaluate the socio-economic, cultural and institutional issues associated with the introduction of control measures at CCPs, and iii) To contribute to a regional policy of Good Practices as a requisite basis for subsequent HACCP plans for mycotoxin prevention and control.

Activity 9. Potential control measures were identified and their advantages/drawbacks were discussed and argued, according to the local context of each South Cone country. Selected control measures were **tested and validated**.

In Argentina, Chile and Uruguay, studies were carried out to assess the resistance of the local wheat varieties to *Fusarium* infection (*Fusarium* Head Blight) responsible for deoxynivalenol production. This was of prime importance for helping the wheat producers through providing advice, recommendations and/or re-orientation of some agricultural practices. *In Argentina*, a study was performed to correlate *Fusarium* Head Blight, DON production and the weather forecasts. A model was tested for development of *Fusarium* index according to the climatic conditions. This work is still under fine-tuning and validation. This computer forecasting approach was also identified *in Uruguay* as being a very important tool in defining strategies for the control of FHB/DON, and future work is foreseen for refining and validating the DonCast system (recommended by a previous FAO programme in Uruguay).

In Uruguay, it was shown that a compromise should be recommended for harvesting date so as to keep DON levels low without high grain losses. *In Chile*, where absence of DON in case-study wheat was confirmed again, it was not possible to validate any control measure as the DON hazard was not present. In North Chile where DON contamination was confirmed for the first time, crop rotation with maize and agro-climatic factors were considered as the main reasons, and were then recommended as control measures. *In Brazil*, the tested and validated control measures were grain drying and cleaning twice, which were favourable to reduction of aflatoxin and fumonisin in corn. The BGYF (Bright Greenish-Yellow Fluorescence) test for grain segregation at the feed mill's entrance and the addition of mycotoxin adsorbent to the feed were suggested as control measures to be tested in the future.

Finally, the use of agro-climatic forecast systems was considered as a priority for mycotoxin control in the Southern Cone region and *this should be stressed with policy makers for future decisions and interventions*.

Activity 10. In addition to the data collected on the CFD in each country (see activity 7 above), **socio-economic participatory studies were done** through questionnaires and surveys with the major stakeholders involved in each selected commodity system. Data was collected on the consumers' perception and awareness of mycotoxin contamination,

their willingness to pay higher price for mycotoxin-free cereal-based products, potential incentives for implementation of quality management systems and local constraints and/or needed services for this implementation, specific institutional and cultural features in each context). The New Institutional Economics approach was applied for extending the single level analysis of players to analysis of a chain system. The costs and benefits of implementing a Quality Management System (QMS) were assessed and the limitations in the cereal chains' governance structure for this implementation were identified. The instruments, e.g. market incentives, regulations and collective action, that are necessary conditions to facilitate the implementation of a QMS were determined. In most cases, the lack of premium price incentives and the need for bottom-up and regulatory interventions were identified as major constraints to the implementation of quality management systems in the respective cereal chains. We should highlight that *the role of socio-economists in the project was clearly efficient in supporting and complementing the technical field activities*.

Activity 11. According to the data collected and collated in each country, and to the local context (technical, socioeconomic, organisational aspects of the cereal chain), a **manual on Good Practices** (Agricultural, Manufacture, Storage) **was jointly elaborated by Argentina, Chile and Uruguay partners for wheat chain, and by Brazil for corn chain**. These manuals compiled advice, recommendations and guidelines on the good practices that should be followed in order to prevent and control efficiently mycotoxin contamination in the Southern Cone region. Recommendations concerned either agricultural practices (choice of resistant variety to *Fusarium*, tillage, fungicide treatment, harvesting date, weather forecasts) or manufacture/storage practices (grain segregation at reception, silo cleaning and emptying, transportation). As the Good Practices are pre-requisite for HACCP plans and Quality Management Systems, the partners agreed upon the need for considering them in an holistic way, and stressed *the need for external interventions driven by authorities and regulatory bodies for ensuring their application by all cereal chain stakeholders*.

The project has used specific case-studies to pioneer the application of a number of methodologies and approaches to the control of mycotoxins in cereal chains in the Southern Cone. The application of HACCP (Hazard Analysis and Critical Control Points), which was initially focusing on technical aspects at lab level, to the whole agri-food chain including stakeholders, and the full integration of socio-economic and technical inputs, has been successfully demonstrated and can therefore be recommended for use throughout the Southern Cone for the control of mycotoxins (and by extension to other types of contaminants) in all cereals and cereal products (and by extension to other types of commodities).

Work Package 6. Development of a Food Quality Management System (FQMS) for mycotoxin prevention and control

> ***Objective of WP 6:*** To develop and implement an integrated and efficient Food Quality Management System along the chain stakeholders to ensure high quality wheat and maize production regarding mycotoxin contamination.

Activity 12. The **generated technical and socioeconomic data were fully integrated** into HACCP plans and the associated pre-requisite Good Agricultural Practices, to elaborate the final architecture of an **efficient Food Quality Management System**, adapted to each local context and helpful for the cereal stakeholders and decision makers. The full integration of technical and socio economic data, along with the identification of gaps and lacks in each country in terms of quality assurance measures, allowed to identify which steps and actions were needed for attaining compliance with the international ISO 9001: 2000 standard and the new Food Safety Management System (FSMS) ISO 22000: 2005. Introduction of traceability systems and certification procedures were also highlighted as actions to be taken on for the same purpose. *This was another challenge of the project as the integration of technical and socioeconomic data generated from field was novel, especially on such a sensitive issue related to food quality and safety*.

Activity 13. To ensure **application of the results and outputs achieved**, the project consortium had a very **dynamic dissemination strategy**, with the strong support of PROCISUR (partner 3) acting as regional platform for output dissemination and organisation of meetings and workshops with the South Cone official bodies (National Ministries for Health and Agriculture, Interprofessional cereal bodies, regulatory offices and the MERCOSUR authorities) and with cereal stakeholders and the academic sector. This was essential according to the expressed needs in the Southern Cone region for considering mycotoxin prevention and control in an holistic way, and for external interventions driven by mandatory regulations and official authorities to ensure the application of Quality Assurance and Management Systems along the cereal chains in the South Cone region. *We should highlight the fact that the project's outputs will serve as decision-making tools for setting an integrated global management of mycotoxin contamination in the Southern Cone of Latin America*.

A great effort was made to disseminate Project findings, including participation in scientific events and production of a large number of scientific documents. The dissemination outputs can be summarised as follows: *scientific peer-reviewed papers* (**14** including **10** published, **2** under revision for publication and **2** submitted, and **12** are under preparation), *book chapters* (**6**), *oral presentations in conferences* (**30**), *posters in congresses* (**42**), *patent applications* (**6** including **3** published and **3** under preparation), *participation in institutional workshops and/or working groups on mycotoxins* (**6**), *dissemination documents* (**4** *bulletins*, **4** *posters*, **5** *flyers and brochures*, **12** *articles in newspapers* and **26** *spots and interviews on TV and radio*), *sectorial meetings with cereal chain stakeholders and official authorities* (**17**), *conferences for dissemination to large audience* (**11**) and *professional training courses dedicated to food inspectors and technologists* (**3)**. In addition, *student training* was a strong component of the project's strategy, with **22** trainees including **8** PhD and **5** Msc, and many exchanges among the partners through scientific stays in the different laboratories for analytical training.

The main publications resulting from the project are the following:

Peer-reviewed scientific papers

1. Samar M.M., Fontan C.F., Resnik S.L., Pacin A.M., Castillo M.D., **2003**. Distribution of deoxynivalenol in wheat, wheat flour, bran and gluten, and variability associated with the test procedure. *Journal of AOAC International*, 86 (3), 551-556.

2. Sarter S., Zakhia N., **2004**. Chemiluminescent and bioluminescent assays as innovative prospects for mycotoxin determination in food and feed. *Luminescence*, 19, 345-351.

3. Brabet C., Salay E., Freitas-Silva O., Alves A.F., Machinski Jr. M., Vargas E.A., Zakhia-Rozis N., **2005**. Gestão integrada de micotoxinas na cadeia produtiva do milho destinado à alimentação de frangos de corte no Brasil. *Cadernos de Ciência & Tecnologia, Brasília*, 22 (2), 439-451.

4. Brabet C., Salay E., Freitas-Silva O., Alves A.F., Machinski Jr. M., Vargas E.A., Zakhia-Rozis N., **2005**. Maîtrise des mycotoxines dans la filière maïs au Brésil. *Cahiers Agricultures*, 14 (1), 164-168.

5. Pacin A., Resnik S., Vega M., Saelzer R., Ciancio Bovier E., Ríos G., Martinez N., **2005**. Occurrence of ochratoxin A in wines in the Chilean and Argentinean markets. *ARKIVOC*, XII, 214-223.

6. Vega M., Castillo D., **2006**. Determination of deoxynivalenol in wheat by validated GC/ECD method: comparison with HPTLC/FLD. *Electronic Journal of Food and Plants Chemistry,* 1(1), 16-20.

7. Muñoz K., Vega M., Ríos G., Muñoz S., Madariaga R., **2006**. Preliminary study of ochratoxin A in human plasma in agricultural zones of Chile and its relation to food consumption. *Food and Chemical Toxicology*, 44, 1884-1889.

8. Samar M., Resnik S.L., González H.H.L., Pacin A.M., Castillo M.D., **2007**. Deoxynivalenol reduction during the frying process of turnover pie covers. *Food Control*, 18 (10), 1295-1299.

9. Broggi L.E., González H.H.L., Resnik S.L., Pacin A.M., **2007**. *Alternaria alternata* prevalence in cereal grains and soybean seeds from Entre Ríos, Argentina. *Revista Iberoamericana de Micología*, 24, 47-51.

10. Broggi L.E., Pacin A.M., Gasparovic A., Sacchi C., Rothermel A., Gallay A., Resnik S., **2007**. Natural occurrence of aflatoxins, deoxynivalenol, fumonisins and zearalenone in maize from Entre Ríos province, Argentina. Accepted for publication in *Mycotoxin Research*, complete references to be assigned.

Oral presentations in conferences

11. Henry G., Salay E., Engler A., **2003**. Integration of socio-economic and food science and technology research in quality management of food supply chains: Mycotoxin control system of grains in the Southern Cone. Invited paper presented at the *V Simposio Latino Americano de Ciencias de Alimentos*, 3-6 November 2003, Campinas-SP, Brazil.

12. Henry G., Iglesias D., Engler A., Salay E., Gutiérrez G., **2004**. Organización de actores alrededor de la gestión de calidad en cadenas agroalimentarias. *In: XIII Seminario Latinoamericano y del Caribe de Ciencia y Tecnología de Alimentos*, 12-15 de Octubre 2004, Montevideo, Uruguay.

13. Vargas E.A., **2006**. Current situation on standardization and validation of analytical methods for mycotoxins in South America. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Cordoba, Argentina.

14. Stewart S., **2006**. Experience from a decision support system approach to reduce DON contamination in Uruguay. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Cordoba, Argentina.

15. Henry G., Engler A., Iglesias D., Gutierrez G., **2006**. Socio-economic constraints and opportunities affecting the implementation of mycotoxin control measures in Southern Cone grain supply chains. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Cordoba, Argentina.

16. Iglesias D., Henry G., Engler A., Gutierrez G., **2006**. Policies for QAS implementation in export chains: mycotoxin management for Mercosur wheat actors. *In: 7th International Conference on Management in AgriFood Chains and Networks*, 31 May-2 June 2006, Ede, The Netherlands.

We should note that strong links and collaborations were made throughout the whole project duration among the consortium members, and especially among the “lab” Work Packages (1&2&3) and “field” Work Packages (4&5&6). The analytical network set-up in WP 1 helped in i) harmonising procedures for sample preparation before analysis and ii) carried out the analysis of the samples collected on-field (within WP 4) to confirm the Critical Control Points (CCPs) identified in the cereal chain. WP 1 & 4 worked together to harmonize the sampling strategy in the 4 countries. The data generated on the human exposure to ochratoxin A (WP 2) and the impact of grain processing on the mycotoxin distribution in the resultant fractions (WP 3) contributed to the hazard analysis

(performed in WP 4) and to the validation of control measures (done in WP 5). WP 6 ensured the full integration of all data generated by the WP 1 to 5 and promoted output dissemination.

We should also highlight the motivation and willingness of all partners to join their efforts for tackling the project's challenges. In spite of the encountered constraints and delays, the partners were very positive and kept strong involvement in the planned activities. Permanent exchanges of information on technical and scientific issues, methods, bibliographic references, relevant websites and scientific events were assured among the consortium members.

INCO: International Scientific Cooperation Projects (1998-2002)

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

Consolidated scientific report

The MYCOTOX project (http://mycotox.cirad.fr) (ref ICA4-CT-2002-10043) entitled "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries" started at the beginning of 2003. It involved partners from France, UK, Argentina, Brazil, Chile and Uruguay.

Context and objectives of the project

Food Safety and Quality is a major research topic due to the increasing concern of consumers with public health related issues, along with the stronger sanitary standards set by the EU for agri product importation. Mycotoxin contamination of food and feed stuffs is among the top priority issues regarding human and animal safety, along with the economic losses they are responsible for. Although several mycotoxin surveys have been performed on cereals in the Latin America South Cone countries, the surveillance data are incomplete and widely dispersed. The control of mycotoxins is currently pursued mainly through quality control and regulatory procedures. However, the analytical methods (mainly chromatographic) currently used for mycotoxin determination need sophisticated equipment and trained analysts, which makes them unsuitable for routine on-field assessment. There is an urgent need for other accurate, simple and cost-effective techniques. There is also an urgent need for a systematic/proactive, cost-effective approach towards the control of mycotoxins throughout the agri-food chains.

The MYCOTOX project addressed the methodological issues associated with the establishment of a Food Quality Management System (FQMS), for controlling mycotoxins in the cereal chains in Latin America South Cone countries. It was based on a multidisciplinary approach, including analytical, technological, socio-economic and chain organisation components in order to ensure, jointly with all stakeholders, mycotoxin prevention and control along the maize and wheat whole chains.

The overall objective of the project was to improve the competitiveness of domestically and internationally traded cereals by controlling the occurrence of mycotoxins in maize and wheat products used as human food and animal feed.

Activities and Results achieved

The project's activities included "lab" activities (within Work Packages 1&2&3) and "field" ones (within Work Packages 4&5&6). The scientific activities carried out, as well as the methodologies used, the major results obtained (and up to mid 2007 regarding dissemination activities and production of scientific papers) are listed below by tasks (within the corresponding Work Package) along the lines of the contract technical annex.

Work Package 1

Development and Standardisation of Effective Analytical Tools for Mycotoxin Determination in Cereals and By-Products

(Leadership: Dr Eugenia Vargas, MAA, Partner 5 (from 2004 to the end of the project) who replaced Dr Tania Corrêa (EMBRAPA, Partner 4) after her retirement (by end of 2003).

Objective of WP 1**:** To develop, validate and make available between all partners accurate, precise, fast, reliable, sensitive, and simple analytical tools, for the detection or determination of mycotoxins, for use during the risk analysis, validation, verification and monitoring components of the HACCP approach to mycotoxin control in maize and wheat.

Task 1. *Inventory of the chromatographic methods available and used at the WP 1 laboratories for mycotoxin determination in wheat and maize*

Information was collected and compiled on the chromatographic analytical procedures used (and/or under implementation or adjustment at the start of project) by each laboratory participant in WP 1 for mycotoxin determination. An inventory of all technical information related to the equipment, method, sampling, extraction, clean-up, analysis and mycotoxin calculation was done. The information was regularly updated throughout the project duration, according to the changes or corrective measures undertaken by those laboratories experiencing low performance. This allowed to build an extensive database on the analytical techniques, experimental conditions, equipments and technical recommendations used by the WP 1 partners.

Task 2. *Harmonisation of the analytical procedures among WP 1 partners*

With the aim to implement inter-laboratory work among WP 1 partners, and given the wide range of analytical methods and procedures used by them, it was essential to standardise and harmonize the procedures for sampling, result reporting and statistical analysis. Protocols were then elaborated for inter-laboratory work between laboratories, internal quality control for each laboratory, and sampling guidance. These protocols were circulated among WP 1 partners and were considered as the work guidelines along the project duration.

Task 3. *Inter-laboratory comparison and proficiency rounds among WP 1 labs using international certified FAPAS reference materials*

Reference FAPAS materials, i.e. standard contaminated matrices with a known mycotoxin concentration, were purchased, by the general project coordinator, in UK (a special discount was obtained for the grouped order) and sent to the partners involved in WP 1. The following reference materials were purchased: i) maize with zearalenone (T 2209), ii) maize with fumonisins B_1&B_2 (T 2208 then T 2211), iii) maize with aflatoxins B_1&B_2&Total (T 0446 then T0453) and iv) wheat flour with DON (T 2210).

An inter-laboratory work was put in place. The WP 1 partners used their own analytical methods and equipments for analysing the FAPAS materials. They reported the results (percentage of recovery and standard deviation) according to the standardised protocols mentioned in task 2. The results were subjected to statistical analysis and calculation of Z score values according to the European Committee for Standardisation (1999). When unacceptable results were obtained, the laboratory concerned made the necessary adjustments and/or modifications for improving its performance, and reported the corrective actions. The partners 9 and 10, even if not initially involved in WP 1, joined the inter-laboratory work in 2004. In addition to the inter-laboratory work within WP 1, the partners 4 (EMBRAPA-Brazil) & 12 (LATU-Uruguay) participated in the international FAPAS rounds, i.e. interlaboratory rounds over the world and showed satisfactory Z

score values.

Task 4. *Inter-laboratory comparison and proficiency rounds among WP 1 labs using home-made reference materials (naturally contaminated & spiked cereals)*
According to the needs expressed many times by the WP 1 partners as well as many Latin American scientists (present at the IV Latin American Congress of Mycotoxicology held in Cuba, September 2003), an essential issue raised within the WP 1 concerned the availability, in the Southern Cone region, of mycotoxin reference materials less expensive than those marketed at the international level such as FAPAS ones. The partner 5, being accredited laboratory, took-up the challenge of preparing homogeneous reference materials, such as i) blank or mycotoxin-free materials, ii) naturally contaminated maize with aflatoxin and zearalenone (by grain mixing in adequate proportions) and iii) spiked cereal samples with DON and ochratoxin A. Note that these reference materials were used internally within the MYCOTOX frame but could be made available to all laboratories within the regional Southern Cone, and by extension to the whole Latin American continent. The WP 1 partners were willing to seek further certification by international and regional scientific authorities for officialising the use of these reference materials in the region.

Specific inter-laboratory work and proficiency rounds were performed in the same conditions than in task 3, on the internally prepared reference materials prepared by partner 5 and sent to all partners involved in WP 1. Instructions were given for analysis in “blind” and “not blind” ways (blind being when the partners analysed the sample without knowing its concentration and not blind when they knew the concentration of the sample prior to analysis. Here again, adjustments were always made for improving lab performance.

The home-prepared reference materials also served for internal laboratory control, with instructions given to the participants for spiking the materials with a fixed amount of mycotoxin and analysing them at the same time than other samples, for internal quality checking.

The inter-laboratory work and proficiency testings helped in setting technical recommendations for mycotoxin determination among the partners. The WP 1 laboratories had a key role in supporting the activities of WP 4&5 through the analysis of samples in-field collected throughout the whole cereal chains.

The project succeeded in setting-up a “small” Southern Cone analytical network with harmonised procedures for aflatoxin, zearalenone, ochratoxin A and fumonisin determination in wheat and maize. The partners were willing to continue this type of networking as it was a priority need for strengthening the regional analytical support to trade and regulatory bodies.

OUTPUTS related to tasks 1 to 4: The deliverable D4 entitled "Standardised and validated analytical chromatographic methods applicable by all partner laboratories for mycotoxin determination in wheat and maize" and the deliverable D12 entitled "Setting up of a network among all partner laboratories for operational exchanges in terms of analytical methods and tools" were achieved. This was one challenge of the project and will help in strengthening the regional analytical support to trade and regulatory bodies.

Task 5*: Prospective research on the inhibition of bacteria luminescence in presence of mycotoxins (as alternative technique for mycotoxin determination)*

The toxicity bioassays, based on the natural ability of some bacteria to emit light, and the potential decrease of bacterial light emission in presence of mycotoxins, were investigated. The bacteria *Vibrio fischeri* was taken as the model and this was incubated either as pure culture or in presence of mycotoxins. The studied mycotoxins were aflatoxin B_1 and deoxynivalenol DON. They were added to the bacterial culture either as diluted standards (at various concentrations) or as mycotoxin-containing cereal extracts. To ensure a security margin for measuring complete light emission, the time 15h was taken as a reaction end-point. The impact of mycotoxin presence was evaluated by calculating the percentage of luminescence inhibition (I%) of *V. fischeri* at the studied concentrations of aflatoxin B_1 and DON standards. The experiments, performed in triplicate showed high reproducibility. The inhibition of *V. fischeri* luminescence was higher with aflatoxin B_1 than with DON. Indeed, aflatoxin B_1 inhibited the luminescence ability by 65% at a concentration of 5 µg/ml, whereas a concentration of 50 µg/ml of DON was needed for inhibiting the *V. fischeri* luminescence by 42%. These results validated our initial hypothesis that the ability of *V. fischeri* to emit light might be used as an indirect means for estimating mycotoxin content.

The toxicity bioassays were also conducted on real cereal matrices such as wheat grain and milling derived fractions (samples collected in Uruguay, Chile and Brazil in the frame of WP 4&5 along with milling fractions collected from the WP 3 PhD of G. Ríos in France). A new degree of complexity was added with the cereal matrix because of the need of an additional step of extraction, which was unnecessary when working with pure mycotoxin standards. Working on real cereal matrices also lowered the reproducibility of the analytical protocol in comparison to the use of mycotoxin standards. A high variability was measured and no direct correlation was evident between the luminescence inhibition (I%) and mycotoxin concentration. This pointed out the difficulty of validating the toxicity bioassays on the real food matrix, which was commonly observed with other analytical methods and various contaminants. This might be due to different reasons: i) the possible interactions between some constituents of the cereal matrix and the mycotoxin itself, ii) the history of the sample between collection, transportation, storage, extraction and toxicity bioassay through luminescence, and iii) the calculation of the inhibition percentage I% as a fraction ratio, aggregated the relative errors of both numerator and denominator, which increased the global uncertainty.

More samples should be analysed and a deeper statistical analysis performed in the future. Experiments will continue at CIRAD (partner 1) beyond the project's end for

determination of EC_{50}, i.e. the mycotoxin concentration that reduces the luminescence by 50%, and a statistical analysis is foreseen according to the Weibull model.

The work performed within WP 1 on toxicity bioassays pioneered the application of this technique to mycotoxins, as most existing literature addressed chemical pollutants in water or wastewater. Our results confirmed the existence of a relationship between the light emission by *V. fischeri* and the mycotoxin concentration. The use of toxicity bioassays through *V. fischeri* luminescence showed to have a potential as semi-quantitative screening tool for rapid discrimination of mycotoxin-contaminated grain bulks or batches. However, the high variability observed should be reduced through further investigation. The MYCOTOX project acted in this sense as an investigation starter.

OUTPUT related to task 5: The deliverable D10 entitled "Implementation of the BCL technique (if successfully validated) in Latin America South Cone laboratories as a rapid tool for monitoring mycotoxin contamination throughout the whole maize and wheat chains" was achieved until a realistic degree. The investigation on this technique took longer than expected for being validated on laboratory scale. The implementation of the technique among the partners was quite ambitious in the duration allocated to the project. This could be achieved through a new proposal to be elaborated by the partners and submitted for further funding. The MYCOTOX project acted in this sense as a starter of investigation.

Task 6: *Prospective research on the application of Near Infrared Reflectance Spectroscopy (NIRS) (as alternative technique for mycotoxin determination)*
The Near Infrared Reflectance Spectroscopy (NIRS) technique is based on the relationship between the chemical composition of the organic matter and its absorption of infrared wavelengths. It is fast and inexpensive but requires a strong calibration phase corresponding to the establishment of a statistical model linking the absorption spectra to the sample chemical composition, and even to some metabolites issued from some mechanisms of sample degradation, by moulds for instance. The aim of this task was to investigate the potential of NIRS for predicting mycotoxin content in cereals and for use as a screening tool in commodity bulk lots.

At the start of project, a preliminary work was carried out by CIRAD (partner 1) and LATU (partner 12) for DON determination in wheat and derived milling fractions using (NIRS). 24 samples were collected by partner 12 in Uruguay: wheat flour (13), ground wheat grain (6), bran (3) and by products (2). Their DON content was determined by HPLC (range 413 to 11322 ppb) and the samples were then sent to France for NIRS analysis. The spectra acquisition was performed in duplicate using a FOSS 6500 spectrometer in reflectance mode, at the wavelength range 400-2500 nm. The high variability of DON content in the samples allowed a good calibration and a clear distinction between the three separate groups of products (grain, flour and by products). The statistical data analysis showed an overfitting of the model because of the limited number of samples. But the NIRS prediction model seemed to be significant and allowed distinguishing the samples with a high DON content from those with a low DON content.

It confirmed the interest in pursuing this investigation, especially by analysing the samples to be collected later in-field within the WP 4&5 activities.

A total number of 113 samples of wheat were then analysed by CIRAD (partner 1) using NIRS method. Those samples included the ones in-field collected (in the frame of WP 4&5) (already analysed by the WP 1 lab in each country using chromatographic techniques) and sent to France by end of 2005, as well as various wheat samples collected in France. The parameterization appeared to have an important effect on the precision of calibrations and the structure of errors. The most suitable model for mycotoxin screening purpose was a 3rd order one with a strong impact of concentration range on the prediction coefficient.

The precision of NIRS calibrations obtained did not allow a precise quantification of DON in wheat samples, but the NIRS technique showed a potential as a tool to be used for sample screening, according to their general contamination level (low, intermediate, high). The main advantages of this technique were rapidity (several hundreds of samples may be analysed per day) and extremely low cost.

The NIRS detection of DON was probably not based on the direct detection of light absorption by the DON molecules themselves since the concentrations were very low (ppb-ppm). It was therefore likely that the detection was made through indirect correlations between DON level and other properties of the sample. This was an interesting finding which should orientate future studies towards the understanding of these relationships and might lead to other rapid screening methods.

Future investigation should also consider higher number of samples for NIRS calibrations, in order to cover the variety of situations that can be met in the field (varieties, growing conditions, post-harvest conditions, etc.) and also type of cereals (others than wheat) and type of mycotoxins (others than DON). We should note that LATU (partner 12), owning an equipment for Near Infra Red measurements, planned to go within further studies on this technique for mycotoxin determination, in collaboration with CIRAD (partner 1).

OUTPUT related to task 6: The deliverable D11 entitled "Validation of the NIRS technique as a rapid tool for quantitative or semi-quantitative monitoring of mycotoxin contamination throughout the whole maize and wheat chains" was achieved until a realistic degree. The investigation on this technique took longer than expected for being validated because of the difficulty to transfer a high number of heavily contaminated cereals from Latin America to Europe. However, the MYCOTOX project confirmed the potential of the technique for the presumed purpose and acted in this sense as a starter of investigation for the future.

Task 7: *Development of the Toximet-T system for rapid and inexpensive tool for routine mycotoxin measurement*

This task was a part of both WP 1 (for analytical aspects) and WP 5 (as a potential control measure throughout the commodity whole agri-chain). The Toximet is a simple,

inexpensive, robust, user-friendly and efficient procedure for accurately detecting and measuring poisonous compounds in foods. A prototype instrument was successfully designed and manufactured which automatically determined the concentration of mycotoxins, immobilised on an especially designed polymer cartridge, by measuring the fluorescence signal generated by the toxin under UV light irradiation. The Toximet-T System (instrument plus cartridge) can be used by non-scientists throughout the cereal production chain and delivers results within 30 minutes of receiving the sample. Preliminary experiments in the laboratory of NRI (partner 2) have shown good levels of accuracy and precision. An international patent, describing the Toximet T System, was published on 23 November 2006. Two UK patent applications were filed, describing polymers for the immobilisation of the aflatoxins (filed on 9 February 2007) and the ochratoxin A (filed on 17 April 2007). Three further UK patents are under preparation. We should note that additional funds to those of the MYCOTOX project were sought for by partner 2 to make possible these achievements. Prof Ray Coker (responsible for Toximet work and leader of WP 4&5) also created the Toximet Ltd. Company (www.toximet.com) for further R&D and commercial exploitation of the Toximet-T technology.

Work Package 2
Risk Assessment of Human Exposure to Ochratoxin A

(Leadership: Dr Ana Pacin, University of Luján, Partner 10)

> ***Objective of WP2*:** To evaluate the risk of human exposure to ochratoxin A (OTA) in Latin America South Cone region

To meet the objective of WP 2, the activities carried out are related to i) OTA determination in blood samples and correlations with the blood donor diet and ii) OTA determination in some foodstuffs (or feed) that could be potential sources with high risk of human exposure to OTA.

Task 8. *Standardisation and implementation of the analytical protocol for OTA determination in human blood*

As decided in the first project meeting at Montevideo, the method of Scott *et al*. (*Survey of Canadian human blood plasma for Ochratoxin A, Food Additives and Contaminants, 15, 555-562*) was selected, slightly modified and applied for OTA determination in human blood samples (collected in two hospitals) and pig serum (collected from a slaughterhouse). This method is based on chromatographic separation (HPLC) with fluorescence detection. Experiments were carried out in Argentina (partners 9 and 10) and in Chile (partner 11) and information was shared for standardizing the method, i.e. extraction, clean-up (using Ochraprep immuno-affinity columns) OTA detection and confirmation, improving the recovery values and method reproducibility, and finally for implementing successfully the method for routine use. Exchanges were made with WP 1 for analytical issues.

OUTPUT related to task 8: The deliverable D5 entitled "A standardised methodology for OTA determination in blood samples implemented in Latin America South Cone Laboratories" was achieved.

Task 9. *OTA analysis in blood samples collected in Argentina*
Within the project, plasma samples were collected from healthy blood donors in two areas of Buenos Aires province. 199 samples (142 male, 57 female) were taken in Mar del Plata area and 236 samples (193 male, 43 female) in General Rodriguez area. All samples collected were analysed for OTA determination by HPLC and OTA confirmation by methyl ester, according to the method validated and implemented in the laboratory. The statistical analysis of data showed that 62.7% from Mar del Plata and 63.8% from General Rodriguez of human plasma samples were positive for OTA; with a median value of 0.11 ng/ml and 0.24 ng/ml respectively (median value is a robust estimator not affected by the extreme values).

The samples from General Rodriguez showed higher OTA levels than those from Mar del Plata. The age group 51-60 showed higher OTA levels than the other groups. The populations of blood donors were homogeneously selected in both regions, in terms of weight, age and race. However, comparison between both areas showed that frequency distributions were different. This might be related to seasonal fluctuations (samples were collected at different seasons) but also to regional or even individual fluctuations. In addition, blood donors in General Rodriguez have lower income than those of Mar del Plata, which could lead to the intake of low-quality and/or more OTA contaminated foodstuffs. The average values encountered in both regions were similar to those reported in other countries.

Task 10. *OTA analysis in blood samples collected in Chile*
88 blood samples were collected from healthy blood donors in the Central-South agricultural area of Chile. 44 samples were collected in Colbún, a rural locality, and 44 in San Vicente de Tagua Tagua, an urban district. OTA was detected in 62 of the 88 samples with a mean level of 0.44 ppb and 0.77 ppb for Colbún and San Vicente de Tagua-Tagua respectively. The levels in the urban district were significantly higher in comparison to those in the rural locality. Both zones showed a different distribution; no significant differences were found in the zone of Colbún while the mean for women was significantly higher than for men in the zone of San Vicente de Tagua-Tagua.

Task 11: *Exploitation of the dietary surveys on food habits and consumption in Argentina and Chile*
OTA-serum albumin conjugate has a half-life that enables exposure over the previous 2-3 months to be estimated. A dietary inquiry consisted of identifying the food intake of the blood donors (in Argentina and Chile) by filling a detailed questionnaire prepared within the project. The questionnaire included information on the age, height, weight of the blood donors along with their food habits (type of foodstuffs consumed, frequency of ingestion, ingested quantities). The data were collated and statistically analysed. The purpose of this work was to identify and quantify those foods known to be susceptible to ochratoxin A contamination and that might be related to OTA presence in blood. This

was to evaluate the risk of exposure to OTA and might help future findings for OTA biomarkers in the blood.

In Argentina, the dietary inquiry showed that cereal-derived products highly contributed to the intake of the population in both areas (Mar del Plata and General Rodriguez). Those products respectively accounted for 82% (bread and bakery stuffs) and 54% (cookies, crackers and confectionery) of the daily intake. Daily intake also was high for beef meat (71%) and infusions (including coffee) (98%). Weekly intake was accounted for 78% (poultry meat), 77% (noodles) and 76% (rice-derived products). Intake of nuts, dried raisins and fruits was generally very low. Beer and wine were more consumed at month-basis in General Rodriguez than in Mar del Plata.

In Chile, correlations with slight significance were found between OTA levels in plasma and food consumption. It was not possible to identify the specific food responsible for the OTA presence in blood, even if cereals were the most likely source of OTA. As OTA levels were higher in the urban San Vicente de Tagua-Tagua, wheat flours were suspected, mainly stored flours. 30 wheat flour samples were collected from different mills in Chile and analysed. The distribution of positive samples showed the highest percentage (56%) for the Northern region of Chile, even the OTA levels were not so high (maximum level of 2 ppb).

The identified foodstuffs most consumed in both countries coincided with those identified as main contributors to OTA intake in Europe (http://europa.eu.int/comm/food/fs/scoop/3.2.7_en.pdf). According to the report of experts (DG Health and Consumer Protection, EC 2002), the Klassen equation was used (with the conversion factor 1.97 from average plasma concentrations) to express the continuous dietary intake of OTA. This latter was respectively 0.21 and 0.47 ng/kg body weight per day in Mar del Plata and General Rodriguez (Argentina) and respectively 0.84 and 1.40 ng/kg body weight/day in Colbún and San Vicente de Tagua-Tagua.

Task 12. *Standardisation and implementation of the analytical protocol for OTA determination in wine*

Because of the high level of wine consumption in Argentina and Chile, the awareness increased about the potential risk of this commodity as a potential source for consumer exposure to OTA. Besides, according to the existing literature on analytical methods for OTA determination in wine, it was easier to start with wine as a first step and to develop further the analytical method for OTA determination in cereals and other commodities.

The chromatographic HPLC method with fluorescence detector was applied in both Argentina and Chile labs, but using different equipments. The analytical protocols were also different in Argentina (*Castellari et al., 2000, J. Of Chromatography A, 888, 129-136*) and Chile (*Visconti et al., 1999, J. Of Chromatography A, 864, 89-101*). To improve recovery, experiments were carried out in both countries on OTA-free wines spiked with different OTA levels. A total of 85 wines were analysed (54 from Argentina, 14 from Chile, 8 from Italy, 5 from Spain, 2 from France and 1 from South Africa). The results showed that none of the red wine produced in Chile or Argentina was

contaminated with OTA. This was in agreement with previous reports showing a low toxigenic capacity for those fungi isolated from grapes grown in the region. Calculations were made to assess the mean daily OTA intake through wine consumption in Argentina and Chile. The values obtained for human exposure to OTA in those countries were compared to the Tolerable Week Intake set by the JECFA (Rome) in 2001 and to the Maximum Tolerable Daily Intake set by the European Commission in 1998. This finding allowed to eliminate wine as potential source for human exposure to OTA in Argentina and Chile.

Task 13. *Standardization and validation of the analytical protocols for OTA determination in wheat, wheat flour and pork*
The chromatographic protocol was standardised among partners 9, 10 and 11 for OTA determination in wheat. Experiments were carried out for optimising the method sensitivity through the elaboration of calibration curves and limits of detection and quantification, along with the method repeatability and recovery. The partner 11 also determined OTA in 30 wheat flour samples which were collected from different mills and retails in Chile and analysed. These samples presented a high incidence of ochratoxin A, but at levels not exceeding 2 ppb. The distribution of positive samples showed the highest percentage (56%) for the Northern region of Chile.

In addition, further to the exploitation of the food diet and habits, pork and derived products were suspected to be a source of human exposure to OTA in Chile. The analytical technique liquid-liquid extraction and HPLC detection) was developed, validated and implemented in the laboratory (partner 11) for OTA determination in pork products. Pork samples (muscle, kidney and liver) were collected from the market and analysed. The OTA levels ranged from 0.11 to 0.24 ppb, which suggested a slight contribution to OTA presence in blood samples. The studies on OTA in pork are continuing in Chile beyond the end of project.

Task 14. *Screening of fungal flora in some grains (cereal, oilseeds) as potential source of contamination with OTA in Argentina*
Studies were undertaken in Argentina for identifying the fungal flora able to contaminate different types of cereals (wheat, maize, soybean, rice, oat and sorghum) in general and OTA-producers in particular, as a means for assessing the probability of OTA occurrence in the derived foods of those cereals. A first screening was done in the principal provinces of the country, and deeper studies were then performed in the Entre Ríos province which was selected as the biggest grain producer in Argentina. The grains were screened for fungal contamination (mycological counts), in order to have an integrated view on the risk of human exposure through their consumption and so the cumulative impact of the intake.

Freshly harvested grains did not appear to be a potential source of OTA, unless in the presence of some toxigenic isolates of *Aspergillus niger.* However, *Alternaria alternata* was the most common fungal species associated with wheat, sorghum, rice and soybean in the Entre Ríos province. This suggested that there was a need for the future to assess the mycotoxigenic ability of these species and the eventual production of *Alternaria* toxins in the grains and derived products.

We should note that this correlation between the fungal flora and mycotoxin production was a very interesting investigation line that was not initially foreseen in the technical annex of the project but which emerged as a investigation priority during the project's running. These studies were initially limited to Argentina, but were later extended to include Uruguay and Chile. They also closely contributed to the hazard analysis performed within Work Package 4.

The pioneering work on the occurrence of ochratoxin A in the Southern Cone region generated original data, contributed to HACCP hazard analysis (WP 4&5), helped in identifying new investigation lines, and contributed to the training efforts in the partner countries. Various papers were produced. The WP 2 outputs provided baseline studies and recommendations for helping the health-related stakeholders in decision-making and action planning to overcome the risk of human exposure to ochratoxin A in the region.

OUTPUT related to tasks 9 to 14: The deliverable D7 entitled "A risk characterization for OTA in Latin America South Cone countries" was achieved.

Work Package 3
Evaluation of Milling Procedures as Potential CCPs
(Leadership: Dr Silvia Resnik, University of Buenos Aires, Partner 9)

> ***Objective of WP 3*:** To evaluate distribution and variability of deoxynivalenol (DON) in wheat and fumonisins in maize in the fractions obtained through wet and dry milling processes in the Southern Cone.

Task 15. *Reporting of the wheat and maize milling diagrams in Argentina and Chile*
As first step of the WP 3, the diagrams of wheat and maize milling *in Argentina and Chile* were reported and fully analysed. It was interesting to point out that the industrial milling processes were different in the two countries. *In Chile*, only dry milling was usually practiced, either for wheat or maize. *In Argentina*, wet milling was very common for maize. The technological unit operations and steps of both process diagrams were fully understood and the mass balance sheets were elaborated. It was pointed out that DON determination was needed in grains and all resultant milling fractions, i.e. flour, starch, gluten, bran and germ.

Task 16. *Standardisation and implementation of the analytical protocol for deoxynivalenol (DON) determination in wheat and derived milling fractions*
In Argentina, the gas chromatography method with electron capture detection (GC-ECD) was under use for DON determination in wheat and derived milling fractions. For standardisation purpose and to optimise the project financial resources, the extraction and clean-up of analytical samples were carried out at the University of Luján (partner 10) according to the slightly modified method of Trucksess *et al*. (*J. AOAC Int., 1996, 79, 883-887*). The further derivatization step, as described by Croteau *et al*. (*J. Agric. Food*

Chem., 1994, 42, 928-934) and the DON quantification by gas chromatography were made at the University of Buenos Aires (partner 9).

In Chile, the University of Concepción (partner 11) first used the planar chromatography HPTLC method for DON determination, according to their available equipment. However, a new GC-ECD apparatus was purchased by the University by end of 2003. The partner 11 implemented then this new analytical tool and the same method was then used by the partners 9, 10 and 11. Here again, the standardisation and validation of the analytical protocol benefited from the links with WP 1 activities through the inter-laboratory work done on DON in wheat.

Task 17. *Standardisation and implementation of the analytical protocol for fumonisin determination in maize and derived milling fractions*
The method for fumonisin determination in maize and derived milling fractions was standardised and implemented in the laboratories of partners 9, 10 and 11. The extraction and clean-up of analytical samples were made according to the slightly modified method of Trucksess *et al*. (*J. AOAC Int., 1996, 79, 883-887*). Fumonisin was determined according to the standard AOAC method (*AOAC Official Methods of Analysis (2000) Chapter 49, Subchapter 5, pp. 44-45. Fumonisins B_1, B_2, B_3 in Corn, AOAC Official Method 995.15*).

Task 18: *Standardisation of procedures for wheat sampling during dry-milling process in Argentina and Chile*
A precise and accurate determination of DON contamination in wheat and derived products is essential to assess the extent of human exposure to this mycotoxin. The difficulty in obtaining a precise value is associated with the variability in the results from the test procedure caused by the heterogeneous distribution of DON contamination. Few grains may be contaminated and some of them might contain high levels of mycotoxin.

Procedures for wheat sampling during dry-milling were tested and optimised *in Argentina and Chile* according to the milling diagrams used in each country. *In Argentina*, samples were taken at regular intervals during the milling of a single 13 ton lot of naturally contaminated wheat (from the 2001 crop) in an Argentinean industrial mill (Santa Fe province). Bulk samples (wheat sample weight = 3 kg, derived milling fractions = 1 kg) were each divided into 6 test samples of equal weight and DON test was performed on an analytical sample of 25 g taken from each sample.

In Chile, samples were taken at regular intervals during the milling of a single 16-ton per hour wheat lot (from the 2003 crop) in the industrial El Globo mill (South Chile). Bulk samples (wheat sample = 3 kg, flour sample = 9 kg, bran = 9 kg and germ = 3 kg) were each divided into 6 test samples of equal weight and DON test was performed on an analytical sample of 25 g taken from each sample. Additional 65 wheat grain samples (from the 2003 crop) were also taken from another industrial mill (Collico mill – Valdivia, South Chile) and their DON content was determined.

The partner 9 tested the same sampling on maize during industrial dry-milling in the Entre Ríos province. Chile was not concerned by maize dry-milling.

This task closely contributed to the WP 4&5 activities (hazard analysis and validation of control measures) through the sampling design and by the fact that partners 9&10 (Argentina) and 11 (Chile) performed the DON analysis on the wheat samples collected in-field within WP 4&5.

Task 19: *Statistical analysis of the DON distribution in wheat and resultant dry-milling fractions*

An ANOVA statistical test was made on the data obtained in Argentina from DON determination in wheat and resultant milling fractions. This showed differences among the samples probably caused by the sampling variability. The mean squares within the samples might be associated with the total variance, i.e. the variance related to combined sampling + sample preparation + sample analysis. On the other hand, the repeatability of the gas chromatography method was shown to be acceptable by calculating the Horrat value for each analytical sample.

The statistical function expressing the DON distribution presented an asymmetric tail for high concentration values in wheat grains and wheat flour. In bran, it showed bimodal curve with 2 separated peaks of different concentrations. In gluten, the normal distribution function gave a reasonably good fit to empirical data. The obtained data on the variability and distribution of DON in wheat and resultant milling fractions would help to improve the design of sampling plans, the selection of sample size or number of samples needed, in order to reduce the total variability. The same statistical tools were later used by the partner 11 in Chile.

OUTPUT related to tasks 15 to 19: the deliverable D1 entitled "Standardised procedures for wheat and maize sampling during milling processes in the Southern Cone region" and the deliverable D17 entitled "Better knowledge of DON and fumonisin variability in wheat and maize in Latin America South Cone" were achieved.

Task 20. *Determination of fumonisins in maize and resultant wet-milling fractions in Argentina*

Chile was not concerned by the work on maize wet-milling, as this process was not usual in Chile. In addition, a few contamination with fumonisin has been reported in the Chilean maize.

Preliminary experiments were carried out in Argentina for fumonisin determination in 100 maize sub-samples. The distribution of fumonisins in maize showed a great asymmetry. The maize wet milling process was then studied at laboratory scale, with a systematical determination of the fumonisin content at every stage of the process. The analytical method for fumonisin determination was adjusted and validated for each type of resultant fraction. The fumonisin distribution was also estimated and the statistical function associated with the variability of total fumonisins, fumonisins B_1 and B_2 in corn and bran was defined.

The maize wet-milling was later studied at pilot scales. 21 samplings were made throughout the process. The 153 collected samples were analysed, they showed to be negative for DON, zearalenone, ochratoxin A and aflatoxins, but positive for fumonisin. The fumonisin (total, B_1 and B_2) content was determined in all resultant fractions, i.e. flour, starch, wash water, gluten meal, steep water, germ, fibre or gluten feed. During wet-milling, in the order of 94% of fumonisin was LOST, literally. Not only was it absent from the flour, but also it could not be found in the bran or steep water. The proposed hypothesis was that fumonisin might bind more strongly to the maize matrix because of heating during milling, which might form derivatives. Fumonisin derivates have been cited in the literature. This opened the way towards a potential investigation on the application of a *in vitro* digestion model to assess the bioavailability of fumonisins from wet-milled maize fractions.

In 2006, the lab-scale study was validated at industrial level, in collaboration with a corn milling plant having a capacity of 1.000.000 ton per year. The fumonisin reduction was then validated, even at lower ratio (around 90%) than at lab-scale, probably because more thorough washings done at lab-scale. Starch and gluten were shown to have very low fumonisin content. This finding suggested that a HACCP corrective action on highly contaminated maize with fumonisin could be to orient its use towards production of starch and gluten, instead of human or animal consumption. This was a very interesting alternative that might be transferred to the corn industry.

The MYCOTOX project acted as a starter for this investigation. The work is still running beyond the project's end. The PhD student, Estela Motta (Research Assistant from the University of Mar del Plata) continues working under the supervision of the partners 9 (University of Buenos Aires) and 10 (University of Luján) and in collaboration with the Bureau of Chemical Safety and Health (Ontario, Canada).

Task 21. *Impact of maize cleaning by sieving before storage and/or dry-milling in Argentina*

The impact of maize cleaning by sieving before storage and/or dry-milling was first evaluated at lab scale. Different sieve mesh sizes (4, 6 and 8) were tested. This resulted in the small particles containing higher levels of mycotoxin than the bulk. This applied when monitoring aflatoxin, zearalenone, DON, and fumonisin. Mycotoxin levels were shown to reduce by maize sieving at the storage entrance. In 2006, the experiments were conducted at semi-industrial scale and confirmed this trend. Sieving might be then considered as an effective segregation control measure for application at a critical control point (CCP) when developing a HACCP Plan (within WP 5). The obtained data helped in setting technical recommendations to the cereal industry. Data validation and transfer to the maize industry is to be done in the near future, so a practical compromise can be found between a satisfactory ratio of mycotoxin reduction and an acceptable grain weight loss, according to the specific end-use of the grain.

Task 22. *Effect of the frying process on the DON reduction in highly-consumed wheat-made pies ("empanadas") in Argentina*

Wheat and derived products are highly susceptible to DON contamination. "Empanadas" are wheat-made, filled and fried, and highly-consumed pies in Argentina. They are fried

using a traditional equipment which allows reaching a temperature ranging from 169°C to 243°C. A study was undertaken for assessing the impact of frying on the DON reduction in the turnover pie cover. The home-made frying conditions and colour of the final pie were taken as reference. The observed DON reduction depended on the frying temperature, ranging from 28% at 169°C, 21% at 205°C and 20% at 243°C. The results allowed to recommend empanadas' frying at the lowest temperature possible with the home-frying equipment.

The daily average consumption of empanadas in Argentina ranges between 135 g and 217 g. The Provisional Tolerable Maximum Daily Intake (PTMDI) according to the JECFA (FAO) is of 1 µg/kg body weight/day; therefore, calculating for a person of 70 kg weight who consumes 217 g of empanadas daily, the maximum DON contamination of the pie should not exceed 322.6 µg/kg. These findings were of prime importance because they stated the critical limits for DON contamination in wheat flour, and by extension, would help in developing HACCP Plans and recommending guidelines to the whole milling process.

Task 23. *Influence of the grain fractioning and its structural properties on the DON distribution in the grain and resultant fractions (joint work Chile-France)*
The PhD student (Gisela Ríos, Research Assistant from University of Concepción, partner 11) continued working with CIRAD (partner 1) in France, in close collaboration with the French INRA (Institut National de Recherche Agronomique), a research institution specialist in cereals. Collaborations were made with INRA Montpellier (for technological aspects), INRA Bordeaux (for molecular biology aspects) and INRA Rennes (for resistance to *Fusarium* infection).

The objectives of the work are:

1 to evaluate the impact of different grain processing (milling and fractioning) on the DON (deoxynivalenol) distribution in the resultant fractions. This will allow identification of the critical limits for each type of wheat grain processing and to propose adapted control measures.
2 to determine the relationship between the wheat grain characteristics (i.e. structural, physical and biochemical) and its sensitivity to *Fusarium* infection. This will allow identification of potential control measures based on cultivar selection for a "protective" effect of the peripherical layers (pericarp, testa, aleurone) against *Fusarium* infection and DON distribution.

Concerning the first objective, experiments were made to explore the impact of different wheat fractioning processes on the DON distribution in the resultant fractions. Two contaminated batches of durum wheat (*Acalou* cultivar), with distinct DON content (400 and 4000 ppb), were used.

Milling was shown to favour the contamination of the inner parts of the grain. Two hypotheses were then stated: i) during milling, the inner parts might be contaminated through strong mechanical contact with the more contaminated outer parts of the grain; ii) *Fusarium* penetration and/or DON migration inside the grain may damage the inner tissues which would be more sensitive to milling and might lead to more contaminated

inner fractions. Experiments on re-milling and sieving (different mesh) the fractions issued from the inner parts of the grain showed that higher DON content was always found in the finest fractions. In experiments on the highly contaminated batch of wheat (DON 4000 ppb), the highest levels of DON were found in the milling fractions corresponding to the inner and central parts of the grain.

In parallel, DNA was extracted from all milling fractions and amplified by qualitative PCR using primers corresponding to the tri5 gene involved in the toxin synthesis. *Fusarium* presence was detected in all milling fractions. This confirmed that DON was present even in the innermost parts of the grain, and validated in some way both hypotheses. Experiments are under way using quantitative PCR for further quantification of *Fusarium* presence in all fractions. However, it can be stated that milling would not be the best process for reducing DON distribution within the flour fractions.

Experiments were also done for studying another grain fractioning process. *De-hulling* was carried out on the same *Acalou* wheat batches through progressive abrasion of the wheat grain for 30 min. De-hulled samples were taken out at time intervals and analysed for DON content. At the same de-hulling time and for the same percentage of mass loss in the grain, higher DON percentage remained in the more contaminated batch (4000 ppb). For a de-hulling yield of 75% (grain remaining after abrasion), only 35% of the initial DON content remained in the grain, whereas for the same milling yield, 50% of DON remained. This finding showed that de-hulling was very useful for fractioning a very contaminated wheat grain, and might find application as a control measure within a HACCP Plan.

Experiments are under way for studying the impact of grain conditioning (water content, duration) prior to dehusking on the DON distribution in the outcoming fractions.

The impact of grain *grading by size* on the DON distribution within the batch was also studied as important step of wheat processing. Grain was sieved on different mesh size (2.2, 2.4, 2.9, and 3.5) and the DON content was determined in the fraction corresponding to each sieving mesh. The grain size was shown as a critical parameter; indeed, removing only 7% of the grain mass, containing grain with the smallest mesh sizes, corresponded to an average reduction of 25% of the DON content (22.4% for the 400 ppb batch and 27.2% for the 4000 ppb batch). Grain grading by size seemed to be a promising control measure technology, with application for the wheat stakeholders (mainly at storage and industry levels) within a HACCP Commodity Flow Diagram (CFD).

Concerning the second objective of the PhD work, experiments are under progress on the relationship between the structural and biochemical characteristics of the wheat grain and its resistance to *Fusarium* infection. Various soft wheat cultivars, with different resistances to *Fusarium,* were artificially infected at different dates of the spike development (collaboration with INRA Rennes). The harvested grains are under analysis with the aim to find out any “protective” effect of some constituents of the grain against *Fusarium*/DON contamination.

Here again, the MYCOTOX project acted as a starter of investigation. The research carried out will continue beyond the project, through the PhD work of Gisela Ríos and the strong collaboration of CIRAD (partner 1), INRA (associate institution) and UDEC (partner 11).

OUTPUT related to tasks 20 to 23: the deliverable D8 entitled "Identification of the impact of different milling processes on the distribution of mycotoxin contamination in the different cereal fractions" was achieved.
The two research lines "impact of wheat processing on the DON distribution in the outcoming fractions" and "bioavailability of fumonisins in the wet milled maize fractions" are continuing beyond the project's end.

Work Package 4: Hazard Analysis of Mycotoxins
(Leadership: Prof Ray Coker, NRI, partner 2)

> ***Objectives of WP 4*****:** i) To identify cereal commodities-mycotoxin combinations that present an unacceptable risk to human health or present a constraint to trade and ii) To construct and verify the corresponding Commodity Flow Diagrams (CFDs).

Task 24. *Establishment of a multidisciplinary HACCP (Hazard Analysis and Critical Control Point) team in each Southern Cone country*
HACCP multidisciplinary Teams were assembled in Brazil, Uruguay, Argentina and Chile, composed of agronomists, socio-economists, HACCP specialists, phyto-pathologists, analysts and representatives of the key players and private sector which got involved in the project. Where necessary, specialist advice and support was provided to the HACCP Teams by CIRAD, France (partner 1), NRI, UK (partner 2) and National Food Administration, Sweden (through the scientific advice of Dr Monica Olsen). In addition, a representative of each multidisciplinary team per country partner followed a FAO training (delivered in Spanish) on the HACCP method and its specific application to mycotoxin prevention and control. The direct benefits of this training were: i) the harmonization of the HACCP glossary and methodological tools among the project partners, ii) the share of Latin American experiences and case studies on HACCP application to mycotoxins, and iii) the access to the same manuals and methodological documents.

Task 25. *Collection and collation of data from literature*
Information on the occurrence of mycotoxins in wheat and corn were collected, gathered and collated in the 4 countries partners. This literature was obtained from a variety of published sources including, for example, refereed papers, learned journals, specialist books, conference proceedings, monographs, institution reports, databases, and PhD theses.

Data were also collected and collated from other sources. Unpublished material and informal information on the occurrence of mycotoxins in wheat and corn was also

obtained from the chain key players (e.g. producers, manufacturers, processors, wholesalers, consumers, etc) along with consultant analysts, government departments and from commercial companies involved in the production and processing of these commodities. For each country, the mycotoxin occurrence data were initially summarised as a tabulated bibliography and were then collated to show key parameters for each data source, including year of study, commodity-mycotoxin(s) combinations, number of samples collected, sampling methodology, mycotoxin level/range and percentage of contaminated samples.

OUTPUT related to tasks 24 and 25: The deliverable D2 entitled "A report documenting mycotoxin surveillance data, both from the literature and from surveillance studies conducted by this project" was achieved.

Task 26. *Identification/selection of the priority mycotoxin-commodity combinations*
The bibliographic and collated data were carefully examined. The priority combinations (mycotoxin-commodity) and the pilot sites were chosen as a selected case-study in each country. These were as follows:

1 Argentina: deoxynivalenol in wheat for flour production;
2 Chile: deoxynivalenol in wheat for flour production;
3 Uruguay: deoxynivalenol in wheat for flour production;
4 Brazil: aflatoxins, ochratoxin A, fumonisins, deoxynivalenol & zearalenone in corn for poultry feed production.

In the following, the selected mycotoxin-commodity combinations will be referred to as "Selected Commodity Systems".

OUTPUT related to task 26: The deliverable D3 entitled "A report describing the hazard analyses conducted and justifying the commodity-mycotoxin combination selected for further study" was achieved.

Task 27: *Establishment of collaborations with the main key players within the Selected Commodity System. This task was also part of Work Package 5.*
In each Southern Cone country partner, contacts were made with the main key players in the selected commodity system, to share the mycotoxin issue with them and convince them to collaborate within the project. It should be noted that, at the start of project, the sensitivity of some of key players, regarding the mycotoxin contamination of their products, delayed (or sometimes cancelled) their effective commitment to participate in the project's activities. The key players were also identified for their motivation and willingness to share activities. Once the key players committed, confidentiality agreements were prepared and validated by all partners to legitimise their involvement in the project and define the mutual activities and outputs. The committed enterprises agreed to receive the project teams and trainees for carrying out joint activities on the CFD (Commodity Flow Diagram) elaboration and the HACCP application.

Task 28: *Construction of the Commodity Flow Diagram (CFD) for each selected commodity system*

In each Southern Cone country partner, the HACCP team elaborated the Commodity Flow Diagram (CFD) for the selected commodity system. The CFDs collected detailed data on the agricultural practices, the raw material supply, the processing and transport channels and stakeholders, the existing quality control procedures, preliminary information on the socio-economic context such as commodity volumes, fluxes and prices, seasonal trade fluctuations and supply chain organisation. The CFDs were regularly reviewed, updated and adjusted, in close collaboration with the key players of the concerned cereal chain, to take into account the evolution of the local context and constraints, and according to the additional information brought by the surveillance studies (see tasks 29 and 30).

OUTPUT related to tasks 27 and 28: the deliverable D6 entitled "A report documenting the verified commodity flow diagrams for each commodity/mycotoxin combination in each of the selected countries" was achieved.

Task 29. *Design and implementation of a surveillance study for each selected commodity system*

This surveillance study aimed to identify at which step in the CFD the mycotoxin hazard entered or increased to unacceptable levels throughout the whole selected commodity system, and hence where additional steps were required to prevent, eliminate, or reduce the hazard to an acceptable level. Each HACCP team defined (with strong support from partner 2, leader of WP 4&5) the main points of the selected commodity system where sampling was essential for monitoring mycotoxin contamination. A sampling plan (procedures, number of samples, sample preparation before analysis) was prepared, shared and harmonised among the consortium members before implementation. The samples collected in-field at different harvests were analysed by the partner laboratories in each country. This close collaboration between "field" (WP 4&5) and "lab" (WP 1&2&3) participants in the same country was very efficient and constructive for the project and strengthened the role of the analytical network set-up within the Work Package 1 (see tasks 1 to 4).

Task 30. *Implementation and follow-up of the surveillance studies in each selected commodity system and update of the Hazard Analysis when needed*

The surveillance studies were implemented in the four Southern Cone countries. They allowed, for each commodity system in each country, identification of the potential Critical Control Points (CCPs), i.e. the steps in the CFD where contamination by moulds and/or mycotoxin production might occur or increase to an unsafe level, and hence where control measures were required.

In Argentina, Chile and Uruguay, surveillance studies found levels of DON in wheat to be very low in the selected case-study catchment areas over the whole study period. This illustrates the variable nature of mycotoxin contamination and the MYCOTOX project sought to identify the factors responsible for this.

In Argentina, surveillance studies were performed to evaluate DON content in different wheat varieties grown at experimental fields of partner 8, as well as wheat samples

collected from local farmers in General Pico (pilot site of the project) and at mill reception. DON was present in most samples, even at low levels. The mycological analysis identified *Alternaria alternata*, *Fusarium graminearum*, *Fusarium poae* and *Fusarium semitectum* as predominant endogenous fungal flora. The presence of nivalenol mycotoxin in these samples was also detected, for the first time in Argentina.

In Chile, because of the absence of DON in the South, the HACCP team extended the hazard study and established for the first time DON contamination in wheat produced in North Chile. Reasons to explain this contamination had been proposed including agro-climatic factors and wheat rotation with maize. Hazard analysis had also identified high contamination with the fungus *Alternaria* even if no detectable levels of *Alternaria* toxins were found.

In Uruguay, because of the low DON levels observed in wheat grain during the 2004 and 2005 harvests, a new surveillance study was designed and implemented on wheat flour contamination to extend the hazard analysis to other mycotoxins than DON. Eighty-four (84) wheat flours samples collected from the market were analysed for different mycotoxins (DON, total aflatoxins, zearalenone, ochratoxin A (which could likely be produced during storage) and ergot alkaloids. All samples showed low mycotoxin content, below the analytical quantification level.

In Brazil, the surveillance studies were performed at the poultry feed mill and the poultry farm. Samples of corn grain, corn flour and feed (end-product) were collected and analysed for aflatoxins, fumonisins and zearalenone. Here it was found that maize in-coming to the feed mill was not significantly contaminated with aflatoxin, an levels did not increase during subsequent steps of the CFD. However, the in-coming maize was found to be contaminated with fumonisin at moderate levels of 3 to 4 µg/g (ppm), and these levels also remained stable.

All these surveillance studies allowed to gather a high number of data on the different steps of the wheat and corn chains. These data served as a basis for elaborating the HACCP plans, the Manual of Good Practices and the Food Quality Management System (FQMS). Those three outputs are of prime importance for further use as recommendations, guidelines and manuals by the cereal chain stakeholders, policy makers and regulatory authorities, in each Southern Cone country.

OUTPUT related to task 29 and 30: The deliverable D14 entitled "Data describing at which steps in the CFD the mycotoxin hazard originates, or at which steps concentrations increase to unacceptable levels" was achieved.

Work Package 5
Identification and Validation of Mycotoxin Control Measures

(Leadership: Prof Ray Coker, NRI, partner 2)

***Objectives of WP 5*:** i) To develop and validate control measures to be applied in

the CFD, ii) To evaluate the socio-economic, cultural and institutional issues associated with the introduction of control measures at CCPs,and iii) To contribute to a regional policy of Good Practices as a requisite basis for subsequent HACCP plans for mycotoxin prevention and control.

Task 31. *Socio-economic overview on the selected commodity systems*
In order to facilitate a thorough understanding of the socio-economic, cultural, organisational and institutional issues related with the introduction of control measures and implementation of Quality Management Systems for mycotoxin control, socio-economic studies were implemented. The socio-economists involved in the 4 HACCP teams jointly worked on the methods required for an expansion of single level analysis of players, to analysis of a chain system had been identified and applied. Questionnaires were elaborated and the key players of each selected commodity system were interviewed. Data was gathered on the supply chain organisation, relationships between stakeholders, price and trade issues, incentives and constraints for further implementation of quality management systems. This data was complementary to that gathered in task 28 (within WP 4) and it served as valuable input for the CFDs validation and for the development of research hypothesis, based on the New Institutional Economics theory.

Surveys were also conducted *in Argentina, Uruguay and Chile* with wheat mills for establishing current Quality Management Systems (QMS) and identifying the constraints (logistics, additional costs) for implementation of HACCP and ISO standards. Consumer surveys were also carried out *in Uruguay* and showed that the consumer might be willing to pay up to 20% more for mycotoxin-free wheat flour. *In Argentina*, real on-farm additional costs for HACCP implementation were calculated. *In Brazil*, surveys were continued along the selected commodity system (corn chain for poultry feeding) to identify the incentives for better corn quality. This case was specific as the chain was vertically integrated, from the corn production to the feed industry.

The costs and benefits of implementing a Quality Management System (QMS) in the cereal commodity chain in each Southern Cone country were assessed. The limitations in the commodity chain's governance structure to implement a QMS were identified. Finally, the instruments, e.g. market incentives, regulations and collective actions, that were necessary conditions to facilitate the implementation of a QMS were determined and recommendations were made.

In most cases, the lack of premium price incentives and the need for bottom-up and regulatory interventions were identified as major constraints to the implementation of quality management systems in the respective cereal chains.

The performed socio-economic studies clearly supported and complemented the technical activities within WP 4&5. The socioeconomic data was fully integrated to the technical data, helping in the elaboration of the final architecture of adequate Food Quality Management Systems (FQMS) for mycotoxin prevention and control in the Latin America Southern Cone (within WP 6).

OUTPUT related to task 31: the deliverable D9 entitled "A report describing the socio-economic studies conducted and the associated findings" was achieved.

Task 32. *Testing and validation of control measures that will prevent, eliminate, or reduce mycotoxin contamination to an acceptable level, when applied to a specified step in the CFD*

The technical and socio-economic inputs along with the CFD's validation allowed the identification of the critical control points (CCPs) for each selected commodity system. These were the points where the contamination by moulds and mycotoxin production might occur or increase to an unsafe level. The CCPs were then the chain steps where control measures should be undertaken. Potential control measures were listed, and their advantages/drawbacks discussed and shared among partners, according to the local context in each South Cone country. With the advice of partner 2 (leader of WP 4&5), the most adequate control measures were selected for testing and further validation.

In Argentina, Chile and Uruguay where the wheat/DON combination was selected as a priority commodity system, studies were carried out to assess the resistance of the local wheat varieties to *Fusarium* infection (*Fusarium* Head Blight) responsible for deoxynivalenol production. This was of prime importance as potential control measure for helping the wheat producers through advice, recommendations and/or re-orientation of some agricultural practices.

In Chile, where absence of DON in wheat was confirmed again, it was not possible to validate any control measure as the DON hazard was unexisting. The HACCP team established for the first time DON contamination in wheat produced in North Chile. Reasons to explain this contamination had been proposed and these included rotation with maize and agro-climatic factors, which were then recommended as control measures. Fungal studies showed high *Alternaria* contamination of wheat. Further studies will focus on this hazard, even if no evident correlation is still established between *Alternaria* and DON-producing *Fusarium*.

In Argentina, a study was performed to correlate *Fusarium* Head Blight, DON production and the weather forecasts. A model was tested for development of *Fusarium* index according to the climatic conditions. This *Fusarium* index could be used as a basis for a control measure strategy (either as CCP or Good Agricultural Practice). This work is still under fine-tuning and validation, and will continue beyond the project's end. This computer forecasting approach was also identified *in Uruguay* as being a very important tool in defining strategies for the control of FHB/DON, and future work is foreseen for refining and validating the DonCast system (recommended by a previous FAO programme in Uruguay).

In Uruguay, the impact of harvesting date on the DON contamination in wheat was studied as a potential control measure. Results showed that delaying the harvest for 3 or 4 days after physiological maturity led to lower DON content in the harvested grain. This allowed to recommend an optimal harvesting date as a compromise between and

technical (physiological maturity of grain and low DON levels) and economic issues (low grain losses). The validation of other identified control measures such as the use of gravity table and grading according to the percentage of pink wheat kernels at mill reception had been cancelled, because of the low levels of DON in wheat at the last harvests. Even though, the grain segregation at reception stage (either for trader or mill) and at silo stage (grain movement transfer between silos or mixture within the same silo) was still recommended as control measure. Mixing flours with different (high and low) DON levels might be also used as control measure.

In Brazil, the tested and validated control measures were grain drying until adequate water content and activity, as well as cleaning twice, which were favourable to reduction of aflatoxin and fumonisin in corn. The BGYF (Bright Greenish-Yellow Fluorescence) test for grain segregation at the feed mill's entrance and the addition of mycotoxin adsorbent to the feed were proposed as additional control measures to be tested in the future.

Finally, the use of agro-climatic forecast systems was considered as a priority for mycotoxin control in the Southern Cone region and this should be stressed with policy makers for future decisions and interventions. The main key issues for mycotoxin control and prevention were shown to be: cultivar selection, crop rotation and agro-climatic factors.

OUTPUT related to task 32: the deliverable D13 entitled "A series of reports documenting studies to develop and evaluate control measures for validating CCPs" was achieved.

Task 33. *Recommendations on Good Practices (Agricultural, Manufacture, Storage) for mycotoxin prevention and control in the Southern Cone*

According to the data collected and collated in each country, and to the local context (technical, socio-economic, organizational aspects of the cereal chain), a manual on Good Practices (Agricultural, Manufacture, Storage) was jointly elaborated by Argentina, Chile and Uruguay partners for wheat chain, and by Brazil for corn chain. Those manuals compiled recommendations on the practices that should be followed in order to prevent and control efficiently mycotoxin contamination in the Southern Cone region. Recommendations concerned either agricultural practices (choice of resistant variety to *Fusarium*, tillage, fungicide treatment, harvesting date, weather forecasts) or manufacture/storage practices (grain segregation at reception, silo cleaning and emptying, transportation). The partners agreed upon the need for considering Good Practices in a holistic way, and stressed the need for external interventions driven by authorities and regulatory bodies for ensuring their application by all cereal chain stakeholders.

OUTPUT related to task 33: the deliverable D16 entitled "A manual describing Good Agricultural Practices for the production of maize and wheat in the Southern Cone" was achieved.

Work Package 6
Development of a Food Quality Management System

(Leadership: Dr Marcelo Masana who replaced Dr Ricardo Rodriguez from 2004, INTA, Partner 8)

> ***Objective of WP 6:*** To develop and implement an integrated and efficient Food Quality Management System along the chain stakeholders to ensure high quality wheat and maize production regarding mycotoxin contamination.

Task 34. *Elaboration of a HACCP plan for mycotoxin control in each Southern Cone country*

The full HACCP teams were composed of: a HACCP specialist; socio-economists; agronomists; crop specialists and mycotoxin analysts; drawn from partners and other organizations within the Public and Private sectors, and including Quality Managers from the collaborating mills. These teams took deliverables produced by work packages 1 to 5 and developed a HACCP Plan specific to their particular case-studies. Each of the 12 HACCP tasks, as defined by Codex 1997, was addressed in turn. Earlier deliverables, such as D3 (Hazard Analysis) and D6 (Commodity Flow Diagram) were updated and steps identified as Good Agricultural Practices (GAPs) or Critical Control Points (CCPs) were finalized. For each CCP, the team established Critical Limits on the Control Measure, a Monitoring System, Corrective Actions should the CCP move out of control, verification procedures and Record Keeping procedures. Of key importance was the integration of technical and socio-economic findings, to allow a realistic assessment of the likelihood that a GAP or CCP could be introduced into the commodity system. In the case of Chile, the DON hazard was under complete control in the case-study location, due to agro-climatic factors and existing Good Agricultural Practices. Nevertheless, a HACCP Plan was developed with a CCP identified at mill reception based on segregation of wheat according to % *Fusarium* Damaged Kernels (%FDK). This CCP could be triggered into action should a computer forecasting system indicate an increased risk of *Fusarium* Head Blight. The specific HACCP Plan could also be used as generic guideline for application elsewhere in Chile, where the risk of DON contamination is now known to be higher. These HACCP plans will serve as methodological guidelines with application throughout the Southern Cone, and specific recommendations can be used by cereal chain stakeholders and decision makers in each country.

OUTPUT related to task 34: The deliverable D15 entitled "HACCP plans for mycotoxin control in the specified commodity for each participating country" was achieved. The HACCP plans are available on the project's website (restricted access area to partners) and will be disseminated by the concerned partners to decision makers in each country.

Task 35. *Recommendations for implementing an efficient Food Quality Management System (FQMS) for mycotoxin control*

The HACCP plan served as output and essential component for setting concrete and adapted recommendations to implement an efficient Food Quality Management System

(FQMS) for mycotoxin control in each Southern Cone country. The full integration of technical and socio economic data, along with the identification of gaps and lacks in each country in terms of quality assurance measures, allowed the identification of steps and actions that were needed for compliance with international ISO 9001: 2000 standard and the new Food Safety Management System (FSMS) ISO 22000: 2005. Introduction of traceability systems and certification procedures were also highlighted as actions to be taken on for the same purpose. It was stressed that there was a need for external interventions driven by mandatory regulations and official authorities for implementing Quality Assurance and Management Systems along the cereal chains in the Southern Cone. Introduction of traceability systems and certification procedures were also highlighted as actions to be taken on for the same purpose.

OUTPUT related to task 35: The deliverable D18 entitled "Implementation of an efficient Food Quality Management System along the chain stakeholders to ensure high quality maize and wheat production regarding mycotoxin contamination" was achieved. The FQMS documents are available on the project's website (restricted access area to partners) and will be disseminated by the concerned partners to decision makers in each country.

Task 36. *Dissemination activities*

To ensure application of the results and outputs achieved, the project consortium had a very dynamic dissemination strategy. In each Southern Cone country, information on the project's activities and outputs was disseminated through brochures, newspapers, radio and TV interviews. Local meetings, workshops and trainings were organized with the cereal chain stakeholders (producers, industries, traders and consumers), extension agents and representatives of the academic sector. A great effort was also made to disseminate the project's findings, including participation in scientific events and production of a large number of scientific documents (see below section Papers and Publications).

PROCISUR (partner 3), acting as regional platform for output dissemination, brought a strong support in the organisation of meetings and workshops with the Southern Cone official bodies and decision makers (National Ministries for Health and Agriculture, Inter-professional cereal bodies, regulatory offices and the MERCOSUR authorities). PROCISUR also designed brochures for regional MERCOSUR dissemination and facilitated access to MYCOTOX website through their own website (www.procisur.org.uy).

This strategy proved to be fruitful according to the expressed needs in the Southern Cone region for considering mycotoxin prevention and control in an holistic way, and for external interventions driven by mandatory regulations and official authorities to ensure the application of Quality Assurance and Management Systems along the cereal chains in the South Cone region. Pertinent official authorities were approached, either in health area (to stress the need for sensitization campaigns oriented towards the consumers upon the negative impact of mycotoxin-contaminated food on human health) or agriculture area (to stress the need for application of Good Agricultural Practices and

integrated management systems to solve mycotoxin problems), as well as regulation area (to stress the need for implementation of adequate regional standards and compliance with international requirements). We should highlight that the project's outputs will serve as a basis to decision-making tools for setting an integrated global management of mycotoxin contamination in the Southern Cone of Latin America.

OUTPUT related to task 36: The deliverable D19 entitled "Training and extension materials including posters, pamphlets, videos and radio and TV broadcasts and Internet web pages, as appropriate" was achieved. All partners planned to continue with output dissemination beyond the end of the project.

Contribution of participants

Work Package 1 (*led by the partner 4 in 2003 and by the partner 5 from 2004*)
The partners 4, 5, 11 and 12 worked on the validation and harmonisation of the classical chromatographic methods for mycotoxin determination. They carried out inter-laboratory works on FAPAS and home-made reference materials (either blanks or spiked cereals). The partner 5 was in charge of leading the WP1 activities from start 2004 after the retirement of the former WP leader (partner 4). The partner 5 elaborated and circulated the adequate protocols for inter-laboratory work, result reporting and guidance for further sampling. The partners 9 & 10 joined some inter-laboratory rounds in 2004 even if not initially involved in WP 1. The partner 1 was in charge of prospecting alternative techniques such as luminescence and NIRS (this latter in collaboration with the partner 12). The partner 2 was in charge of prospecting the Toximet System as alternative technique for mycotoxin determination.

Work Package 2 (*led by the partner 10*)
The partners 9, 10 and 11 were involved in WP 2. The partners 9&10 were in charge of standardizing the method for OTA determination in human blood. The three partners worked later on standardisation of analytical protocols for wine, cereals and animal products. The partner 10 elaborated a questionnaire for surveying the diet of blood donors and circulated it to the partner 11 (Chile) for application. Fruitful exchanges were done among the partners 9, 10 and 11 for validating analytical protocols, sharing and harmonising work on OTA, through meetings and scientific stays of their analysts in the respective laboratories.

Work Package 3 (*led by the partner 9*)
The partners 1, 9, 10 and 11 were involved in WP 3. The partners 9&10 studied, at pilot and semi-industrial scales, the fumonisin distribution in corn and resultant fractions from wet-milling process. They also studied the effect of maize sieving before dry-milling on the distribution of various mycotoxins, as well as the impact of frying on the DON content in wheat-made locally consumed pies. The four partners studied the impact of wheat dry-milling on the DON distribution, the partners 9 and 10 in Argentina, and the partners 1 and 11 through the joint supervision of a Chilean PhD student in France, focusing on the influence of grain structural properties and processing steps on the distribution of mycelium and DON in wheat and derived milling fractions.

Work Packages 4&5 (*led by the partner 2*)
All partners were involved in WP 4&5, which illustrated a gook linkage and strong collaboration between "lab partners" and "field partners". The participation in WP 4&5 activities was extended to the key players in each country (which got involved in the project) and to some Brazilian associate institutions (Universities of Campinas and Maringa, Institute for Regional Development). The partners 4, 6, 7 and 8 constituted multidisciplinary HACCP teams and were responsible for field visits and permanent contacts with the chain actors for i) collection and validation of the data on Commodity Flow Diagrams, ii) socio-economic surveys on the cereal chain and incentives for implementation of a Quality Management System, iii) surveillance studies and field sampling, and iv) elaboration of HACCP plans and the architecture of the Quality Management System in each country. The partners 1 and 2 participated in the above mentioned activities and data analysis. The partners 5, 9, 10 and 11 brought analytical support to WP 4&5 activities, through participation in the sampling design and further analysis of the samples collected from the field. The partner 1 also brought support for officialising the relationships with the private sector through meetings, project presentation and elaboration of confidentiality agreements.

Work Package 6 (*led by the partner 8*)
The partners 4, 6, 7 and 8 were participants in WP 6, with a support from partner 3 (responsible for regional dissemination). Partners 1 and 2 brought support to the development of the integrated quality management systems and advice on the design of dissemination documents. Indeed, all partners participated in the dissemination WP 6 strategy through publication of scientific papers, oral presentations in congresses, dissemination conferences participation in workshops and working groups dealing with mycotoxins, and communication spots on local media.

Problems encountered along the project duration (2003-2006)

For WP 1
In the first year of the project, technical adjustments and corrective actions were needed in some laboratories to improve their analytical performance. However, this was one of the benefits of the proficiency tests done within WP 1 and ensured a reliable implementation of the protocols in all WP 1 laboratories. Some problems occurred with the reception of the homogeneous corn samples prepared by MAA (partner 5 – leader of WP 1) and sent to all WP 1 participants. Some delays also occurred in the reception of laboratory feedback to the WP 1 leader.

For WP 2
The major problem encountered was the high complications for material clearance from Argentinean customs and the 8-month delay in delivery of Ochraprep immuno-affinity columns for OTA determination. These were purchased in Europe for benefiting from special price offer.

For WP 3
No major problem encountered.

For WP 4&5
At the project start, some constraints and delays occurred for getting key players involved in the project and finalize confidentiality agreements. Other constraints and unexpected problems and/or issues related to the local contexts were also experienced, such as the difficulty to find naturally contaminated wheat with high DON content in Chile and low incidence of *Fusarium* Head Blight in Uruguay during the last harvests, some changes in the local strategies for cultural practices, and grain harvesting and storage throughout the project duration, climatic risks in some selected regions. However, adjustments were always made by the consortium and studies were re-oriented to overcome those problems. On the contrary, they were considered as instructive examples for the elaboration of global quality management systems for cereal chains in the Southern Cone region.

For WP 6
No major problem was encountered.

Technology Implementation Plan
The main outputs of the project concerned with "use of knowledge" and further exploitation are the following:

1. The Toximet-T system, a simple, inexpensive, rapid and robust tool (instrument plus cartridge) for mycotoxin measurement (owner of the results: partner 2). An international patent, describing the Toximet T System, was published on 23 November 2006. Two UK patent applications were filed, describing polymers for the immobilisation of the aflatoxins (filed on 9 February 2007) and the ochratoxin A (filed on 17 April 2007). Three further UK patents are under preparation. This knowledge is being exploited immediately after the project completion via Toximet Limited, a University of Greenwich spin-out company. Toximet is currently seeking appropriate licensing arrangements with appropriate companies. The IP is currently owned by the University and an exclusive license for its exploitation has been granted to Toximet.

2. The results on maize sieving before dry-milling, owned by the partners 9 and 10 through trials at lab and semi-industrial scales, are under validation before further transfer to the cereal industry in Argentina. However, it is difficult yet at this stage, to evaluate the time needed for this transfer and the name(s) of company(ies) to which this transfer will be effective. The contractors 9 and 10 commit to inform the EC in due time.

3. The results on impact of wheat structure and fractioning on DON distribution in the resultant fractions, owned by partners 1 and 11 (shared with the French "Institut National de Recherche Agronomique" (INRA) and in collaboration with the French Consortium on Mycotoxins) may come up in the future with a transfer to the industry. However, as far as the on-going PhD is not finished and the results under preparation for publication, it is too early to forecast this transfer. The contractors 1 and 11 commit to inform the EC in due time.

Publications and papers

A great effort was made to disseminate the project's findings. The dissemination outputs can be summarised as follows:

- *scientific peer-reviewed papers* (**14** including **10** published, **2** under revision for publication and **2** submitted, **12** are under preparation),
- *book chapters* (**6**),
- *oral presentations in conferences* (**30**),
- *posters in congresses* (**42**),
- *patent applications* (**6** including **3** published and **3** under preparation),
- *participation in institutional workshops and/or working groups on mycotoxins* (**6**),
- *dissemination documents* (**4** *bulletins*, **4** *posters*, **5** *flyers and brochures*, **12** *articles in newspapers* and **26** *spots and interviews on TV and radio*),
- *sectorial meetings with cereal chain stakeholders and official authorities* (**17**),
- *conferences for dissemination to large audience* (**11**),
- *professional training courses dedicated to food inspectors and technologists* (**3)**.

In addition, *student training* was a strong component of the project's strategy, with **22** trainees including **8** PhD and **5** Msc, and many exchanges among the partners through scientific stays in the different laboratories for analytical training.

Most publications listed below were annexed to the previous reports (annual reports 2003, 2004, 2005 and 2006). Some missing papers in those reports are annexed in the section "Papers and Publications" of this final report.

Publications in peer-reviewed scientific journals

1. Samar M.M., Fontan C.F., Resnik S.L., Pacin A.M., Castillo M.D., **2003**. Distribution of deoxynivalenol in wheat, wheat flour, bran and gluten, and variability associated with the test procedure. *Journal of AOAC International*, 86 (3), 551-556.

2. Sarter S., Zakhia N., **2004**. Chemiluminescent and bioluminescent assays as innovative prospects for mycotoxin determination in food and feed. *Luminescence*, 19, 345-351.

3. Brabet C., Salay E., Freitas-Silva O., Alves A.F., Machinski Jr. M., Vargas E.A., Zakhia-Rozis N., **2005**. Gestão integrada de micotoxinas na cadeia produtiva do milho destinado à alimentação de frangos de corte no Brasil. *Cadernos de Ciência & Tecnologia, Brasília*, 22 (2), 439-451.

4. Brabet C., Salay E., Freitas-Silva O., Alves A.F., Machinski Jr. M., Vargas E.A., Zakhia-Rozis N., **2005**. Maîtrise des mycotoxines dans la filière maïs au Brésil. *Cahiers Agricultures*, 14 (1), 164-168.

5. Pacin A., Resnik S., Vega M., Saelzer R., Ciancio Bovier E., Ríos G., Martinez N., **2005**. Occurrence of ochratoxin A in wines in the Chilean and Argentinean markets. *ARKIVOC*, XII, 214-223.

6. Vega M., Castillo D., **2006**. Determination of deoxynivalenol in wheat by validated GC/ECD method: comparison with HPTLC/FLD. *Electronic Journal of Food and Plants Chemistry,* 1(1), 16-20.

7. Muñoz K., Vega M., Ríos G., Muñoz S., Madariaga R., **2006**. Preliminary study of ochratoxin A in human plasma in agricultural zones of Chile and its relation to food consumption. *Food and Chemical Toxicology*, 44, 1884-1889.

8. Samar M., Resnik S.L., González H.H.L., Pacin A.M., Castillo M.D., **2007**. Deoxynivalenol reduction during the frying process of turnover pie covers. *Food Control*, 18 (10), 1295-1299.

9. Broggi L.E., González H.H.L., Resnik S.L., Pacin A.M., **2007**. *Alternaria alternata* prevalence in cereal grains and soybean seeds from Entre Ríos, Argentina. *Revista Iberoamericana de Micología*, 24, 47-51.

10. Broggi L.E., Pacin A.M., Gasparovic A., Sacchi C., Rothermel A., Gallay A., Resnik S., **2007**. Natural occurrence of aflatoxins, deoxynivalenol, fumonisins and zearalenone in maize from Entre Ríos province, Argentina. Accepted for publication in *Mycotoxin Research*, complete references to be assigned.

11. Sarter S., Métayer I., Zakhia N. A luminescence-based bioassay using *Vibrio fischeri* for mycotoxin determination: the case of Deoxynivalenol DON and Aflatoxin B_1. *Under revision for publication*.

12. Vega M., Madariaga R., Aranda M., Morlock G. Application of HPTLC/MS: confirmation of deoxynivalenol presence in Chilean wheat. *Under revision for publication*.

13. Pacin A.M., Ciancio Bovier E.V., Motta E., Resnik S.L., Villa D., Olsen M. Survey of Argentinean human plasma for ochratoxin A. *Submitted to Food Additives and Contaminants*.

14. Muñoz K., Vega M., Jahn E., Madariaga R. Experimental study for fumonisins and fungi screening in maize for silage and its relation with cut height and harvest date. *Submitted for publication in "Animal Feed Science and Technology"*.

Oral presentations in conferences and congresses

1. Resnik S.L., Pacin A.M., **2003**. Micotoxinas en nuestros países. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

2. Pacin A.M., Taglieri D., Cano G., Resnik S.L., **2003**. Reducción de la contaminación por fumonisinas durante la limpieza del maíz. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

3. Vega M., Saelzer R., Rios G., Herlitz E., Bastis C., **2003**. Chile, micotoxinas, globalización. Las micotoxinas son un problema emergente? *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

4. Corrêa T., Vargas E., Cea J., Vega M., Resnik S., Souza M.L.,Freitas-Silva O., Zakhia N., **2003**. Sistema de gerenciamento de la calidad para el control de micotoxinas en las cadenas de producción y procesamiento de cereales de los países del Cono Sur. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

5. Zakhia N., **2003**. MYCOTOX: una colaboración entre América Latina y Europa sobre el manejo global de la contaminación por micotoxinas en las cadenas productivas de trigo y maíz. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

6. Henry G., Salay E., Engler A., **2003**. Integration of socio-economic and food science and technology research in quality management of food supply chains: Mycotoxin control system of grains in the Southern Cone. Invited paper presented at the *V Simposio Latino Americano de Ciencias de Alimentos*, 3-6 November 2003, Campinas-SP, Brazil.

7. Vargas E.A., Castro L., Corrêa T.B.S., Freitas-Silva O., Brabet C., Cea J., Vega M.A.H., **2004**. Desenvolvimento e padronização de ferramentas analiticas efetivas para determinação de micotoxinas em cereais e subprodutos. *In: XI Encontro Nacional de Micotoxinas*, LAN/ESALQ-USP, 30th June-2nd July 2004, Piracicaba, Brazil.

8. Zakhia N., **2004**. Overview on mycotoxins and emerging challenges towards innovative food safety management. *In: Food Safety Under Extreme Conditions*, 6-8 September 2004, Jaén, Spain.

9. Henry G., Iglesias D., Engler A., Salay E., Gutiérrez G., **2004**. Organización de actores alrededor de la gestión de calidad en cadenas agroalimentarias. *In: XIII Seminario Latinoamericano y del Caribe de Ciencia y Tecnología de Alimentos*, 12-15 de Octubre del 2004, Montevideo, Uruguay.

10. Madariaga R., Engler A., Vega M., Villegas R., Saelzer R., Ríos G., **2004**. Flora fungosa de los granos de trigo cosechado en el sur de Chile. *In: 55th Congress of the Chilean Agronomical Society*, 19 October 2004, Valdivia, Chile.

11. Zakhia N., **2004**. A new challenge for cereal production and processing chains: development of a food quality management system for the control of mycotoxins. *In: MYCO-GLOBE Launch Conference*, 22 October 2004, Brussels, Belgium.

12. Engler A., Henry G., Iglesias D., Alves A., Gutiérrez G., Salay E., **2005**. Actor organization for QAS along agro supply chains: the case of mycotoxin reduction in Southern Cone grains. *In*: *92nd Seminar of the European Association of Agro-Economists (EAAE) on Quality Management and Quality Assurance in Food Chain,* 2-4 March 2005, Göttingen, Germany.

13. Resnik S., **2005**. Manejo de la contaminación por micotoxinas después de la cosecha y durante la industrialización. *In: X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías,* 18-20 de Mayo del 2005, Mar del Plata, Argentina.

14. Engler A., Gutiérrez G., Henry G., Iglesias D., **2005**. Adoption constraints of QAS implementation in Argentina and Uruguay wheat supply chains. *In*: *V International PENSA Conference on Agri-food Chains/Networks Economics and Management,* 27-29 July 2005, Ribeirão Preto, Brazil.

15. Madariaga R., Bustamante S., Engler A., Vega M., **2005**. Flora fungosa de los granos de trigo cosechado en el sur de Chile. I. *Fusarium*. *In: 56 Congreso Agronómico de Chile,* 12 de Octubre del 2005, Chillán, Chile.

16. Pacin A., **2005**. Evaluación del riesgo a la ingesta de maíz contaminado por micotoxinas. *In: VIII Congreso Nacional de Maíz*, 16-18 de Noviembre del 2005, Rosario, Argentina.

17. Masana M., Ricca A., **2005**. La prevención de riesgos en las cadenas agroalimentarias. *In: Conferencia Regional FAO/OMS sobre Inocuidad de los Alimentos para las Américas y el Caribe*, 6-9 de Diciembre del 2005, San José, Costa Rica.

18. Cea J., **2006**. Update on worldwide regulations for mycotoxins. The MERCOSUR harmonization of limits on mycotoxins with the international regulations. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

19. Zakhia N., **2006**. The MYCOTOX project: an EC-funded project in partnership with Latin America South Cone countries. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

20. Resnik S., **2006**. Food processing to reduce the entry of mycotoxins to the food and feed chains. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

21. Pacin A., **2006**. Ochratoxin A occurrence and significance in human blood samples in South America. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in*

South America Ensuring Food and Feed Safety in a Myco-Globe Context, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

22. Vargas E.A., **2006**. Current situation on standardization and validation of analytical methods for mycotoxins in South America. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

23. Stewart S., **2006**. Experience from a decision support system approach to reduce DON contamination in Uruguay. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

24. Henry G., Engler A., Iglesias D., Gutierrez G., **2006**. Socio-economic constraints and opportunities affecting the implementation of mycotoxin control measures in Southern Cone grain supply chains. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

25. Villegas R., **2006**. Sistemas de aseguramiento de calidad en cereales: ejemplo para la cadena de trigo en Latinoamérica. *In: Calidad y Seguridad Alimentaria, una estrategia para competir en mercados exigentes*. International seminar organised by the Chilean Government, INIA and University of Bío-Bío, 27 April 2006, Chillán, Chile.

26. Iglesias D., Henry G., Engler A., Gutierrez G., **2006**. Policies for QAS implementation in export chains: mycotoxin management for Mercosur wheat actors. *In: 7th International Conference on Management in AgriFood Chains and Networks*, 31st May-2nd June 2006, Ede, The Netherlands.

27. Iglesias D., Henry G., Engler A., Gutierrez G., **2006**. Adoption of QAS and impact from norms in export chains: mycotoxin management for Mercosur wheat actors. *In: 16th Annual World Forum; Symposium and Case Conference,* International Food and Agribusiness Management Association (IAMA), 10-13 June 2006, Buenos Aires, Argentina.

28. Resnik S., González H.H.L., Pacin A., **2006**. Evaluación de brotes de tricotecenos en Argentina y Uruguay. *In: V Congreso Latinoamericano de Micotoxicología - IV Simposio en Almacenaje Cualitativo de Granos del MERCOSUR*, 18-21 de Junio del 2006, Florianópolis, Brazil.

29. Resnik S., **2006**. Calidad de granos almacenados versus actividad de agua y hongos. *In: V Congreso Latinoamericano de Micotoxicología - IV Simposio en Almacenaje Cualitativo de Granos del MERCOSUR*, 18-21 de Junio del 2006,

Florianópolis, Brazil.

30. Resnik S., **2006**. Prácticas para la reducción de micotoxinas en la cadena de producción e industrialización de los alimentos. *In: Congreso Internacional de Ciencia y Tecnología de los Alimentos*, 15-17 de Noviembre del 2006, Córdoba, Argentina.

Book Chapters

1. Pacin A., Resnik S., **2006**. Regulaciones nacionales e internacionales, perspectivas de la producción de cereales y alimentos a base de cereales en la provincia de Córdoba. *In: Micotoxinas: Impacto en la Producción y Salud Humana y Animal*, Héctor R. Rubinstein (ed.), capítulo 1, pp. 29-47, ISBN 987-530-068-3.

2. Resnik S., Pacin A., Funes G., **2006**. Identificación y cuantificación de micotoxinas en maíz cosechado en la provincia de Córdoba y en productos de molienda. *In: Micotoxinas: Impacto en la Producción y Salud Humana y Animal*, Héctor R. Rubinstein (ed.), capítulo 8, pp. 199-220, ISBN 987-530-068-3.

3. Pacin A., Resnik S., Ciancio Bovier E., **2006**. Detección de OTA en muestras biológicas provenientes de humanos. *In: Micotoxinas: Impacto en la Producción y Salud Humana y Animal*, Héctor R. Rubinstein (ed.), capítulo 10, pp. 255-269, ISBN 987-530-068-3.

4. Resnik S., Pacin A., **2007**. Toxinas T-2 y HT-2. *In: Micotoxinas en Alimentos*, José Miguel Soriano del Castillo (ed.), capítulo 15, pp. 293-312, Editorial Díaz de Santos, Madrid, Spain.

5. Pacin A., Resnik S., **2007**. Acido ciclopiazónico. *In: Micotoxinas en Alimentos*, José Miguel Soriano del Castillo (ed.), capítulo 18, pp. 335-356, Editorial Díaz de Santos, Madrid, Spain.

6. Zakhia-Rozis N., Catala A.I., Soriano J.M., **2007**. Trazabilidad y descontaminación/ detoxificación de las micotoxinas. *In: Micotoxinas en Alimentos*, José Miguel Soriano del Castillo (ed.), capítulo 6, pp. 119-132, Editorial Díaz de Santos, Madrid, Spain.

Patent Applications

1. International Patent Publication N° WO 2006/123189 A2 – Device for detection and measurement of a target compound such as food toxins, 23 November **2006**.

2. UK Patent Application N° 0702489.6 – Solid phase extraction of aflatoxins, filed on 9 February **2007**.

3. UK Patent Application N° 070375.2 – Solid phase extraction of ochratoxin A, filed on 17 April **2007**.

Posters presented in congresses

1. Resnik S.L., Taglieri D., Cano G., Pacin A.M., **2003**. Aflatoxinas en las fracciones obtenidas durante la limpieza del maíz. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

2. Pacin A.M., Cano G., Resnik S.L., Villa D., Taglieri D., Ciancio E., **2003**. Incidencia de la contaminación por aflatoxinas en maíz argentino, periodo 1995-2002. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

3. Resnik S.L., Villa D., Pacin A.M., **2003**. Distribución de fumonisinas en el maíz y en las fracciones obtenidas durante la limpieza del maíz. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

4. Pacin A.M., Resnik S.L., Ciancio E., Cano G., Taglieri D., **2003**. Estudio preliminar sobre la contaminación por ocratoxina A en vinos argentinos. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

5. Pacin A., Cano G., Resnik S.L., Villa D., Taglieri D., Ciancio E., **2003**. Incidencia de la contaminación por aflatoxinas en maíz. *In: Jornadas Bonaerenses de Ciencia y Tecnología*, 17 de Diciembre del 2003, La Plata Provincia de Buenos Aires, Argentina.

6. Resnik S.L., Taglieri D., Ciancio E., Cano G., Pacin A.M., **2003**. Reducción de micotoxinas en maíz: limpieza. *In: Jornadas Bonaerenses de Ciencia y Tecnología*, 17 de Diciembre del 2003, La Plata Provincia de Buenos Aires, Argentina.

7. Resnik S.L., Villa D., Pacin A.M., **2003**. Muestreo de fumonisinas en maíz: función de distribución. *In: Jornadas Bonaerenses de Ciencia y Tecnología*, 17 de Diciembre del 2003, La Plata Provincia de Buenos Aires, Argentina.

8. Pacin A.M., Resnik S.L., Ciancio E., Cano G., Taglieri D., Martinez N., **2003**. Contaminación por ocratoxina A en vinos argentinos. *In: Jornadas Bonaerenses de Ciencia y Tecnología*, 17 de Diciembre del 2003, La Plata Provincia de Buenos Aires, Argentina.

9. Vega H.M., Muñoz S.K., Ríos G., **2004**. Determinación de ocratoxina A en sangre humana, estudio preliminar para estimar riesgo de exposición. *In: COLACRO X*, 20-23 de Octubre del 2004, Campos da Jordao, São Paulo, Brazil.

10. Ríos G., Muñoz S.K., Vega H.M., **2004**. Ocratoxina A en cereales, estudio de dos procedimientos de análisis. *In: COLACRO X*, 20-23 de Octubre del 2004, Campos da Jordão, São Paulo, Brazil.

11. Pacin A.M., González H.H.L., Resnik S.L., Moltó G.A., Masana M., **2005**. Micoflora contaminante y presencia de tricotecenos tipo A y B en trigo cosechado en la provincia de Buenos Aires. *In: X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías,* 18-20 de Mayo del 2005, Mar del Plata,

Argentina (published in the proceedings, volume III, 2006, pp. 936-942).

12. Funes G.J., Taglieri D., Cano G., Pacin A., Resnik S.L., **2005**. Contaminación por fumonisinas en fracciones obtenidas en la molienda húmeda de maíz. *In: X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías,* 18-20 de Mayo del 2005, Mar del Plata, Argentina (published in the proceedings, volume III, 2006, pp. 929-935).

13. Motta E., Ciancio Bovier E., Pacin A., Resnik S.L., Villa D., **2005**. Estimación de la ingesta de alimentos en 210 donantes de sangre en la ciudad de Mar del Plata. *In: X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías,* 18-20 de Mayo del 2005, Mar del Plata, Argentina (published in the procedings, volume III, 2006, pp. 1197-1203).

14. Zelaya M.J., González H.H.L., Resnik S.L., Martínez M.J., **2005**. Micoflora contaminante en soja cosechada en la principal zona de producción de la República Argentina. *In: X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías,* 18-20 de Mayo del 2005, Mar del Plata, Argentina (published in the proceedings, volume V, 2006, pp. 1788-1794).

15. Broggi L.E., Pacin A.M., González H.H.L., Resnik S.L., Cano G., Taglieri D., **2005**. Micoflora contaminante y ocurrencia natural de micotoxinas en el maíz almacenado y los subproductos del proceso de industrialización por molienda seca. *In: II Jornadas de Difusión de Proyectos de Investigación-Extensión* UNER-INEX, Junio del 2005, Concordia, Argentina.

16. Muñoz K.S., Vega M., Ríos G., Madariaga R., **2005**. Ochratoxin A in cereals. Study of two procedures of analysis. *In: 27 Mykotoxin-Workshop Ifado,* 13-15 de Junio del 2005, Dormund, Germany.

17. Muñoz K.S., Vega M., Ríos G., Madariaga R., **2005**. Determination of ochratoxin A in human blood. Preliminary study to estimate risk exposure in Chile. *In: 27 Mykotoxin-Workshop Ifado,* 13-15 de Junio del 2005, Dormund, Germany.

18. Sekiyama B.L., Brabet C.J., Lemes R.O., Dalpasquale V.A., Silva O.F., Vargas E.A., França R.C.A., Machinski Jr. M., **2005**. Desenvolvimento dos pontos críticos de controle para prevenir a contaminação por micotoxinas ao longo da cadeia produtiva do milho. *In: XIV Congresso Brasileiro de Toxicologia,* 9-12 de outubro de 2005, Recife, Pernambuco, Brazil.

19. Zelaya M.J., González H.H.L., Resnik S.L., Pacin A., **2005**. Aislamiento e identificación de la micoflora contaminante en cereales y oleaginosas: un caso de estudio en soja cosechada en la República Argentina. *In: Bienal de Ciencia y Tecnología 2005 de la provincia de Buenos Aires*, 8-10 de Noviembre del 2005, La Plata, Argentina.

20. Cea J., Cammarota L., **2006**. Study of clean-up procedures using charcoal-alumina-celite column, immunoaffinity column and StrataX column to determine deoxynivalenol by high performance liquid chromatography in wheat. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

21. Cea J., Martinez M.O., **2006**. Relationship between the level of deoxynivalenol contamination in wheat and the fungal infection. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

22. França R.C., Dos Santos E.A., De Castro L., Vargas E.A., **2006**. The development of a food quality management system for the control of mycotoxins in cereal production and processing chains in Latin America South Cone countries. Part I. Reference material preparation. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

23. De Castro L., Dos Santos E.A., França R.C., Vargas E.A., Cea J., Vega M., Freitas-Silva O., **2006**. The development of a food quality management system for the control of mycotoxins in cereal production and processing chains in Latin America South Cone countries. Part II. Interlaboratory control. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

24. Castillo M.D., Ciancio Bovier E.V., Pacin A.M., Resnik S.L., Villa D., **2006**. Food intake estimation in 236 blood donors in General Rodriguez, Buenos Aires province, Argentina. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

25. Motta E., Ciancio Bovier E.V., Pacin A.M., Resnik S.L., Villa D., **2006**. Ochratoxin A in human plasma in Buenos Aires province, Argentina. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

26. Castillo M.D., Pacin A.M., Molto G.A., Resnik S.L., **2006**. Contamination by aflatoxins, zearalenone and deoxynivalenol in corn and the fractions obtained in the wet milling process. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

27. Frusteri L.M., González H.H.L., Zelaya M.J., Resnik S.L., Pacin A.M., Martínez M.J., **2006**. Insecticide effect on the mycoflora of soybean RR isolated from the Pampean region in Argentina. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

28. Zelaya M.J., González H.H.L., Resnik S.L., Martínez M.J., **2006**. Contaminant mycoflora of soybean RR seeds harvested in different production areas in Argentina. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

29. Madariaga R., Bustamante S., **2006**. Fungal flora on wheat grains harvested on Southern Chile. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

30. Vega M., Madariaga R., Saelzer R., Villegas R., Ríos G., Muñoz K., Carrillo D., Bastias C., Torres O., **2006**. Deoxinivalenol en Chile. Estudio de 3 años. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil.

31. Vega M., Madariaga R., Ernesto J., Muñoz K., Villegas R., Sepúlveda C., Torres O., **2006**. Estudio de hongos y fumonisina B_1 y B_2 presentes en maíz para ensilaje y su relación con la altura de corte en el campo. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil.

32. Muñoz K., Färber P., Vega M., **2006**. Producción de ocratoxina A por algunas especies fúngicas en trigo, café y otros medios de cultivo. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil.

33. Vargas E.A., De Castro L., Dos Santos E.A., França R.C.A., Cea J., Moriyama C., Vega M., Freitas-Silva O., Souza M.L.M., **2006**. Interlaboratory control among INCO-DEV MYCOTOX project laboratories. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil.

34. Souza M.L.M., Vasconcelos M.G., Teixeira A.S., Farias A.X., Freitas-Silva O., Costa S.S., **2006**. Desenvolvimento e validação de método para análise de zearalenona em milho por CLAE. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil.

35. Souza M.L.M., Farias A.X., Freitas-Silva O., Montello A.P., Cunha F.Q., Brabet C., Dalpasquale V., Machinski Jr. M., Sekiyama B.L., Costa S.S., **2006**. Avaliação da qualidade do milho utilizado no processamento de rações quanto a contaminação por aflatoxinas. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil.

36. Sacchi C.A., Broggi L.E., Resnik S.L., González H.H.L., Pacin A.M., **2006**. Micoflora contaminante y ocurrencia natural de micotoxinas en avena cosechada en la provincia de Entre Ríos, Argentina. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil.

37. Muñoz K., Färber P., Vega M., **2006**. HPTLC como herramienta para el estudio de producción de ocratoxina A, por algunas especies fúngicas en trigo, café y otros medios de cultivo. *In: COLACRO XI, Congreso Latino Americano de Cromatografía y Ciencias Afines*, 26-30 de Junio del 2006, Mérida, Mexico.

38. Ríos G., Zakhia-Rozis N., Chaurand M., Samson M.F., Richard-Forget F., Abecassis J., Lullien-Pellerin V., **2006**. Assessment of wheat grain fractionation process involvement in the product contamination with deoxynivalenol (DON). *In: Food is Life, IUFOST, 13th World Congress of Food Science & Technology*, 17-21 September 2006, Nantes, France.

39. Frusteri L.M., Molto G.A., Pacin A.M., González H.H.L., Resnik S.L., Masana M.O., **2006**. Tricotecenos tipo A y B y micoflora contaminante asociada en el trigo cosechado en la provinicia de Buenos Aires. *In: Congreso Internacional de Ciencia y Tecnología de los Alimentos*, 15-17 de Noviembre del 2006, Córdoba, Argentina.

40. Cea J., Stewart S., Gutiérrez G., **2007**. *A HACCP plan along the wheat chain to prevent DON in wheat flour in Uruguay. In: XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins*, 21-25 May 2007, Istanbul, Turkey (www.atal.tubitak.gov.tr/iupac2007-mycotoxin).

41. De Castro L., Santos E.A., França R.C., Cea J., Herrera M.V., Freitas-Silva O., Vargas E.A., **2007**. Interlaboratory control involving participant laboratories within the INCO-DEV MYCOTOX project. *In: XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins*, 21-25 May 2007, Istanbul, Turkey (www.atal.tubitak.gov.tr/iupac2007-mycotoxin).

42. Souza M.L.M., Freitas-Silva O., Brabet C., Dalpasquale V., Machinski Jr M., De Castro L., Vargas E.A., Nagler M., Costa S.S., **2007**. Minimizing risks by mycotoxins in maize and poultry feed using the HACCP plan. *In: XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins*, 21-25 May 2007, Istanbul, Turkey (www.atal.tubitak.gov.tr/iupac2007-mycotoxin).

Dissemination documents and conferences, sectorial meetings with cereal stakeholders and authorities, institutional workshops and/or working groups on mycotoxins

1. Argentina desarrollará un sistema de gestión de calidad para el Cono Sur. *Information Bulletin* published by INTA (partner 8) in the institutional "*INTA Informa*", Number 233, May **2003**.

2. The development of a food quality management system for the control of mycotoxins in cereal production and processing chains in Latin America South Cone countries. *Poster* presented by the general coordinator *at the Third European Mycotoxin Cluster Workshop*, 2-4 June **2003**, Uppsala, Sweden.

3. Control de micotoxinas en alimentos derivados de maíz y trigo en países del Cono Sur. *Bulletin* published by INTA (partner 8) in the institutional "*INTA Informa Internacional*", Number 1, June **2003**.

4. Desarrollo de un sistema de manejo de calidad de alimentos para el control de micotoxinas en la cadena de producción y procesamiento de cereales en los países del Cono Sur de América. *Flyer* distributed at the *IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del **2003**, La Habana, Cuba. This documents was elaborated with the logistic support of PROCISUR (Programa Cooperativo para el Desarrollo Tecnológico Agroalimentario y Agroindustrial del Cono Sur) (partner 3) for translation to Spanish and edition.

5. S. Resnik (partner 9) participated in the *workshop* on soybean quality organised by INTA (partner 8), 9 September **2004**, Marcos Juárez, Argentina.

6. S. Resnik (partner 9) participated in the *workshop* "Biosafety, surveillance and segregation of grains and seeds modified and not modified genetically", 22-23 September **2004**, Buenos Aires, Argentina.

7. S. Resnik (partner 9) and A. Pacin (partner 10) participated in a *working group* on mycotoxins organised by the Argentinean Agency for Scientific and Technological Promotion (SECyT), 29 September **2004**, Buenos Aires, Argentina.

8. PROCISUR (partner 3) designed, printed and presented a *poster* for presenting MYCOTOX project at the International Alliances Pavilion, *"INTA Expone" Fair,* Santa Fé, Argentina, October **2004**.

9. A. Pacin and Marcelo Castillo (partner 10) participated as lecturers in the 10hr *training course* "Similitudes y diferencias entre las micotoxinas más conocidas" organised by the Asociación Argentina de Tecnólogos en Alimentos, Buenos Aires, 23-24 June **2005**.

10. A. Pacin (partner 10) presented the *dissemination conference* "Evaluación de riesgo a la intoxicación por micotoxinas" at the *Jornadas Interdisciplinarias de Toxicología Alimentaria*, Buenos Aires, on 9th and 20-21 September **2005**.

11. The University of Concepción (partner 11) organised and participated in the lectures given within the *training course* "Actualisation in Technology, Control and Regulation on Foods" dedicated to 40 food inspectors and responsibles of Health Services in Concepción and Talcahuano (September-November **2005**).

12. Resnik S. (partner 9), Pacin A. (partner 10), **2005**. Micotoxinas, un enemigo presente en los alimentos. *Conference* presented at the *IV Congreso Nacional de*

Estudiantes de Bioquímica y Biotecnología, Universidad Nacional del Litoral, 1 de Octubre del 2005, Santa Fé, Argentina.

13. S. Resnik (partner 9) and A. Pacin (partner 10) participated in the "*Food Security and Contaminants in Food*" workshop, Mycotoxins and Pesticides *working group*, organised within the frame of National Strategic Programs, 7 October **2005**, Buenos Aires, Argentina.

14. Madariaga R.B. (partner 7), **2005**. Hablemos de micotoxinas. *Internal Seminar*, 21 November 2005, Instituto de Investigaciones Agropecuarias INIA, Chile.

15. Pacin A. (partner 10), **2005**. Implicancias de las micotoxinas en la salud animal y humana. *Seminar organised by INTA (partner 8) with the wheat chain stakeholoders and representatives of the academic sector and official bodies*, 1st December 2005, General Pico, Argentina.

16. Resnik S. (partner 9), **2005**. Que son las micotoxinas? Modificaciones de la contaminación natural por micotoxinas por efecto del procesamiento. *Seminar organised by INTA (partner 8) with the wheat chain stakeholders and representatives of the academic sector and official bodies*, 1st December 2005, General Pico, Argentina.

17. Zakhia N. (partner 1), **2005**. Micotoxinas en los alimentos: panorama actual, legislación europea y perspectivas de manejo. *Seminar organised by INTA (partner 8) with the wheat chain stakeholders and representatives of the academic sector and official bodies*, 1st December 2005, General Pico, Argentina.

18. Zakhia N. (partner 1), **2005**. Micotoxinas en los alimentos: panorama actual, legislación europea y perspectivas de manejo. Talk presented at *different seminars* (4-7 December 2005) *organised with the wheat chain stakeholders* (Semillas von Baer, Molinos Collico, ALISUR Osorno, Agromaster S.A.) in the localities of Valdivia, Osorno, and Temuco in Chile.

19. Ricardo Madariaga (INIA Chile, partner 7) gave a *conference to large audience* in the frame of the World Food Day (December **2005**) celebrated by the University of Concepción (partner 11).

20. Pacin A., 2006. Existe un diagnóstico sobre micotoxinas en soja en Argentina? Dissemination conference at the workshop "*Calidad de la producción y granos con valor agregado*", *Tercer Congreso de Soja del MERCOSUR*, 27-30 de junio del 2006, Rosario, Argentina,

21. Cooperação: o projeto europeu Mycotox em fase de finalização. *França Flash* 48, out-nov **2006**, CenDoTec, São Paulo, Brazil. This *bulletin* was prepared by CIRAD (partner 1).

22. Resnik S., assisted by Cano G., Taglieri D. and Zelaya M. (UBA, partner 9) and A. Pacin (UNLU, partner 10) participated as lecturers in the 10hr *training course*

"Micotoxinas en alimentos", organised by the Asociación Argentina de Tecnólogos en Alimentos, Luján, 15 June **2006.**

23. PROCISUR (partner 3), **2006**. El desarrollo de un sistema de calidad de alimentos para el control de las micotoxinas en la cadena de proceso y producción de cereales en los países del Cono Sur de Latinoamérica. *Flyer* distributed by PROCISUR at the *International Fair "INTA Expone"*, November 2006, Río Negro, Argentina.

24. PROCISUR (partner 3), **2006**. Control de micotoxinas en la cadena de cereales. Poster presented by PROCISUR at the *International Fair "INTA Expone"*, International Strategies Alliances Pavilion, November 2006, Río Negro, Argentina.

25. Madariaga R. (partner 7), **2006**. Ausencia de micotoxinas y de hongos micotoxigénicos en la cadena de trigo, *conference* presented at the *meeting organised with farmers and wheat producers*, June 2006, Temuco, Chile.

26. Madariaga R. (partner 7), **2006**. Micotoxinas y problemas de hongos, *conference* presented at the first seminar *(Granos Almacenados: Situación de Mercado y Almacenaje)* organised by a group of private companies, August 2006, Temuco, Chile.

27. Meetings were jointly organised by INIA Uruguay (partner 6), LATU (partner 12) and PROCISUR (partner 3) with representatives of the National Board for Wheat (Mesa Nacional de Trigo) and the academic sector for discussion of mycotoxin management in the Uruguayan wheat chain, Colonia (November **2006**) and Montevideo (December **2006**).

28. Mendes de Souza M.L. and Freitas-Silva O. (EMBRAPA, partner 5) participated in several meetings with the Brazilian Technical Group of Contaminants in Foods within the regulatory sector (ANVISA, Ministry of Health, Ministry of Agriculture and Academy). This group is a formal team working on a discussion paper on the maximum levels of mycotoxins in food for the *Codex Alimentarius* Brazil. The meetings were held in Brasilia in August, November and December **2006**, and other meetings were planned for February and March **2007**.

29. A field day (video, script and images) was planned on 29 June **2007** for dissemination on TV Rural, a Brazilian cable channel, through the "Food Safety and Maize Quality to Broiler Feed" programme. This programme was prepared and produced by EMBRAPA (partner 4). The first part of the programme was dedicated to the theoretical approach of food safety, including technological aspects and applications. The second part was planned as an interview with the maize chain stakeholders, including the Brazilian team involved in the MYCOTOX project.

30. INTA (partner 8) organised a internal *workshop* for sharing mycotoxin awareness and issues, in order to elaborate a pertinent research agenda to be integrated within the National Wheat Programme, Training Centre of Pergamino, Buenos Aires, May **2003**.

31. INTA (partner 8) presented a *dissemination conference* entitled "Desarrollo de un sistema de gestión con base HACCP para cadenas de cereales seleccionados", at the *Primer Congreso Argentino y Primer Congreso Mercosur de BPM-POES-HACCP*, 27-28 November **2003**, University of Río Cuarto, Argentina.

32. INTA (partner 8) disseminated the MYCOTOX activities and increased awareness on the mycotoxin contamination among the cereal stakeholders in the General Pico (pilot site of the project in Argentina). A meeting was organised for increasing awareness on mycotoxin problem among large audience including farmers in General Pico area (December **2005**) and another one was held at the Rural Trade Fair in Santa Rosa area (October **2006**). Dissemination brochures were delivered.

33. INTA (partner 8) gave various interviews in **2004**, **2005** and **2006** to present the MYCOTOX project in Argentina in *newspapers* (La Reforma Diario, La Tranquera, La Nueva Era de Tandil, Democracia de Junín, Informe Rural Pampeano, La Arena Newspaper), *TV* (Multicanal TV Channel, Multicanal circuit regional cable), and *radio* (FM La Isla, LU37 Radio General Pico, LU33 Radio Emisora Pampeana de Sta Rosa). A total of 12 articles in newspapers and 25 spots on TV and radio were produced.

34. The main outputs of the project were also disseminated at the global regional level, through meetings with the cereal chain stakeholders and with official bodies (Ministries for Health and Agriculture, Interprofessional cereal bodies, regulatory offices) in the 4 partner countries. PROCISUR (partner 3), the regional platform in the Southern Cone, strongly contributed to the output dissemination, through their website (www.procisur.org.uy), and by designing and editing documents and policy notes that were presented to the national and MERCOSUR authorities.

Papers under preparation

1. González H.H.L., Moltó G., Pacin A., Resnik S., Zelaya M., Masana M., Martinez A.
Tricothecenes type A and B and related mycoflora in commercial wheat cultivars harvested in 9 locations of Buenos Aires province, Argentina.

2. Funes G.J., Bello M.O., Resnik S.L., Pacin A.M., Cano G.
Fumonisin behaviour on laboratory scale corn wet milling process.

3. Funes G., Castillo M.D., Molto G.A., Pacin A.M., Resnik S.L.
Effect of industrial wet milling process on the distribution of aflatoxins, deoxynivalenol, fumonisin B_1 and B_2, and zearalenone in corn fractions.

4 Resnik S., Pacin A.
Effect of maize cleaning by sieving before storage.

5. Sacchi C., González H.H.L., Broggi L.E., Pacin A., Resnik S.L., Cano G., Taglieri D.
Mycoflora and mycotoxin natural occurrence in oats from Entre Ríos and Buenos Aires provinces, Argentina.

6. Vega M., Madariaga R., Muñoz K., Jahn E., Villegas R.
Determination of fumonisins and mould screening in maize for silage and its relation with the height cut.

7. Sepúlveda C., Vega M., Villegas R., Muñoz K.
Validation of an analytical methodology for determination of ochratoxin A in cereals and derivatives.

8. Opazo A., Muñoz K., Vega M., Villegas R.
Validation of a methodology for determination of ochratoxin A and its metabolites in pork's muscle, liver and kidney.

9. A paper is under preparation on the *influence of dry milling operations on the distribution of DON in wheat grain and outcoming fractions*.

10. A paper is under preparation on the global *MYCOTOX methodology integrating technical and socioeconomic features for quality management along the food supply chain*.

11. A paper is under preparation on the *mycotoxin contamination through the whole supply chain of corn destined to poultry feeding in Brazil*.

12. A literature review is under preparation on the *occurrence of mycotoxins in the Brazilian corn supply chain*.

Conclusion

The project was successful in achieving the commitments of the technical annex, it also was pioneer in launching innovative investigation. The main benefits arising, directly or indirectly from the project, and which contributed to considering it as "success story", may be summarised as follows:

1. A regional Southern Cone analytical network was set-up with harmonised procedures for aflatoxin, zearalenon, ochratoxin A and fumonisin determination in wheat and maize. After the project completion, this network will be a pillar for strengthening the regional analytical support to trade and regulatory bodies.

2. A challenge took-up by the project was the preparation of internal reference materials (mycotoxin-free and naturally or spiked contaminated cereals) that were used among the partners for inter-laboratory proficiency rounds. These reference materials could be made available to all laboratories within the Southern Cone, and by extension to the whole Latin American continent. The partners are willing to seek further certification by international and regional scientific authorities for officialising the use of these reference materials in the region. This can then answer the need expressed many times by scientists in different conferences on mycotoxins, for available reference materials less expensive than those marketed at the international level such as the FAPAS ones.

3. The project has used specific case-studies to pioneer the application of a number of methodologies and approaches to the control of mycotoxins in cereal chains in the Southern Cone. The application of HACCP (Hazard Analysis and Critical Control Points), which was initially focusing on technical aspects at lab level, to the whole agri-food chain including stakeholders, and the full integration of socio-economic and technical inputs, has been successfully demonstrated and can therefore be recommended for use throughout the Southern Cone for the control of mycotoxins (and by extension to other types of contaminants) in all cereals and cereal products (and by extension to other types of commodities).

4. This approach allowed the constitution in each country partner of a multidisciplinary HACCP team including agronomists, phytopathologists, analysts, HACCP specialists, socio-economists, and representatives of the cereal chain stakeholders, and involving EU partners. This was a successful achievement as the basis for collaborative research between Europe and Latin America, in the frame of the EC international cooperation.

5. Strong involvement of the cereal stakeholders, especially the private sector, was ensured in each country partner, as well as linkages with the official authorities, regulatory bodies and decision makers. This will help for further impact of the project in the Southern Cone region and give more persuasive arguments to the involved partners for pushing the local and regional policies in terms of quality management systems within the cereal chains.

6. The project initiated innovative investigation which will continue beyond the end of project. These concern: the toxicity bioassays using Vibrio fischeri luminescence and the Near Infra Red Spectroscopy as alternative tools for semi-quantitative mycotoxin assessment, the Toximet-T system as simple, inexpensive and robust rapid tool for mycotoxin measurement, the human exposure to ochratoxin A in the Southern Cone region, the impact of grain processing and fractioning on the mycotoxin distribution in the resultant fractions, and the identification/correlation of the endogenous fungi and related mycotoxin production in each South Cone country partner.

7. Strong linkages and collaborations were achieved throughout the whole project duration among the “lab” and “field” work packages. In each country, the laboratories involved in the project analysed the samples in-field collected by the HACCP teams, for confirmation of the Critical Control Points identified. The data generated on the human exposure to ochratoxin A and the impact of grain processing on mycotoxin distribution in the resultant fractions contributed to the hazard analysis and validation of control measures. Permanent exchanges of information and bibliographic references were assured among the consortium members.

8. A very dynamic strategy was used to disseminate the project findings, including participation in scientific events and production of a large number of scientific documents. The dissemination outputs can be summarised as follows: scientific peer-reviewed papers (**14** including **10** published, **2** under revision for publication and **2** submitted, **12** are under preparation), book chapters (**6**), oral presentations in

conferences (**30**), posters in congresses (**42**), patent applications (**6** including **3** published and **3** under preparation), participation in institutional workshops and/or working groups on mycotoxins (**6**), dissemination documents (**4** bulletins, **4** posters, **5** flyers and brochures, **12** articles in newspapers and **26** spots and interviews on TV and radio), sectorial meetings with cereal chain stakeholders and official authorities (**17**), conferences for dissemination to large audience (**11**) and professional training courses dedicated to food inspectors and technologists (**3**).

9. Student training was a strong component of the project's strategy, with 22 trainees including 8 PhD and 5 Msc. Exchanges were also done among the partners through scientific stays of their analysts in the different laboratories.

10. Most Southern Cone partners reinforced the priority on mycotoxin issues and continuation of the MYCOTOX project approach within their research agenda for the next few years.

INCO: International Scientific Cooperation Projects (1998-2002)

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

Title:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries

Management report

General Management Issues

- The rules of project running and management as initially stated in the proposal submitted to the EC were discussed and validated by the whole consortium members at the project launching. The general coordination brought permanent administrative support to the partners in order to ensure a fluid and smooth cooperation within the consortium and compliance with the EC rules.

- Because of local administrative rules and constraints, the first fund (advance) transfer to Brazilian and Argentinian institutions was complicated and took longer than provided for. The general project coordinator did the best for managing this situation, in concertation with the partners and in accordance with the above mentioned difficulties.

- In the specific case of Brazil, all institutions usually commit, through contractual modalities, external foundations for managing their financial resources, especially the budgets coming from outside donors and international projects. We had to follow those modalities otherwise it was impossible to transfer the funds. To this end, a joint agreement was prepared and signed between the general project coordination, the Brazilian partners (EMBRAPA, partner 4 and MAA, partner 5) and the FUNARBE Brazilian foundation committed to manage the financial resources of these partners and to prepare their cost statements. Meanwhile this process was running, and in order to let the Brazilian partners carry out their planned activities in good conditions, the general coordinator (partner 1) advanced, on its own funds, the needed budget for the first-year expenses of partners 4&5. This advance was reimbursed to the general coordinator by deduction from the next fund transfer to these partners.

- The general coordination informed the EC about this agreement and modified the contract technical annex as required by the EC. The additional clause to the contract technical annex was the following (in italic):

In Brazil, all institutions usually commit, through contractual modalities, external foundations for managing their financial resources, especially for budgets coming from outside donors and international projects. The project's coordination had to follow those modalities otherwise it was almost impossible to transfer the funds. To this end, a joint agreement was elaborated and signed (25th May 2004) by the general project coordination (CIRAD, partner 1), the Brazilian partners (EMBRAPA, partner 4 and MAA, partner 5) and the Brazilian FUNARBE foundation committed to manage the financial resources of partners 4&5 and to submit the annual cost statements for these institutions. The cost incurred by the FUNARBE foundation will be eligible according to the definition of article 22 of the general conditions annexed to the Contract Technical Annex.

- However, the process of approval and validation of this additional clause to the contract technical annex by the EC juridical office took longer than expected, and was only completed by end of November 2005. This procedure delayed the effective fund transfer from the EC to the general coordinator and so to the whole consortium. The

general coordinator managed this complicated situation by transferring funds to the partners on its own budget and the partners invested some own resources in order to complete the activities of the project.

- Because of the following reasons:
    - The delayed start of project after contract signature (January and February being holiday period in the Southern Cone);
    - The adjustments needed for financial management with the Brazilian partners (4 and 5) and the additional clause to the Contract Technical Annex (as explained above);
    - Some delay in the validation of the first cost statements and the second fund transfer;
    - Some field problems encountered during the last cereal harvests and the need for more samples and data collection.

The general coordination gratefully requested a 9-month extension of the project's duration, until 30 September 2006. This request was examined by the EC Scientific Officer, Dr M.J. Fernandes, and officially approved by end of November 2005. According to this extension, the project terminated by end of September 2006. Before final reporting, the general coordination planned to meet all partners but this was only possible by end of 2006, due to the work schedule and availability of the partners. As January-February was summer holiday period in the Latin America Southern Cone, it was negotiated with the EC officer to deliver final reports early 2007.

- The consortium was very active and positive in terms of permanent exchanges of technical and scientific information, methods, bibliographic references, relevant web sites, scientific events, etc. These fruitful exchanges were effective among all project partners (in different countries), and between the partners participating in the same work package (WP), as well as with the work package leaders and the general coordination. Information was regularly exchanged between the partners, the work package leaders and the general coordination. Electronic discussions raised upon essential issues such as sampling (at lab or in field), sample preparation before analysis, integration of socio-economic aspects and chain actor organization.

- At the project start, CIRAD (partner 1) has outposted two scientists (Dr C. Brabet and Dr G. Henry) to Brazil. By the end of 2003, Dr G. Henry moved to Argentina and was based at INTA (partner 8) offices. This allowed to strengthen the cooperation and enabled permanent interaction with the South Cone partners. The two outposted CIRAD scientists ensured an efficient regional coordination of the project's activities and permanent support to the Southern Cone partners. They also ensured a frequent feedback to the WP leaders and the general coordination. Several visits and meetings were conducted in the 4 countries in order to (i) seek and promote further institutional support and adoption for the project, (ii) planning of field activities and (iii) trouble shooting (administrative, financial and managerial) on behalf of the project coordination in France.

- Scientific links were done with some institutions who were not direct partners of MYCOTOX project but able to bring relevant support to the project activities through methodological development, student supervision and relationship with the private sector and the cereal chain stakeholders concerned by mycotoxins. These institutions were either universities (University of Campinas and University of Maringá in Brazil, University of República in Uruguay) or technology transfer centres (Institute for Regional Development in Brazil). The general coordination negotiated then with those institutions and signed agreements with them for involvement in the WP 4&5 ("field" technical and socio economic activities) of the project.

- An important step at the project beginning was the constitution of 4 multidisciplinary teams, one in each Southern Cone country member of the project, for carrying out the "field" activities of WP 4&5, i.e. commodity chain study (product flows, relations between actors, critical points, local socio-economic environment, etc). These teams included agronomists, socio-economists, HACCP specialists and analysts, which enabled them to take up the project's challenge, by adopting an integrated approach for mycotoxin control throughout the whole wheat and maize chains, according to the specific context in each country. This was one of the project's challenges and highlights.

- Seven members of the consortium (at least one from each South Cone country) followed a FAO training (La Havana, Cuba, 22-26 September 2003) in Spanish on the HACCP (Hazard Analysis and Critical Control Points) method and its specific application to mycotoxin prevention and control. The direct benefits of this training for MYCOTOX project were numerous: i) harmonization of the HACCP glossary and methodological tools among the partners, ii) share of Latin American experiences and case studies on HACCP application to mycotoxins, and iii) access to the same manuals and methodological documents. As initially discussed between the partners, the representative of each multidisciplinary team (per Southern Cone country partner) who followed the HACCP training was responsible for the application of this method, as a member of the multidisciplinary team in the frame of MYCOTOX project and subsequently to diffuse the information and capitalize it within his own institution and to a larger scale in his country.

- Strong relationships and partnerships were built with the private sector as one of the wheat and maize chain stakeholder. This approach was essential to ensure a close collaboration with the professionals involved in wheat and maize chains in each South Cone country, and to help further output dissemination. Once the location of pilot sites chosen in each Southern Cone country, different grain stakeholders were contacted to join the project. All expressed their concern with the impact of mycotoxin and their need for an integrated quality management system throughout the whole grain chain. Specific confidentiality agreements were elaborated and proposed by the general project coordination, then validated by the whole consortium for use as an official commitment between the MYCOTOX project and the private sector in the 4 Southern Cone countries (Argentina, Brazil, Chile and Uruguay). However, the negotiation and confidentiality agreement signing took longer than planned because of the sensitivity of some to the likely implications of their product contamination. After discussion and signing of confidentiality agreements, the project succeeded in involving a representative of each

stakeholder of the cereal chain (seed and grain producers, mills, transporters, poultry farms, agro-groups and extensionnists for technical field support) in each Southern Cone country. Those participated in the activities of WP 4&5, along with the four MYCOTOX HACCP teams.

- A strong collaboration was ensured between the "lab" (WP 1&2&3) and "field" (WP 4&5&6) work packages, which helped in the achievement of the project's challenges. The technical and socioeconomic data were fully integrated, according to the local context of each Southern Cone country. Besides, in each country, the laboratories involved in the project performed the mycotoxin analyses of all samples collected in the field, they then succeeded in setting a local network for mycotoxin analysis with harmonized methods and procedures. This was another challenge taken up and a highlight of the project.

- A website was designed by the end of 2004 in replacement of the former Intranet site "Quick Place" implemented at the project start. In addition, links were put in place between the MYCOTOX website (http://mycotox.cirad.fr) and that of PROCISUR (partner 3) (www.procisur.org.uy) (both sections "Procisur Informa" and "Projects with external funding"). PROCISUR being responsible for the regional output dissemination and coordination among Southern Cone partners, the link between both sites favoured and promoted the project's activities and dissemination towards the cereal stakeholders in the Latin America South Cone.

- With the aim of optimising financial resources and project running, we could get, with the help of Dr Monica Olsen (scientific advisor of the MYCOTOX project, NFA, Sweden) a special price offer for purchasing Ochraprep immuno-affinity columns (for OTA determination). These lab materials were ordered (by end of 2003) in Europe (R-Biopharm company) by the general coordinator and sent to the University of Luján (partner 10, Argentina) and the University of Concepción (partner 11, Chile). The columns were well delivered to partner 11 (Chile); however, due to high complications for material clearance from the Argentinean customs, the columns were received by the Argentinean partner only in August 2004.

- In order to benefit from interesting price offers, the general coordinator also purchased (for the WP 1 partners) in Europe the reference FAPAS materials used as standards for mycotoxin determination. These reference materials were essential for the purpose of WP 1 aiming at harmonization and standardization of the analytical methods used by the different laboratories of the project in the 4 Southern Cone countries.

- Dr Tania Barreto Corrêa, leader of Work Package 1, (EMBRAPA-partner 4) retired from her institution in October 2003 but kept in contact with the project partners until the end of the year for pursuing the current activities and supervising the annual project writing. Discussions were made with Dr Eugenia Vargas (MAA, partner 5) who agreed for effectively taking over the leadership of the WP 1. Dr Vargas and her team updated the agenda for WP 1 activities and ensured efficiently this leadership.

- Because of institutional changes at INTA (partner 8), the leadership of WP 6 was transferred in 2004 from Dr Ricardo Rodriguez to Dr Marcelo Masana.

- In spite of his retirement from NRI (partner 2), Prof Raymond Coker, leader of WP 4&5, kept strongly involved in the project and ensured the leadership of the mentioned work packages, strongly seconded and supported by Dr Martin Nagler.

- We should highlight that, in spite of some encountered problems during the project lifetime, all partners showed high enthusiasm and strong willingness to join their efforts and tackle the project's challenges, and specifically the Latin American partners through their strong regional cooperation.

Meetings

During the whole project duration, several meetings were held in order to ensure good and fluid communication, information exchange and discussion of the advances. The meetings are listed below:

1. *Kick-off (First Annual) meeting held in Montevideo (Uruguay), 17-19 February 2003.*
The meeting was hosted by PROCISUR (partner 3).
First meeting between all partners for project launching. The activities stated in the work packages were deeply discussed and the actions to be undertaken in 2003 were planned, in total concertation among partners. The rules for project running and management were discussed and validated. General and transversal issues such as sampling (either at lab or in field) were raised.

2. *Mid-year progress meeting specific for WP 4&5 held in Buenos Aires (Argentina), 20-22 August 2003.*
The meeting was hosted by INTA (partner 8).Dr. Monica Olsen (NFA, Sweden), scientific advisor of the project, attended this meeting.

This meeting was initially planned in the beginning of year 2 (2004) but was held in 2003 to let the WP 4&5 move forward. Indeed, the WP 4&5 partners and leader expressed the need for a joint discussion and planning of the preliminary actions (e.g. constitution of multidisciplinary teams, training on HACCP methods, discussions about socio economic methods and tools) to be undertaken before carrying out the "field" activities of WP 4&5, i.e. the diagnosis of the whole cereal chains, the analysis of the critical control points and the comprehension of the socio economic context and relationships between the agrichain actors. This meeting was very fruitful and the objectives were reached.

Benefiting from our presence in Argentina, specific meetings were hold between the general coordinator, the scientific advisor and partners involved in the WP 2&3, respectively led by UnLu (partner 10) and UBA (partner 9). This allowed to discuss the advances of WP 2&3. Dr Olsen brought support and advise on the analytical problems faced by the concerned laboratories. We should note that a regional meeting was initially planned for WP 2 in 2003 but taking the opportunity of Argentina WP 4&5 meeting for

discussing the WP 2 activities allowed optimising the financial resources and kill two birds with one stone.

3. *Informal meetings for WP 1, 3, 4&5 held in la Habana (Cuba), 22-26 September 2003.*
Benefiting from a regional scientific event, the IV Latinamerican Congress on Mycotoxicology, the project partners had an interesting joint strategy for optimising time and financial resources. Indeed, almost all institutional partners were represented in this event and some of them presented posters and conferences. Apart from the congress, the specific FAO training (in Spanish) on the application of HACCP method to mycotoxin prevention and control was followed by at least one person from each multidisciplinary team (one per country). Finally, the opportunity was taken by the general coordinator for informal meetings and discussions on the advances and the next activities of WP 1&3 with the present leaders and partners.

4. *Internal regional workshop on the "formulation of socio economic approach, methods and instruments", held in Campinas (Brazil), 1-2 December 2003.*
The meeting was hosted by the University of Campinas, associated to the project through research linkages and interaction with the outposted scientists of partner 1 (CIRAD). Two invited experts participated in the meeting and exchanged experiences and tools with the partners (Dr Elisabeth Farina from the University of São Paulo (Brazil) and Dr Benoit Daviron from CIRAD (France).

This meeting was not initially planned for year 1 (2003) but was held because of the need for discussing and sharing methodological inputs and tools between the socio economists involved in WP 4&5. We should remind that one of the MYCOTOX project challenges is the real and deep integration of socio economic issues to the technical ones to allow a global and adequate approach for mycotoxin prevention and control throughout the whole wheat and maize chains. So it was essential to hold a specific meeting for socio economic issues, as it was done on technical issues (the HACCP training for instance). A fruitful output of this workshop was the elaboration of a CFD (Commodity Flow Diagram) model to be applied in the subsequent activities of WP 4&5.

5. *Second Annual Meeting held in Montevideo (Uruguay), 4-7 October 2004.*
Objectives: discussion of the project's advances and planning of the next activities. The scientific adviser of the project, Dr Monica Olsen, participated in the meeting.

6. *Specific meeting of the socio-economists involved in the project, Mar de Plata, (Argentina), 4-5 November 2004.*

Objectives: to (i) progress on research methods, (ii) divide tasks among the team, (iii) discuss possible research papers to write and submit.

7. Other *informal meetings* were also held among the *socio-economists* involved in the project during participation in regional scientific events and conferences (12-15 October 2004 in Montevideo (Uruguay), 27-29 July 2005 in Ribeirão Preto (Brazil), 15-17 March 2006 in Villa Carlos Paz (Argentina) and 10-13 June 2006 in Buenos Aires (Argentina).

8. *Regular visits were done by the general coordinator to the project's pilot sites* and meetings were then held with the consortium members, and the associate institutions and stakeholders involved in the project:

- *Argentina, August 2003*. Visit of the experimental INTA sites (Buenos Aires province) for wheat cultivar selection. Meetings with INTA (partner 8), UBA (partner 9) and UnLu (partner 10).

- *Uruguay, October 2004*. Visit of the Molino Rio Uruguay mill (Montevideo) (stakeholder which collaborated with the project in Uruguay. Meetings with INIA Uruguay (partner 6), LATU (partner 12), PROCISUR (partner 3) and the University of República (associate institution involved in the socioeconomic work of the project).

- *Brazil, February 2005*. Visit of the Frangos Canção Company (poultry producer which collaborated with the project in Brazil). Meetings with EMBRAPA (partner 4), MAA (partner 5) and the associate institutions (University of Maringa, Institute for Regional Development) involved in the project.

- *Argentina, November 2005.* Visit of the General Pico (pilot site of the project) and meetings with the stakeholders involved in the wheat chain in this region and collaborating with the project (grain producers, Don Antonio mill). Meetings with INTA (partner 8), UBA (partner 9) and UNLU (partner 10). The general coordinator also met a representative of PROCISUR (partner 3) travelling to Buenos Aires at the same period.

- *Chile, December 2005*. Visit of the Valdivia pilot site of the project and meetings with the stakeholders involved in the wheat chain in this region (Seed producer, Collico Mill, Group of Wheat Producers, Supplier of agrimaterials and inputs). Meetings with INIA Chile (partner 7) and University of Concepción (partner 11).

9. *Regional meetings* were regularly held between the two CIRAD (partner 1) scientists (Catherine Brabet and Guy Henry) respectively outposted in Brazil and Argentina with the partners in the 4 South Cone countries (2003, 2004, 2005 and 2006).

10. *Specific meetings* were regularly held among the partners (from "analytical" WP 1&2&3 and "field" WP 4&5&6) in each Southern Cone country, according to the needs of each WP advances and activities.

11. A *final trip* was done by the general coordinator to the Southern Cone by the end of the project (23 November to 3 December 2006) in order to meet all partners and discuss the last outputs, final reporting and further valorisation through scientific papers and conferences. Meetings were held in Belo Horizonte (Brazil), Buenos Aires and Luján (Argentina), Santiago (Chile) and Colonia (Uruguay).

Training

Training was a strong component of the project's strategy, with 22 trainees throughout the whole project duration, including 8 PhD and 5 MSc. The following students participated in the project's activities under the supervision of different partners:

The following students participated in the activities of the project under the supervision of different partners:

1. *Rodolfo Osório de Oliveira*, Institute of Economics, University of Campinas, Brazil (associate institution to the project), March to July 2003, under supervision of CIRAD (partner 1). *Topic*: *socio-economic activities of WP 4&5*, through literature reviews on the wheat and maize agrichain organization in Brazil.

2. *Ariel Wilder*, Department of Economics, Agricultural High School Luis de Queiroz, Brazil, March to August 2003, under supervision of CIRAD (partner 1). *Topic*: *socio-economic activities of WP 4&5*, through literature reviews on the wheat and maize agrichain organization in Brazil.

3. *Julio Paredes Guzman*, Faculty of Engineering, University of Campinas, Brazil (associate institution to the project), April to May 2003, under supervision of CIRAD (partner 1). *Topic*: *technical activities of WP 4&5*, through literature reviews on the mycotoxin contamination levels in wheat and maize in Brazil.

4. *Maria Ines Abecia Soria*, Faculty of Engineering, University of Campinas, Brazil (associate institution to the project). April to November 2003, under supervision of CIRAD (partner 1). *Topic*: *technical activities of WP 4&5*, through literature reviews on the mycotoxin contamination levels in wheat and maize in Brazil.

5. *Leticia Broggi*, University of Buenos Aires, Argentina (partner 9). PhD student (start in 2003). *Topic*: *Potential contamination by molds and mycotoxins of the agricultural products in the Entre Rios province and influence of regional milling processes* ("Productos agricolas de la provincia de Entre Rios: contaminacion potencial por hongos y micotoxinas e influencia de los procesos de molienda regional").

6. *Maria Margarita Samar*, University of Buenos Aires, Argentina (partner 9). PhD student (start in 2003). *Topic*: *Influence of some steps of wheat processing on the tricothecene persistence or modification* ("Persistencia y/o transformacion de tricotecenos durante algunas etapas del procesamiento de trigo").

7. *Gisela Ríos*, University of Concepción, Chile (partner 11). PhD student in France under the supervision of partners 1 and 11, start of PhD by end of 2004. Participation in WP 3. *Topic: Study of the influence of grain structural properties and milling steps on the mycotoxin distribution in the grain and outcoming fractions.*

8. *Paulina Riquelme*, University of Concepción, Chile (partner 11), Master of Pharmaceutical Sciences degree (2004-2005). Participation in WP 1&3. *Topic:*

Analytical protocol for fumonisin determination in maize.

9. *Katherine Muñoz*, University of Concepción, Chile (partner 11), Master (2004-2005) and start of PhD (by end of 2005) in Pharmaceutical Sciences degree. Participation in WP 2. *Topic: OTA determination in human blood samples and relationships with the Chilean food diet*.

10. *Gabriela Torezan*, University of Campinas, Brazil, under the supervision of CIRAD (partner 1). *Participation in WP 4&5 Brazil* (2004-2005). *Topic: updating the literature data on the natural occurrence of mycotoxins in Brazilian corn and corn-based products.*

11. *Beatriz Leiko Sekiyama*, MSc, Health Sciences, Maringa State University, Brazil. *Participation in WP 4&5 Brazil* (2004-2005). Joint supervision of partners 1&2 and the Brazilian institutions associated to the project (University of Campinas, University of Maringa and the Institute for Regional Development)

12. *Robson de Oliveira Lemes*, Pre-graduated, Geographical Sciences, Maringa State University, Brazil. *Participation in WP 4&5 Brazil* (2004-2006). Joint supervision of partners 1&2 and the Brazilian institutions associated to the project (University of Campinas, University of Maringa and the Institute for Regional Development)

13. *Laura Biasotti*, Licenciatura de Economía, INTA, Argentina (partner 8). PhD student (2004-2005). *Participation in WP 4&5, socio-economic study in Argentina*. Surveys with the whole wheat chain actors. Joint supervision of partner 8 (INTA) and partner 1 (CIRAD).

14. *Estela Motta* , Faculty of Natural Sciences, University of Buenos Aires, Argentina (partner 9). PhD student under the joint supervision of partners 9 and 10. Participation in WP 3 and WP 2. *Topic: Degradation of fumonisins through maize wet milling and assessment of exposure to ochratoxin A* ("Degradación de fumonisinas en la molienda húmeda de maíz y evaluación de la exposición por ocratoxina A")

15. *Emilia Ciancio Bovier*, Food Engineering student (2004-2006), University of Luján, Argentina (partner 10). Participation in WP 2. *Topic: Ochratoxin A determination in blood and correlation to the donor diet in Argentina*.

16. *Gustavo Funes*, Faculty of Natural Sciences, University of Buenos Aires, Argentina (partner 9). PhD student (start in 2005). Participation in WP 3. *Topic: Fumonisin derivates in wet corn milling fractions*.

17. *Lucila Frusteri*, Faculty of Natural Sciences, University of Buenos Aires, Argentina (partner 9). Magister student (2005-2006) under joint supervision of partners 9&10. Participation in WP 2 and support to WP 4&5. *Topic: Agrochemical effects on DON and fungal contamination on wheat and soybean*.

18. *Manuel Zelaya*, Faculty of Natural Sciences, University of Buenos Aires, Argentina (partner 9). Magister student in Food Science (2005-2006) under joint supervision of

partners 9&10. Participation in WP 2. *Topic: Identification of the mycoflora of soybean germoplasm in Argentina* ("Estudio de la micoflora contaminante del germoplasma de soja en Argentina").

19. *Alexandre Iwahashi*, Maringa State University, Brazil. Participation in WP 4&5 field activities in Brazil (2005-2006). Joint supervision of partners 1&2 and the Brazilian institutions associated to the project (University of Campinas, University of Maringa and the Institute for Regional Development).

20. *Carolina Sepúlveda*, University of Concepción, Chile (partner 11). Pre-graduated student (2006). Participation in WP 2. *Topic: Implementation of an analytical technique for OTA determination in wheat and derived products*.

21. *Alejandra Opazo*, University of Concepción, Chile (partner 11). Pre-graduated student (2006). Participation in WP 2. *Topic: Validation of an analytical technique by liquid-liquid extraction for OTA determination in pork muscle, liver and kidney*.

22. *José Mouat*, INIA Chile (partner 7). Pre-graduated student in Agricultural Engineering, University of Concepción (start in 2006). Participation in WP 4&5 socio-economic activities. *Topic: Assessment of the concept of quality for wheat milling industry in Chile*.

In addition, exchanges of scientists were made among the partners, especially for analytical aspects within WP 1&2&3.

- M. O. Martínez (LATU, partner 12) had a scientific stay in 2005 at UBA (partner 9) for exchanges on the identification of mycotoxigenic fungi.

- E. Ciancio Bovier (UNLU, partner 10) had a scientific stay in 2005 at University of Concepción (partner 11) for sharing methodological aspects on determination of human exposure to OTA.

- J. Cea (LATU, partner 12) had a scientific stay in 2006 at University of Concepción (partner 11) for sharing analytical methods on mycotoxin determination by chromatography.

- Exchanges were made between analysts of MAA (partner 5) and EMBRAPA (partner 4) in 2004 and 2005 for implementation of analytical methods for mycotoxin determination by chromatography.

Main problems encountered for project management

- Because of the local administrative rules and constraints, the fund transfer to Brazilian and Argentinean institutions was complicated and took longer than provided for. The general project coordinator did the best for managing this situation, in concertation with

the concerned partners and in accordance with the above mentioned difficulties. In Brazil, all institutions usually commit, through contractual modalities, external foundations for managing their financial resources, especially the budgets coming from outside donors and international projects. We had to follow those modalities otherwise it was almost impossible to transfer the funds. To this end, a joint agreement was elaborated between the general project coordination, the Brazilian partners (EMBRAPA, partner 4 and MAA, partner 5) and the FUNARBE Brazilian foundation committed to manage the financial resources of partners 4&5. The EC required an additional clause to the contract technical annex, which was done but the processr required time for validation and approval. This procedure delayed the effective fund transfer to the partners. However, the general coordinator managed this complicated situation by transferring funds to the partners on its own budget and the partners invested by advance some own resources in order to complete the activities of the project.

- The cost statements provided by the partners for the first year (2003) were not all completely eligible by the EC. Many modifications were brought according to the request of the EC project administrative officer, in order to comply with the EC requirements. This process took longer than planned and was not completely solved by the end of 2004. The project's running suffered from the delay in fund transfer, especially that we were entering a crucial phase, i.e. the sample collection during the December-February grain harvests. The general coordination managed this delay by transferring funds to the partners on its own budget and the partners invested by advance some own resources in order to pursue the planned activities.

However, we should recognize that this delay in validating cost statements served as a lesson to the whole consortium, for the elaboration of the next ones. In order to avoid this problem, we required from the whole consortium to send a first expense draft to the general coordination for checking before sending the original signed cost statements to the EC administrative officer.

- There was a delay (eight months) in the custom clearance and delivery of the Ochraprep immuno-affinity columns (purchased in Europe by the general coordinator for benefiting from interesting price offers) to the University of Luján (partner 10, Argentina). This problem was finally solved.

- We also had to face some unexpected problems and/or issues related to the local contexts (some changes in the local strategies for cultural practices, grain harvesting and storage, climatic risks in some selected regions, feedback from the socio-economic surveys and the chain stakeholders, etc.). This made us revise and adapt some planned activities according to the needed adjustments to closely meet the local context in each country partner. The delivery of some outputs was postponed; however, all work was finalised as committed in the contract technical annex.

However, we should highlight the motivation and willingness of all partners to join their efforts for tackling the project's challenges. In spite of the encountered constraints and delays, the partners were very positive and kept strong involvement in the planned

activities. Permanent exchanges of information on technical and scientific issues, methods, bibliographic references, relevant websites and scientific events were assured among the consortium members.

INCO: International Scientific Cooperation Projects (1998-2002)

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

Title:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries

Individual Partners Reports

Partner 01 - CIRAD

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

The activities carried out by CIRAD (partner 1) within the whole duration (2003-2006) of MYCOTOX project were the following:

General coordination

Task 1: Management of administrative and financial issues

CIRAD managed the fund transfer to the consortium members. Some complications occurred throughout the project's running such as: i) some delays in the validation of first cost statements and ii) the later EC approval (by end of November 2005) of the additional clause to the technical contract annex with the Brazilian partners. However, the general coordinator managed this complicated situation by transferring funds to the partners from its own budget and the partners invested some own resources in order to run the activities of the project as planned, meanwhile the EC transfers were done.

The general coordinator ensured permanent relationships with the EC officers. An annual meeting was held with the EC scientific officer in Brussels for presenting the project's advances.

Because of the administrative delays mentioned above and some field constraints locally encountered, the general coordinator requested a 9-month extension of the project, until 30 September 2006, which was approved by the EC Scientific Officer. Before final reporting, the general coordinator planned to meet all partners but this was only possible by end of 2006, due to the work schedule and availability of the partners. As January-February was summer holiday period in the Latin America Southern Cone, it was negotiated with the EC officer to deliver final reports early 2007.

Task 2: Organisation and participation in the project meetings and visits to the partners

In collaboration with the partners, the general coordinator organised and participated in three annual meetings (February 2003, October 2004 and March 2006) and specific meetings of the different Work Packages. The general coordinator visited the 4 pilot sites of the project and met the partners very regularly within the whole project duration.

Task 3: Regional coordination in the Southern Cone region

The two CIRAD scientists, Dr C. Brabet (Brazil) and Dr G. Henry (Argentina), ensured an efficient regional coordination of the project's activities and permanent support to the Southern Cone partners. They also ensured a frequent feedback to the WP leaders and the general coordination. Several visits and meetings were conducted in the 4 countries in order to (i) seek and promote further institutional support and adoption for the project, (ii) planning of field activities and (iii) trouble shooting (administrative, financial and managerial) on behalf of the project coordination in France.

Task 4: Design of the project website

CIRAD designed the project's website (http://mycotox.cirad.fr) and favoured links with the regional PROCISUR website (www.procisur.org.uy) to facilitate wider dissemination of the project in the Southern Cone region.

Task 5: Support for analytical material purchase in Europe
When needed by the partners, the general coordinator brought help for purchasing materials in Europe, either because of their unavailability in the partner country or with the aim to take benefit of a special price offer and optimise the use of the project's financial resources. Certified reference FAPAS materials and Ochraprep immuno-affinity columns were ordered by the general coordinator respectively at CSL (UK) and Biopharm company (UK) for WP 1&2&3 partners.

Task 6: Formalization of confidentiality agreements with the private sector
Contacts and visits were done to identify, discuss and negotiate concrete collaborations (within WP 4&5 field activities) with pertinent cereal stakeholders from private and public sectors, in each Southern Cone country. These visits also served to better understand the constraints and opportunities of the wheat and maize commodity systems in the 4 countries. Confidentiality agreements were prepared by the general coordination, validated by the whole consortium and signed to formalise commitments with the stakeholders who got involved in the project.

Task 7: Extension of the project's partnership to associate institutions
Scientific collaborations were done with Brazilian institutions who were not direct partners of MYCOTOX project but who brought relevant support to the project activities through methodological development, student supervision and relationship with the private sector and the cereal chain actors concerned by mycotoxins. These institutions were either universities (University of Campinas UNICAMP and University of Maringa) or technology transfer centres (Institute for Regional Development). Specific cooperation agreements were elaborated and formalized between the partner 1 and those institutions.

Participation in Work Package 1

Task 8: Prospective research on the inhibition of bacteria luminescence in presence of mycotoxins *(Work done by Samira Sarter, Isabelle Métayer and Nadine Zakhia)*
The "toxicity bioassays", based on the natural ability of some bacteria to emit light, and the potential decrease of bacterial light emission in presence of mycotoxins, were pursued. The bacteria *Vibrio fischeri* was taken as model. The toxicity bioassays consisted of incubating (Bactomarine medium, continuous stirring, 25°C for 24h) the *V. fischeri* culture alone (referred to as "*control*") and in the presence of mycotoxins (referred to as "*mycotoxin bioassay*") and to measure and compare the quantity of emitted light (in RLU). For "*mycotoxin bioassays*", the mycotoxins added to the bacterial culture (10 ml) were either diluted standards or mycotoxin-containing cereal extracts (a volume of 100 µl). The studied mycotoxins were aflatoxin B_1 and deoxynivalenol DON.

During the lag phase (which lasted 6h), the light emission decreased drastically for the mycotoxin assays - with aflatoxin (10 µg/ml) or DON (20 µg/ml) - and for the corresponding controls. At the end of lag phase, the light emission reached a minimal value and then started rising exponentially until reaching a peak after 12h of incubation.

The absorbance followed the same behavior confirming then the bacterial growth.

When used as **pure standards**, mycotoxins were diluted respectively in dimethyl sulfoxide (DMSO) for aflatoxin B_1 or in methanol for DON. They were tested at various concentrations (aflatoxin 5, 10, 20 µg/ml; and DON 10, 20, 50 µg/ml) and at two different cell concentrations (absorbance of 0.1 and 0.01 at 550 nm). The value of 0.01 was later adopted for bacterial cell absorbance in all our experiments. Experiments were conducted in triplicate. Bacterial cell was assessed by absorbance measurement and the light emission (referred to as "*luminescence*") was measured using a luminometer (Berthold S.A.) and expressed in Relative Light Units (RLU).

To ensure a security margin for measuring complete light emission, the time 15h was taken as end-point for determining the inhibition percentage (I%) of the emitted light in presence of the two studied mycotoxins. The "inhibition percentage" was calculated, according to Froehner *et al*., by the equation $I\ (\%) = [(C_{t15} - S_{t15})\ 100]/C_{t15}$ where C_{t15} was the average of control luminescence at 15h and S_{t15} was the average of sample (either pure mycotoxin standard or cereal extract) luminescence at 15h.

The impact of mycotoxin presence was evaluated by calculating the percentage of luminescence inhibition of *V. fischeri* (absorbance 0.01) at the different concentrations of aflatoxin B_1 and DON mentioned above. The experiments done in triplicate showed high reproducibility. Table 1 showed that aflatoxin B_1 totally inhibited the luminescence ability of *V. fischeri* at both 10 and 20 µg/ml concentrations, and by an average I% of 65% at 5 µg/ml. On the contrary, the initially tested concentration of 10 µg/ml for DON was not able to inhibit the *V. fischeri* luminescence. Higher concentrations were tested and the concentration of 50 µg/ml proved to reduce the luminescence by an average ratio of 42%. The obtained inhibition percentages I% were satisfactory in the sense that the range 40-60% was in the middle of the 0-100% inhibition scale. These results validated our hypothesis that the ability of *V. fischeri* to emit light might be used as an indirect means for estimating mycotoxin content.

The "*mycotoxin bioassays"* were also conducted in duplicate **upon real food matrices**, such as wheat grain and outcoming fractions of wheat milling (coarse flour, fine flour, bran). Two lots were used with two contamination levels (average of 4000 ppb for Lot A and 400 ppb for Lot B). All samples (grain and milling fractions) were also analysed by HPLC (classical reference technique) for DON determination. A new degree of complexity was added with the cereal matrix because of the need of an additional step of extraction. The extraction procedures were optimized: samples were ground and extracted with a 84% solution of acetonitrile, stirred for 1h30 and filtered. The *"mycotoxin bioassay"* was then conducted according to the same protocol than described above, the wheat extract acting as a "source" of mycotoxin instead of the mycotoxin standard itself.

According to the previous results with mycotoxin standards, we considered the range of 40-60% for inhibition percentage (I%) as a good indicator for estimating the impact of mycotoxin presence on the *V. fischeri* ability to emit light. When the I% value was over 90%, the experiment was repeated after dilution of the wheat extract with methanol, so as to reduce the I% value. Working on real food matrices – in our case, wheat extracts -

lowered the reproducibility of the analytical protocol in comparison to the use of mycotoxin standards. This phenomenon was often observed in different types of analytical procedures dealing with real food matrices *vs* standards, because of the impact of the food matrix constituents and possible biochemical interactions or interferences with mycotoxins.

All wheat extracts (either from grain or milling fraction) showed around 100% inhibition of light emission, when no diluted. Different dilutions were then tested. The most contaminated fractions such as bran (14416 ppb) and fine flour (7737 ppb) for Lot A needed higher dilution ratio to obtain an average I (%) values below 50%. This logically agreed with the hypothesis of our work, that higher was the mycotoxin level in the cereal, higher should be the extract dilution to get the same range for inhibition percentage I%. We should note that the reproducibility was good (low standard deviations) except for some dilutions. These experiments served to optimize the extraction procedure and initiate the *"mycotoxin bioassays"* on food matrices.

The toxicity bioassays using *V. fischeri* luminescence were also carried out on some **cereal (either wheat or maize) samples on-field collected in Brazil, Uruguay and Chile** (in the frame of WP 4&5) and analysed by classical HPLC technique by the partner laboratories of these countries. The samples were ground before extraction with acetonitrile (for DON/wheat) and methanol/water (for aflatoxin B_1/maize). The bioassay was conducted according to the same protocol described above. The inhibition percentage (I%) was calculated for aflatoxin B_1 in maize samples collected in Brazil, and for DON in wheat samples collected in Uruguay.

A high variability was shown among samples collected from Brazil. No evident direct correlation was found between the I% values and the aflatoxin concentration in the microbial culture (expressed in µg/ml) or the one measured by HPLC (expressed in µg/kg or ppb). For instance, the less contaminated samples, i.e. n°74 (1.5 ppb) and 49 (1.2 ppb) showed high I% values (respectively 90.5 and 72%) even if they presented a similar concentration of aflatoxin B_1 in the microbial culture (respectively 0.08 x 10^{-3} and 0.06 x 10^{-3} µl). A high variability was also evidenced among samples collected from Uruguay. For the samples A_3A_2 (28430 ppb by HPLC) and A_4A_1 (14215 ppb by HPLC), dilution ratios of 1/100 and 1/50 were respectively used; this was logical as A_3A_2 was more contaminated than A_4A_1. However, after conducting the *"mycotoxin bioassay"*, both samples showed the same value of 0.021 µg/ml as DON concentration in the microbial culture but showed different inhibition percentages, respectively 50.5% and 74%. Most samples collected in Chile showed to be negative for DON by classical HPLC technique or presented very low levels, so that no justification was evident for conducting a *"mycotoxin bioassay"* on those samples, unless the variability problem was solved.

The high variability of I% and the lack of correlation between I% and mycotoxin concentration in the microbial culture, which were generally observed on the cereal extracts, pointed out the difficulty of validating the *"mycotoxin bioassays"* on the real food matrix. This might be due to different scenarios: i) the possible interactions between some constituents of the cereal matrix and the mycotoxin itself, ii) the history of the sample between collection, transportation, storage, extraction and toxicity bioassay

through luminescence, and iii) the calculation of the inhibition percentage I% as a fraction ratio, aggregated the relative errors of both numerator and denominator, which increased the global uncertainty. More samples should be analysed and a deeper statistical analysis performed in the future. Experiments are currently under way for determination of EC_{50}, i.e. the mycotoxin concentration that reduces the luminescence by 50%, and a statistical analysis is foreseen according to the Weibull model.

This work on toxicity bioassays was pioneer for application to mycotoxins, as most existing literature dealing with *V. fischeri* bioassays concerned chemical pollutants in water or wastewater. The use of toxicity bioassays through *V. fischeri* luminescence showed a potential as semi-quantitative screening tool for rapid discrimination of mycotoxin-contaminated grain bulks or batches. Our results confirmed the existence of a relationship between the light emission by *V. fischeri* and the mycotoxin concentration. However, the high variability observed should be reduced through further investigation; this research line will be continued by CIRAD. The MYCOTOX project acted in this sense as an investigation starter.

Task 9: Prospective research on the application of Near Infrared Reflectance Spectroscopy (NIRS) technique for mycotoxin determination

(Work done by Denis Bastianelli and Laurent Bonnal)

Near Infrared Spectroscopy is widely used to determine the chemical composition of raw materials, food and feed. This technique is based on the absorption of infrared light by the chemical bonds of the organic matter. It is generally admitted that only major compounds can have sufficient effect on the spectrum to be detected and quantified. However several studies have shown that when the presence (or level) of a trace compound is linked to other changes in the sample composition, the NIRS technique may detect this compound through indirect relationships and correlations. This is typically the case of mould detection and it was the hypothesis that was tested in this study. Mould induces major biochemical modifications in the grain composition, which definitely have an effect on the grain's spectra. The mould count is not directely correlated with DON level but our hypothesis was that the conditions in which mould produce mycotoxins might be traced by NIRS spectra.

Wheat samples collected on-field (in the frame of WP 4&5) and analysed for DON by classical chromatographic techniques in the partner laboratories (Uruguay and Chile) were submitted to NIRS measurement. They were already ground and the characteristics of the powder (granulometry) allowed direct use in NIRS analysis without additional sample preparation. The maize samples collected in Brazil were not submitted to NIRS measurements as they where i) spectrally too different from wheat grain, which impeded them to be included in the database under construction; ii) they were analysed by chromatography for aflatoxin and not for DON; and iii) the number of those maize samples was not enough high for creating a separate database.

So, the total wheat database included samples from Uruguay and Chile. Wheat samples from France with known DON levels were included to increase the database size and variability. The French wheat samples were coherent with the South American ones from a spectral point of view. A total number of 113 samples was submitted to NIRS

measurement.

Reference DON values were provided by the partner laboratories (Uruguay and Chile) and by the French cereal supplier. Methods were different between laboratories and especially between South America and France. Indeed, the samples from South America were analysed by chromatographic techniques whereas those from France were analysed by ELISA technique as routine survey on field samples and not from research laboratories. The samples sent from Chile and Uruguay were mainly samples showing positive DON level. The samples which showed negative for DON (by HPLC) or were under the analytical detection level were not transported from South America to CIRAD, in order to optimize transportation costs. The histogram of DON levels did not follow a normal distribution. There were much more values around the “low” DON levels than the “high” ones.

The NIR spectra were collected in reflectance mode in ring cups on a FOSS NIRSystem 6500 spectrometer (Foss NIRSystems, Silver Spring, MD, USA). Readings were made in duplicate (with 2 different cup fillings) and the spectra were averaged. The wavelength range was 400-250 nm, *i.e.* visible (400-800 nm) + near infrared (800-2500 nm). The spectral database was homogeneous and no sub-population structure or major spectral outliers were detected.

Partial least square regression was used for calibration procedure, as it was the most adapted statistical method for quantitative determinations on medium size databases as ours. There was no initial hypothesis on the best mathematical pre-treatment to be used on this kind of calibration based on indirect relationships between DON (ppb-ppm concentrations) and other structural/compositional properties of samples. So a wide range of mathematical treatments was tested.

The parameterization appeared to have an important effect on the precision of calibrations and the structure of errors. In some cases, the error was more concentrated on the samples with low DON levels while in other cases it was more distributed between samples with high DON levels. This was due to the high number of samples with low DON concentrations. The models which appeared to be the most suitable for our screening purpose were those with a better prediction throughout the concentration range, even if the accuracy on low values was somewhat lower. The best models were obtained with high order derivatives (3^{rd} order). Residual standard deviation was around 2000. This value was considered as very high for low values but it was more acceptable for the samples with very high DON concentrations. R^2 values appeared to be high because of the high variability of the population. The synthetic quality criterion RPD (= SD/SECV) was greater than 2 which indicated a reasonable possibility of using the calibrations for the purpose of grain batch screening.

According to the graphical interpretation (vertical and horizontal lines), the calibration allowed to predict to which of the 3 categories (low, intermediate and high DON level) the sample belongs. To check this statistically, a discriminant analysis (FDA) was performed on the spectra. 4 categories were designed for practical use. The FDA was based on the spectral information (first 12 variables of the principal component analysis

of spectra). The rate of success in cross validation was 89%. All samples, except 1, were assigned to the right category or to the immediate neighbour. 94% of samples with measured value <1750 ppb were predicted as such and also 94% of samples classified as <1750 ppb were indeed below this threshold value.

This study allowed defining the potential role of NIRS for mycotoxin management. It clearly appeared that the precision of NIRS calibrations obtained did not allow a precise quantification of DON in wheat samples. But the NIRS technique showed a potential as a tool to be used for sample screening, according to their general contamination level (low, intermediate, high). The main advantages of this technique are rapidity (several hundreds of samples may be analysed per day) and extremely low cost.

The NIRS detection of DON was probably not based on the direct detection of light absorption by the DON molecules themselves since the concentrations were very low (ppb-ppm). It was therefore likely that the detection was made through indirect correlations between DON level and other properties of the sample. This is an interesting finding which should orientate future studies towards the understanding of these relationships and might lead to other rapid screening methods.

Future investigation should also consider higher number of samples for NIRS calibrations, in order to cover the variety of situations that can be met in the field (varieties, growing conditions, post-harvest conditions, etc.). This would also help developing calibration on samples with low DON values, which would probably improve the precision obtained on the studied samples. Then a 2 step–analysis could then be proposed: a first step with a general calibration followed by a second step with a calibration adapted to the DON range of the sample.

Participation in Work Package 3

Task 10: *Co-supervision of a PhD on the influence of grain structural properties and milling steps on the mycotoxin distribution in the grain and resultant fractions*

CIRAD (*Nadine Zakhia*, partner 1) and UDEC (*Mario Vega*, partner 11, Chile) co-supervised the PhD of Ms Gisela Rios (Research Assistant from UDEC) in Montpellier (France), in close collaboration with the French INRA (Institut National de Recherche Agronomique), a research institution specialist in cereals.

The objectives were:

2 to evaluate the impact of different wheat processing (milling and fractioning) on the DON (desoxynivalenol) distribution in the resultant fractions. This aimed at identifying the critical points (where mycotoxin content is at unsafe level) for each process and proposing adapted control measures.

3 to determine the relationship between the wheat grain characteristics (structural, physical and biochemical) and its sensitivity to *Fusarium* infection. This aimed at identifying such a “protective” effect of the peripherical layers (pericarp, testa, aleurone) against *Fusarium* infection and DON distribution within the grain.

Two contaminated batches of durum wheat (*Acalou* cultivar), with distinct DON content (400 and 4000 ppb), were used.

Milling was shown to favour the contamination of the inner parts of the grain. Two hypothesis were then stated: i) during milling, the inner parts might be contaminated through strong mechanical contact with the more contaminated outer parts of the grain; ii) *Fusarium* penetration and/or DON migration inside the grain may damage the inner tissues which would be more sensitive to milling and might lead to more contaminated inner fractions. Experiments on re-milling and sieving (different mesh) the fractions issued from the inner parts of the grain showed that higher DON content was always found in the finest obtained parts. The highest was the average DON level in the grain (4000 ppb), the highest was DON content in the milling fractions corresponding to the inner and central parts of the grain.

In parallel, DNA was extracted from all milling fractions and amplified by qualitative PCR using primers corresponding to the tri5 gene involved in the toxin synthesis. *Fusarium* presence was detected in all milling fractions. This confirmed that DON was present even in the innermost parts of the grain, and validated in some way both hypothesis. Experiments are under way using quantitative PCR for further quantification of *Fusarium* presence in all fractions. However, it can be stated that milling would not be the best process for reducing DON distribution within the resultant fractions.

Experiments were also done for studying another grain fractioning process. *De-hulling* was carried out on the same *Acalou* wheat batches through progressive abrasion of the wheat grain for 30 min. De-hulled samples were taken out at time intervals and analysed for DON content. At the same de-hulling time and for the same percentage of mass loss in the grain, higher DON percentage remained in the more contaminated batch (4000 ppb). For a de-hulling yield of 75% (remaining grain after abrasion), only 35% of the initial DON content remained in the grain, whereas for the same milling yield, 50% of DON remained. This finding showed that de-hulling was very interesting for fractioning a very contaminated wheat grain.

The impact of grain *grading by size* on the DON distribution within the batch was also studied as important step of wheat processing. Grain was sieved on different mesh size (2.2, 2.4, 2.9, 3.5) and the DON content was determined in the fraction corresponding to each sieving mesh. The grain size was shown as a critical parameter, as removing up only 7% of the grain mass led to an average reduction of 25% of DON content (22.4% for the 400 ppb batch and 27.2% for the 4000 ppb batch). Grain grading by size seems to be a promising technological alternative for the wheat stakeholders (mainly storage and industry levels).

Concerning the second objective of the PhD work, experiments are under progress on the relationship between the structural and biochemical characteristics of the wheat grain and its resistance to *Fusarium* infection. Various soft wheat cultivars, with different resistances to *Fusarium,* were artificially infected at different dates of the spike development (collaboration with INRA Rennes). The harvested grains are under analysis

with the aim to find out any "protective" effect of some constituents of the grain against *Fusarium*/DON contamination.

The MYCOTOX project acted as a starter of this investigation. The research will continue beyond the end of project, through the PhD work of Gisela Ríos and the strong collaboration of CIRAD (partner 1), INRA (associate French institution) and UDEC (partner 11).

Participation in Work Packages 4&5

(Work done by Catherine Brabet for technical aspects and Guy Henry for socio-economics)

Task 11: Support to the establishment of multidisciplinary HACCP teams

The formation and organization of the 4 project multidisciplinary teams (one per country) was assisted. These teams, called HACCP teams (as they applied the Hazard Analysis and Critical Control Points method) included agronomists, socio-economists, analysts, HACCP specialists). CIRAD participated in their constitution, identification of needed competences from non-partner institutions in the 4 countries and formalisation of collaborations.

Task 12: Participation in the HACCP team activities in Brazil

A literature review on mycotoxin occurrence in Brazilian maize and wheat was performed for the period 1975-2003 using international databases. A hundred references were collected and analysed. This allowed to select the Paraná state as pilot site, the maize for poultry feeding as commodity system with a wide range of mycotoxins of interest (aflatoxins, fumonisins, zearalenone and ochratoxin A).

Task 13: Support to elaboration and updating of the Commodity Flow Diagram (CFD) in Brazil

CIRAD (partner 1) participated in the elaboration and regular updating of the Commodity Flow Diagram of the selected commodity system (corn for poultry feeding) in Brazil. A representative population of key players, composed of corn suppliers (producers or cooperatives or traders), transporters, feed plant, poultry farms, slaughter houses, was identified and interviewed on the basis of questionnaires which were elaborated by the Brazilian HACCP team. Frangos Canção, a poultry feed factory and slaughterhouse, was selected for getting involved in the project activities in Brazil, according to their concern with mycotoxins and their motivation. The filled questionnaires compiled a lot of information on the corn supply, volumes and fluxes, transportation channels, quality control, process diagram in the Frangos Canção company, and basic information on socio-economic aspects.

Task 14: Elaboration of a surveillance programme in Brazil

CIRAD participated in the elaboration of the surveillance programme in Brazil, jointly with the HACCP team and in strong collaboration with NRI (partner 2 and leader of WP 4&5). A protocol was prepared for corn sampling, precising the points and populations of

the selected corn supply chain where sampling should be done, as well as the number of batches and replicates. Sample handling and preparation prior to analysis were also shared among partners and in collaboration with WP 1. Four studies were initiated in 2005 within this surveillance programme in Brazil, three of them were conducted at the poultry feed plant (impact of the corn supply at reception, impact of cleaning and drying and impact of grain storage in silo) and the fourth one at the poultry farm level (impact of on-farm feed storage and handling). Corn samples were collected at different harvests, they were pre-dried prior to analysis for moisture content and water activity, as well as mycotoxin determination (aflatoxin, fumonisin and zearalenone; ochratoxin A was determined in some samples).

Task 15: Implementation of field surveys for tracing the "history" of the sampled corn

Three questionnaires were elaborated for tracing the "history" of the sampled corn, i.e. agricultural and technical itineraries, transportation, storage, etc, which can allow matching this information with the analytical measurements performed on the corn samples and can give indication of potential control measures and good practices to be recommended. The different stakeholders of corn supply chain were interviewed on the basis of those questionnaires. Data was also collected on climatic factors within the sampling period.

Task 16: Testing and validation of potential control measures in Brazil

In Brazil, the tested and validated control measures were grain drying until adequate water content and activity, as well as cleaning twice, which were favourable to reduction of aflatoxin and fumonisin in corn. The BGYF (Bright Greenish-Yellow Fluorescence) test for grain segregation at the feed mill's entrance and the addition of mycotoxin adsorbent to the feed were proposed as additional control measures to be tested in the future.

The surveillance studies allowed to gather a high number of data on the different steps of the corn chain for poultry feeding in Brazil. These data served as a basis for elaborating the HACCP plan, the Manual of Good Practices and the Food Quality Management System (FQMS). Those three outputs are of prime importance for further use as recommendations, guidelines and manuals by the Brazilian corn chain stakeholders, policy makers and regulatory authorities.

Task 17: Coordination of the socio-economic studies in the 4 Southern Cone countries and scientific support to the socio-economists in each country partner

The HACCP teams in the 4 countries were regularly visited with the aim to i) discuss the work advances and adjust the surveys and questionnaires and (ii) further develop socio-economic research approach proposal, theory and instruments. All survey questionnaires, stakeholder interviews and research methods were discussed jointly and harmonised among the project's socio-economists, to allow further effective and significant data analysis. The New Institutional Economics approach was used, taking into account the transaction costs analysis, the existing or not incentives, the possible governance structures between the chain actors. This study added to the preceding literature in the sense that it analyses structure and costs necessary to implement a

HACCP system throughout the supply chains.

Participation in Work Package 6

CIRAD (partner 1) participated in WP 6 through advices and support to the WP 6 leader (INTA, partner 8) for strategy implementation and to PROCISUR (partner 3) for conception and design of dissemination documents, as well as participation in the specific WP 6 meetings.

Co-supervision of student work

- Julio Paredes Guzman, Faculty of Engineering, University of Campinas (Brazil), April to May 2003.

- Maria Ines Abecia Soria, Faculty of Engineering, University of Campinas (Brazil), November 2003.
These two students contributed to the technical part of WP 4&5, through literature reviews on the mycotoxin contamination levels in wheat and maize in Brazil.

- Rodolfo Osório de Oliveira, Institute of Economics, University of Campinas (Brazil), March to July 2003, contribution to the socio economic activities of WP 4&5.

- Ariel Wilder, Department of Economics, Agricultural High School Luis de Queiroz (Brazil), March to August 2003.
These two students contributed to the socio economic activities of WP 4&5 through literature reviews on the wheat and maize agrichain organization in Brazil.

- Gisela Ríos, from University of Concepción, Chile (partner 11). PhD in the framework of WP 3, start by end of 2004.

- Beatriz Leiko Sekiyama, MSc, Health Sciences, Maringa State University, Brazil. Support to WP 4&5 field activities in Brazil (2004-2005).

- Robson de Oliveira Lemes, Pre-graduated, Geographical Sciences, Maringa State University, Brazil. Support to WP 4&5 field activities in Brazil (2004-2006).

- Gabriela Torezan, Unicamp-Fea, Brazil. Support to the update of the literature data on the natural occurrence of mycotoxins in Brazilian corn and corn-based products (2004-2005).

- Alexandre Iwahashi, Maringa State University, Brazil. Participation in WP 4&5 field activities in Brazil (2005-2006).

All Brazilian students were jointly supervised by partners 1&2 and the Brazilian partners (partners 4 and 5) and the Brazilian institutions associated to the project (University of

Campinas, University of Maringa and the Institute for Regional Development).

Participation in scientific events

- Encontro Nacional de Analistas de Alimentos, 22-25 June 2003, Rio de Janeiro, Brazil.

- IV Congreso Latinoamericano de Micotoxicología, 24-26 de Septiembre del 2003, La Habana, Cuba.

- V Simposio Latino Americano de Ciencias de Alimentos, 3-6 November 2003, Campinas-SP, Brazil

- FAO training on the application of HACCP method for mycotoxin prevention and control, 22-23 September 2003, La Habana, Cuba.

- XI Encontro Nacional de Micotoxinas, 30th June–2nd July 2004, Piracicaba, SP, Brazil.

- Food Safety Under Extreme Conditions, 6-8 September 2004, Jaén, Spain.

- XIII Seminario Latinoamericano y del Caribe de Ciencia y Tecnología de Alimentos, 12-15 de Octubre del 2004, Montevideo, Uruguay.

- Dissemination Day of the European Mycotoxin Prevention Cluster, 21st October 2004, Brussels, Belgium.

- Launch Conference of the MYCO-GLOBE project (EC-funded FP6 Specific Support Action), 22nd October 2004, Brussels, Belgium.

- Reducing Impact of Mycotoxins in Tropical Agriculture with Emphasis on Health and Trade in Africa, MYCO-GLOBE Conference, 13-16 September 2005, Accra, Ghana.

- Congresso Brasileiro de Toxicologia, 9-12 de outubro de 2005, Recife, Pernambuco, Brazil.

- 92nd Seminar of the European Association of Agro Economists (EAAE) on Quality Management and Quality Assurance in Food Chains, 2-4 March 2005, Göttingen, Germany.

- V International PENSA Conference on Agri-food Chains/Networks Economics and Management, 27-29 July 2005, Ribeirão Preto, Brazil.

- Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context, International Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz (Cordoba), Argentina.

- 7th International Conference on Management in AgriFood Chains and Networks, 31st

May-2nd June 2006, Ede, The Netherlands.

- 16th Annual World Forum, Symposium and Case Conference, International Food and Agribusiness Management Association, 10-13 June 2006, Buenos Aires, Argentina

- Food is Life, IUFOST, 13th World Congress of Food Science & Technology, 17-21 September 2006, Nantes, France

- Advances on genomics, biodiversity and rapid systems for detection of toxigenic fungi and mycotoxins, International Conference of the EC MYCO-GLOBE project, 26-29 September 2006, Monopoli (Bari) Italy.

Publications and papers

Publications in peer-reviewed scientific journals

- Sarter S., Zakhia N., **2004**. Chemiluminescent and bioluminescent assays as innovative prospects for mycotoxin determination in food and feed. *Luminescence*, 19, 345-351.

- Brabet C., Salay E., Freitas-Silva O., Alves A.F., Machinski Jr. M., Vargas E.A., Zakhia-Rozis N., **2005**. Gestão integrada de micotoxinas na cadeia produtiva do milho destinado à alimentação de frangos de corte no Brasil. *Cadernos de Ciência & Tecnologia, Brasília*, 22 (2), 439-451.

- Brabet C., Salay E., Freitas-Silva O., Alves A.F., Machinski Jr. M., Vargas E.A., Zakhia-Rozis N., **2005**. Maîtrise des mycotoxines dans la filière maïs au Brésil. *Cahiers Agricultures*, 14 (1), 164-168.

- Sarter S., Métayer I., Zakhia N. A luminescence-based bioassay using *Vibrio fischeri* for mycotoxin determination: the case of Deoxynivalenol (DON) and Aflatoxin B_1. *Under revision for publication in "Luminescence"*.

Book chapter

- Zakhia-Rozis N., Catala A.I., Soriano J.M., **2007**. Trazabilidad y descontaminación/detoxificación de las micotoxinas. *In: Micotoxinas en Alimentos*, José Miguel Soriano del Castillo (ed.), capítulo 6, pp. 119-132, Editorial Díaz de Santos, Madrid, Spain.

Oral presentations in conferences and congresses

- Zakhia N., **2003**. MYCOTOX: una colaboración entre América Latina y Europa sobre el manejo global de la contaminación por micotoxinas en las cadenas productivas de trigo y maíz. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, La Habana, Cuba.

- Henry G., Salay E., Engler A., **2003**. Integration of socio-economic and food science and technology research in quality management of food supply chains: Mycotoxin control system of grains in the Southern Cone. Invited paper presented at the *V Simposio Latino Americano de Ciencias de Alimentos*, 3-6 November 2003, Campinas-SP, Brazil.

- Vargas E.A., Castro L., Corrêa T.B.S., Freitas-Silva O., Brabet C., Cea J., Vega M.A.H., **2004**. Desenvolvimento e padronização de ferramentas analiticas efetivas para determinação de micotoxinas em cereais e subprodutos. *In: XI Encontro Nacional de Micotoxinas*, LAN/ESALQ-USP, 30th June-2nd July 2004, Piracicaba, Brazil.

- Zakhia N., **2004**. Overview on mycotoxins and emerging challenges towards innovative food safety management. *In: Food Safety Under Extreme Conditions*, 6-8 September 2004, Jaén, Spain.

- Henry G., Iglesias D., Engler A., Salay E., Gutiérrez G., **2004**. Organización de actores alrededor de la gestión de calidad en cadenas agroalimentarias. *In: XIII Seminario Latinoamericano y del Caribe de Ciencia y Tecnología de Alimentos*, 12-15 de Octubre del 2004, Montevideo, Uruguay.

- Zakhia N., **2004**. A new challenge for cereal production and processing chains: development of a food quality management system for the control of mycotoxins. *In: MYCO-GLOBE Launch Conference*, 22 October 2004, Brussels, Belgium.

- Engler A., Henry G., Iglesias D., Alves A., Gutiérrez G., Salay E., **2005**. Actor organization for QAS along agro supply chains: the case of mycotoxin reduction in Southern Cone grains. *In*: *92nd Seminar of the European Association of Agro Economists (EAAE) on Quality Management and Quality Assurance in Food Chains*, 2-4 March 2005, Göttingen, Germany.

- Engler A., Gutiérrez G., Henry G., Iglesias D., **2005**. Adoption constraints of QAS implementation in Argentina and Uruguay wheat supply chains. *In*: *V International PENSA Conference on Agri-food Chains/Networks Economics and Management*, 27-29 July 2005, Ribeirão Preto, Brazil.

- Sekiyama B.L., Brabet C.J., Lemes R.O., Dalpasquale V.A., Silva O.F., Vargas E.A., França R.C.A., Machinski Jr. M., **2005**. Desenvolvimento dos pontos críticos de controle para prevenir a contaminação por micotoxinas ao longo da cadeia produtiva do milho. Pôster, XIV Congresso Brasileiro de Toxicologia, Recife, Pernambuco, 9-12 de outubro de 2005.

- Zakhia N., **2006**. The MYCOTOX project: an EC-funded project in partnership with Latin America South Cone countries. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

- Henry G., Engler A., Iglesias D., Gutierrez G., **2006**. Socio-economic constraints and opportunities affecting the implementation of mycotoxin control measures in Southern Cone grain supply chains. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina.

- Iglesias D., Henry G., Engler A., Gutierrez G., **2006**. Policies for QAS implementation in export chains: mycotoxin management for Mercosur wheat actors. *In: 7th International Conference on Management in AgriFood Chains and Networks*, 31st May-2nd June 2006, Ede, The Netherlands.

- Iglesias D., Henry G., Engler A., Gutierrez G., **2006**. Adoption of QAS and impact from norms in export chains: mycotoxin management for Mercosur wheat actors. *In: 16th Annual World Forum; Symposium and Case Conference,* International Food and Agribusiness Management Association (IAMA), 10-13 June 2006, Buenos Aires, Argentina.

Posters presented in congresses

- Sekiyama B.L., Brabet C.J., Lemes R.O., Dalpasquale V.A., Silva O.F., Vargas E.A., França R.C.A., Machinski Jr. M., **2005**. Desenvolvimento dos pontos críticos de controle para prevenir a contaminação por micotoxinas ao longo da cadeia produtiva do milho. *In: XIV Congresso Brasileiro de Toxicologia,* 9-12 de outubro de 2005, Recife, Pernambuco, Brazil.

- Souza M.L.M., Farias A.X., Freitas-Silva O., Montello A.P., Cunha F.Q., Brabet C., Dalpasquale V., Machinski Jr. M., Sekiyama B.L., Costa S.S., **2006**. Avaliação da qualidade do milho utilizado no processamento de rações quanto a contaminação por aflatoxinas. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil.

- Ríos G., Zakhia-Rozis N., Chaurand M., Samson M.F., Richard-Forget F., Abecassis J., Lullien-Pellerin V., **2006**. Assessment of wheat grain fractionation process involvement in the product contamination with deoxynivalenol (DON). *In: Food is Life, IUFOST, 13th World Congress of Food Science & Technology*, 17-21 September 2006, Nantes, France.

- Souza M.L.M., Freitas-Silva O., Brabet C., Dalpasquale V., Machinski Jr M., De Castro L., Vargas E.A., Nagler M., Costa S.S., **2007**. Minimizing risks by mycotoxins in maize and poultry feed using the HACCP plan. *In: XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins*, 21-25 May 2007, Istanbul, Turkey (www.atal.tubitak.gov.tr/iupac2007-mycotoxin).

Dissemination documents and/or sectorial meetings with cereal stakeholders

- The development of a food quality management system for the control of mycotoxins in cereal production and processing chains in Latin America South Cone countries. Poster presented by the general coordinator *at the Third European Mycotoxin Cluster Workshop*, 2-4 June **2003**, Uppsala, Sweden.

- Desarrollo de un sistema de manejo de calidad de alimentos para el control de micotoxinas en la cadena de producción y procesamiento de cereales en los países del Cono Sur de América. *Flyer* distributed at the *IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre **2003**, La Habana, Cuba. This documents was elaborated with the logistic support of PROCISUR (Programa Cooperativo para el Desarrollo Tecnológico Agroalimentario y Agroindustrial del Cono Sur) (partner 3) for translation to Spanish and edition.

- Zakhia N., **2005**. Micotoxinas en los alimentos: panorama actual, legislación europea y perspectivas de manejo. *Seminar organised by INTA (partner 8) with the wheat chain stakeholoders and representatives of the academic sector and official bodies*, 1st December 2005, General Pico, Argentina.

- Zakhia N., **2005**. Micotoxinas en los alimentos: panorama actual, legislación europea y perspectivas de manejo. Talk presented at *different seminars* (4-7 December 2005) *organised with the wheat chain stakeholders* (Semillas von Baer, Molinos Collico, ALISUR Osorno, Agromaster S.A.) in the localities of Valdivia, Osorno, and Temuco in Chile.

- Cooperação: o projeto europeu Mycotox em fase de finalização. *França Flash* 48, out-nov **2006**, CenDoTec, São Paulo, Brazil. This bulletin was prepared by CIRAD (partner 1).

Papers under preparation

- A paper is under preparation on the *influence of dry milling operations on the distribution of DON in wheat grain and outcoming fractions*.

- A paper is under preparation on the global *MYCOTOX methodology integrating technical and socioeconomic features for quality management along the food supply chain*.

- A paper is under preparation on the *mycotoxin contamination through the whole supply chain of corn destined to poultry feeding in Brazil*.

- A literature review is under preparation on the *occurrence of mycotoxins in the Brazilian corn supply chain*.

Partner 02 - NRI

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

Co-ordination activities:

1. Ongoing discussions with the Project Co-ordinator and project partners concerning key technical, socio-economic and financial issues

2. The project teams were closely monitored in order to strongly encourage the timely production of deliverables

3. Assistance was provided with the design of meeting programmes, presentations were prepared and delivered, and leadership was provided in specific areas

Work Package 1: Development and standardisation of effective analytical tools for mycotoxin determination in cereals and by-products

A prototype instrument was successfully designed and manufactured which automatically determined the concentration of mycotoxins, immobilised on an especially designed polymer cartridge, by measuring the fluorescence signal generated by the toxin under UV light irradiation. The Toximet T System (instrument plus cartridge) can be used by non-scientists throughout the cereal production chain and delivers results within 30 minutes of receiving the sample. Preliminary experiments in the partner's laboratory have shown good levels of accuracy and precision. An international patent application has been published together with the filing of a UK patent application.

Publications:

1 International Publication No. WO 2006/123189 A2 – Device for detection and measurement of a target compound such as a food toxins; 23 November 2006
2 UK Patent Application No. 0702489.6 – Solid phase extraction of aflatoxins; 9 February 2007

Work Package 4: Hazard Analysis of Mycotoxins

Deliverable D2: A report documenting mycotoxin surveillance data, both from the literature and from surveillance studies conducted by this project:

Collaboration with project partners in order to: (a) assemble HACCP teams; and, (b) summarise and collate the collected data

Deliverable D3: Report describing the hazard analyses conducted, justifying the commodity-mycotoxin combination selected for further study:

- In collaboration with partners, the commodity-mycotoxin(s) combinations selected for further study were confirmed.

- In collaboration with partners, appropriate sampling and sample preparation procedures were designed and implemented for the selected commodity-mycotoxin(s) combination.

Deliverable D6: Report documenting the verified CFDs for each commodity-mycotoxin combination in each of the selected countries
All partners were assisted in the construction of a final, verified CFD for the relevant component of their commodity system

Deliverable D14: Report describing at which steps in the CFD the mycotoxin hazard originates, or at which steps concentrations increase to unacceptable levels

Partners were advised on the design and implementation of surveillance, sampling and sample preparation methods

Work Package 5: Identification and Validation of Mycotoxin Control Measures

Deliverable D9: Report describing the socio-economic studies conducted and the associated findings. These data will provide a thorough understanding of the stakeholders within the commodity system and will help identify the constraints & opportunities affecting the implementation of proposed mycotoxin control measures

- Partners were assisted with the identification of key players within the CFDs; and measures that will facilitate a thorough understanding of those socio-economic, cultural & institutional issues associated with the introduction of control measures at Critical Control Points (CCPs) were discussed with the partners.

- Special attention was paid to the integration of the technical and socio-economic aspects of the HACCP team activities.

Deliverable D13: A series of reports documenting studies to develop and evaluate control measures, and studies to validate CCPs

Advice was provided to partners on the identification of control measures and CCPs. Detailed advice was given on the design of studies to evaluate the efficiency of control measures that could be applied at steps in the CFD where control was deemed necessary. Advice was also given on the validation of CCPs in the case-study HACCP Plans.

Deliverable D16: A manual describing Good Agricultural Practice for the production of maize and wheat in the Southern Cone

Much time was spent in discussing how to achieve this deliverable. It was agreed that the ‘Manual on GAP’ would be more a handbook describing potential pre-harvest control measures, and their validation. Each country would then consider which of these were appropriate for inclusion in national GAP.

Work package No. 6: Development of Food Quality Management System

Advice was provided on the format for these over-arching Deliverable reports.

Deliverable D15: HACCP Plans for mycotoxin control in the specified commodity for each participating country

Ongoing support was provided to the WP 6 Co-ordinator. Particular emphasis was placed upon the integration of the technical and socio-economic studies, and integration of all of the work-packages to yield the outstanding deliverables. Importantly, the socio-economic inputs complemented the technical inputs when describing the commodity chain, as well as providing an understanding of the mechanisms by which control measures might be introduced. Much support was also provided to the project partners in order to enable them to achieve these deliverables.

Deliverable D18: Implementation of an efficient Food Quality Management System along the chain stakeholders to ensure high quality maize and wheat production regarding mycotoxin contamination

Advice was provided to the WP 6 Co-ordinator and the project partners on the incorporation of D15 HACCP Plans into Food Quality Management Systems (FQMS) such as ISO 9001:2000 and the new Food Safety Management System ISO 22000:2005. Further socio-economic studies were recommended to determine the current status of FQMS in the commodity system under study and to assess the opportunities and constraints for full implementation.

Deliverable D19: Training and extension material including posters, pamphlets, videos and radio and TV broadcasts and internet web pages, as appropriate

It was agreed that the training & extension documents would be targeted at policy makers and would be disseminated by brochure, web-site and meetings.

Field Visits

- In 2003, field visits were undertaken to Argentina, Uruguay, Brazil and Chile in order to advise the HACCP teams on final selection of case studies and on the construction of associated Commodity Flow Diagrams.

- In 2004, field visits were undertaken to Argentina, Uruguay, Brazil and Chile in order to advise the HACCP teams on (a) the design of surveillance procedures, (b) the construction & verification of CFDs and (c) the performance of Hazard Analysis.

Progress Meetings

Progress Meetings (in order to review progress and to agree the next steps with the project partners):

- Uruguay (2004, PROCISUR, Montevideo).

- Argentina (13–14 March 2006); third MYCOTOX Meeting at Villa Carlos Paz, Cordoba, Argentina.

- Argentina (15-17 August 2006); final MYCOTOX Meeting at INTA, Buenos Aires.

Meetings with Project Co-ordinator

Meetings with Project Co-ordinator (In order to discuss progress and constraints associated with all Work Packages. Appropriate action agreed and executed):
- UK, NRI (1st February 2006);

- UK, NRI (15–16 February 2007) in order to discuss the final project reports

Scientific Meetings

- Argentina (15–17 March 2006; Mr Nagler participated in the MYCO-GLOBE Meeting: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food Safety in a Myco-Globe Context, Villa Carlos Paz, Cordoba, Argentina).

Partner 03 - PROCISUR

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

PROCISUR (Programa Cooperativo para el Desarrollo Tecnológico Agroalimentario y Agroindustrial del Cono Sur) (partner 3), regional platform in the Southern Cone and responsible for the relationships with the agri-food chain stakeholders and the MERCOSUR official authorities, was involved in the MYCOTOX project within WP 4&5&6 for supporting field activities, favouring relationships with stakeholders and promoting output dissemination in the South Cone region.

The activities performed by PROCISUR are listed below:

Activity 1. Participation and support to the organisation of the project's meetings

1. Participation in and logistic support to the first and second annual meetings of the project

PROCISUR participated in and provided logistic support to the organisation of the kick-off (first annual) and second annual meetings of the project which were held in Montevideo (Uruguay), respectively on 17-19 February 2003 and 4-7 October 2004.
PROCISUR was responsible for the logistics of the meetings held at NH Columbia Hotel (first annual meeting) and MERCOSUR building (second annual meeting), which included:

- preparation of the folders with all necessary documents for the participants
- hotel reservations
- airport-hotel-airport transportation of participants
- fellowship dinner
- audio-visual equipment and recording of the event
- secretary support during the whole meeting.

2. Participation in and support to the preparation on the specific meeting of Work Packages 4&5

Eng. Cecilia Gianoni, PROCISUR Technical Assistant in Programming and Management, representing PROCISUR Executive Secretary and involved in the MYCOTOX project, provided support for the preparation of the specific meeting of Work Packages 4&5 held in Buenos Aires (20-22 August 2003). She held meetings with Dr Guy Henry (CIRAD, partner 1) in Montevideo (May 2003) in order to precise the profile of needed socio-economist for integrating the multidisciplinary HACCP team in Uruguay. She also had meetings with INTA (partner 8) hosting the meeting in Buenos Aires for preparation of the meeting's organisation.

Eng. Cecilia Gianoni, representing PROCISUR, participated in the meeting where the first advances achieved by WP 4&5 partners were discussed. She participated in the planning of the socio-economic and technical activities to be done in Uruguay.

3. Participation in the third annual meeting of the project

Cecilia Gianoni and José Olavarría (consultant hired by PROCISUR for additional support to WP 6 dissemination activities) participated, as representatives of PROCISUR, in the third annual meeting of the project which was held in Villa Carlos Paz (Argentina), 13-14 March 2006.

During this meeting the main advances of the project were shared and the agenda was defined until the end of the project. A specific meeting was also held amont the partners of WP 4&5&6 to discuss the progress of the last deliverables D15, D16 and D18 and the integration of socio-economic issues until the end of project.

They also participated in the conference organised by the MYCO-GLOBE project *"Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context"* held on 15-17 March 2006, in Villa Carlos Paz, Córdoba, Argentina.

Activity 2. Participation in WP 6 and support to the WP 6 leader (INTA, partner 8) for regional dissemination

- Support to the elaboration of the work plan of WP 6, in close collaboration with INTA (partner 8, leader of WP 6).

- Contacts with the wheat supply chain stakeholders in Uruguay and with cereal stakeholders in the South Cone countries, for promoting the project's activities and foreseeing future dissemination actions.

- Promotion of the project's activities and outputs with the regional entities and/or decision makers responsible for the Agricultural Development and Sanitary Issues, such as Comisión directiva del PROCISUR, Consejo Ministros del MERCOSUR ampliado CAS, Consejo de Decanos a Agronomía, Organismos Internacionales como el Comité de Sanidad Vegetal, COSAVE, etc.

- Logistic support for the conception of the dissemination documents coming out from the project, mainly i) support to the elaboration of manuals on Good Practices (agricultural and industrial) for controlling mycotoxin contamination in the region, and ii) support to the design of a synthetic document on the main outputs of the project, to be presented to the COSAVE, organisation depending from the Ministry of Agriculture and grouping the regulatory bodies in each Southern Cone country.

- Meeting with the leader of WP6 (INTA, partner 8) in Buenos Aires, Argentina (20-21 February 2006)
Eng. Cecilia Gianoni met Drs Marcelo Masana and Daniel Iglesias from INTA (partner 8) in order to refine the dissemination strategy for the last period of the project, and preparation of the forthcoming third annual meeting of the project (13-14 March 2006) where a specific meeting was planned with WP 6 participants.

Activity 3. Design and edition of dissemination documents for the project

PROCISUR as regional platform in the Southern Cone, helped in designing, editing, printing and disseminating documents and policy notes that were presented to the national and MERCOSUR authorities.

- PROCISUR helped with the Spanish translation, edition and printing of the dissemination brochure "*Desarrollo de un sistema de manejo de calidad de alimentos para el control de micotoxinas en la cadena de producción y procesamiento de cereales en los países del Cono Sur de América*". This flyer was distributed at the *IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre **2003**, La Habana, Cuba.

- PROCISUR designed, edited and printed a dissemination poster for presenting the MYCOTOX project at the "INTA Expone" Fair, held at the International Alliances Pavilion, Oliveros, Santa Fé, Argentina in October **2004**. This fair, focusing on agribusinesses, was visited by around 43 000 professionals, students, farmers, entrepreneurs, government representatives and international institutions such as delegations from embassies and from the European Commission.

- PROCISUR designed, edited and printed the poster entitled "Control de micotoxinas en la cadena de cereales" for output dissemination. The poster was presented in November 2006 at the International Fair "INTA Expone", International Strategies Alliances Pavilion (PROCISUR stand), held in Allen, Río Negro (Argentina). The fair focusing on agribusiness was visited by around 54 000 professionals, students, farmers, entrepreneurs, government and international institutions, among others.

- PROCISUR also designed a flyer entitled "El desarrollo de un sistema de calidad de alimentos para el control de las micotoxinas en la cadena de proceso y producción de cereales en los países del Cono Sur de Latinoamérica" and distributed it at the same International Fair "INTA Expone" mentioned in the previous paragraph and held in November 2006 in Río Negro, Argentina.

- By the end of the project, PROCISUR prepared a policy note with suggestions for actions to be taken by policy makers, based on the national reports made by each country team. This was essential according to the expressed needs in the Southern Cone region for external interventions driven by official authorities and regulatory bodies to ensure the application of Quality Assurance and Management Systems along the cereal chains in the region.

- PROCISUR elaborated also a final regional document containing the following information:
i) the methodological framework of the project, ii) the main results obtained within the different Work Packages of the project on the basis of each country contribution, and iii) a global synthesis with information on the convergences and divergences between the 4 countries' results and its impact on the South Cone region.

Activity 4. Promotion of the project activities and outputs in the Southern Cone region

- PROCISUR worked very closely with all partners and Work Package leaders to collect and update information for further dissemination at the global regional level, through meetings with the cereal chain stakeholders and with official bodies (National Ministries

for Health and Agriculture, Interprofessional cereal bodies, regulatory offices, and the MERCOSUR authorities) in the 4 partner countries.

- PROCISUR, as regional platform in the Southern Cone, regularly promoted the activities and outputs of the MYCOTOX project through their website (www.procisur.org.uy), Procisur Online section, in both “PROCISUR Informa” and “Projects with external financing” blocks. Besides, a specific link was created on PROCISUR website towards the website of MYCOTOX project (http://mycotox.cirad.fr), in order to ensure updated information on the project. The link between both sites favoured and promoted the project’s activities and dissemination towards the cereal stakeholders and official bodies in the Latin America South Cone. In addition, a section was progressively included in which the dissemination materials prepared by each country as well as the extension activities (seminars, meetings, etc.) carried out were uploaded.

Partner 04 - EMBRAPA

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

Embrapa was involved in the WP 1 and WP 4, 5 & 6 activities of the Mycotox project. This report presents a summary of the Embrapa activities developed from 2003 to 2006.

WP 1: "Development and standardisation of effective analytical tools for mycotoxin determination in cereals and by-products"

WP 4: "Hazard analysis of mycotoxins"

WP 5: "Identification and Validation of Mycotoxin Control Measures"

WP 6: "Development of a Food Quality Management System"

EMBRAPA WP 1 ACTIVITIES FROM 2003 TO 2006

WP 1 Activities in 2003/2004

Deliverable D4: "Standardised and validated analytical chromatographic methods applicable by all partner laboratories for mycotoxin determination in wheat and maize".

- As WP 1 coordinator in 2003 (before the retirement of Dr Tania Corrêa), the inventory of the analytical chromatographic methods currently used by the different WP 1 partners for mycotoxin determination in wheat and maize, as well as the organization and result synthesis of interlaboratory works between the Mycotox partners using FAPAS materials with known mycotoxin contamination. Description of the analytical chromatographic methods currently used by the WP 1 partners for mycotoxin determination in wheat and maize.

- Interlaboratory works: In order to evaluate the performance of the analytical chromatographic methods currently used by the WP 1 partners for mycotoxin determination in corn and wheat, a first serie of interlaboratory works was organized by the EMBRAPA and performed by the different WP 1 partners, using FAPAS materials of known mycotoxin contamination and following the protocol of the interlaboratory control elaborated by the MAA - Step 1 - (see MAA individual annual report 2003).

- Implementation and validation of analytical chromatographic methods for zearalenone determination in corn, in its laboratory.

  Material and methods: A literature review was first performed on the methodologies used for the extraction, purification, separation, detection and quantification of the following mycotoxins in corn: zearalenone, deoxynivalenol, fumonisin and aflatoxin. Based on this review, nine analytical chromatographic methods for zearalenone determination were selected. Six HPLC mobile phase/wave-length combinations were first tested using Sigma zearalenone standard, then two clean-up methods (Romer column and immunoaffinity column) for zearalenone determination in corn.

- Participation in FAPAS proficiency tests: EMBRAPA mycotoxin laboratory participated in the following FAPAS rounds in aflatoxin analysis: series 4 round 53 (maize), round 49 (dried material) and round 56 (peanut powder), and the results were satisfactory within the accepted ≤ 2 Z score values.

  Results: The results were reported according to the Report Resulting Sheets elaborated by the EMBRAPA and the MAA .In the case of the EMBRAPA, the AFB2 determined value was outside the FAPAS satisfactory range after the correction by the recovery percentage, which, even acceptable, is low (77 %). This result could be explained by the lack of experience of the analyst who was just joining the laboratory. Indeed, the mycotoxin laboratory of the EMBRAPA demonstrated good performance with higher recovery percentage (more than 90 %) in FAPAS rounds using the same methodology.

WP 1 Activities in 2005

- Since 2005 the WP 1 co-ordination was transferred to Eugenia Vargas from MAA, Brazil.

- It was started the implementation of analytical method for zearalenone in corn by HPLC. To indoor validation

WP 1 Activities in 2006

Implementation of HPLC method for zearalenone determination in corn

Before initiating the Mycotox project, TLC methods for aflatoxins determination were already implemented and used by EMBRAPA mycotoxin laboratory. It was finalised the implementation of analytical method for zearalenone in corn by HPLC. The indoor validation was also made using statistical analyses.

Participation in FAPAS proficiency tests

It was continued by FAPAS reference material acquisition directly from CSL, since this project collaboration was established.

WP 4&5 Activities in 2003/2004

EMBRAPA contribution in the workpackages WP 4&5 consisted of the participation in the multi-disciplinary HACCP team, and the execution of the activities related with the technical aspects.

Deliverable D2: "Report documenting mycotoxin surveillance data, both from the literature and surveillance studies conducted by this project".

EMBRAPA participated in the literature review on mycotoxin occurrence in Brazilian corn and wheat which was performed for the period 1975-2003 using international databases (Agricola, Agris, Biological abstracts, Cab abstracts, Food and human nutrition, FSTA, Medline), as well as thesis and books databases of the Unicamp-Fea and Embrapa.

A total of 60 bibliographic references on mycotoxin occurrence in Brazilian corn and wheat were collected: 50 on corn and 10 on wheat.

Data were synthesised and organised in tables including for each published work, information on the Brazilian state(s) and region(s) studied, year(s) of sampling, product(s) and step(s) of supply chain concerned, sampling and mycotoxin analytical methods used, and mycotoxin levels of contaminated samples.

The list of the bibliographic references on sampling and analytical methods used in the published works was also established. A report reviewing and discussing these data was drafted (see Publications).

Deliverable D3: "A report describing the hazard analyses conducted and justifying the commodity/mycotoxin combination selected for further study".

The commodity/mycotoxin combination selected for the case study in Brazil was identified and justified in the report completed for deliverable D2.

- Corn selected as commodity.
- Aflatoxins, fumonisins and zearalenone identified as the most important toxins, OTA for some control analysis.
- Paraná state selected as area to be studied.
- Corn production to poultry corn-based feed ration, selected as supply chain.

Participation in the organization of the multi-disciplinary HACCP team

- Institutional team members assembled, including members from Cirad, Embrapa, as well as from non-project partners (UEM, IDR and Unicamp) and MAA.

- Participation of the private sector in the project: Industry Abatedouro de Aves Frangos Canção - Gonçalvez & Tortola LTDA: Poultry slaughter-house, Maringa, PR and feed ration plant, Indianapolis, PR.

Participation in the formalisation of the collaboration with the private sector

WP 4&5 Activities in 2005

- Elaboration of a protocol with the procedure for receiving and preparation of samples of maize and ration for mycotoxins analyses to guarantee the homogeneity of the samples of maize in grain, rations and maize sub-products for mycotoxin analyses (reception of samples sent by the UEM; reception of the samples in the Embrapa; storage; milling; homogenisation; division; packing; storage). All samples had been prepared for all analysis in Embrapa. It was analysed all maize samples for Aflatoxins, in accordance with the methodology for determination of Aflatoxins B_1, B_2, G_1 and G_2, according with Brazilian Official method (Diario Oficial da União, section 1, n°62, p.41, 30, 2000). Of the 77 maize samples analysed, 24 had were positives, being 23 contaminated with the aflatoxin B_1 and one with the aflatoxin B_1 and G_1. It was not

detected the Aflatoxins B_2 and G_2. The major value for aflatoxin B_1 was 12,85 μg/Kg.

- However, in these same samples the values found of AW and moisture, in general had been lower to the critical levels for the growth of aflatoxigenic fungi.

- All the samples analysed were according the acceptable levels by Brazilian and Mercosul Regulations.

- The ration samples were analysed and all samples were sent to CIRAD and MAA for complementary analysis according to the workplan.

EMBRAPA WP 4, WP 5 and WP 6 ACTIVITIES IN 2006

Scientific activities

EMBRAPA contribution to the workpackages WP 4&5 consisted of the participation in the multi-disciplinary HACCP team, and the execution of the activities related with the technical aspects.

Deliverables D15, D16, D18: "A report describing the GAP including CCPs and HACCP plan for mycotoxin control in maize"

The commodity/mycotoxin combination selected for the case study in Brazil was identified and justified in the report completed for deliverable D15 and D16.

- Maize selected as commodity.
- Collected samples in Parana by CIRAD, UEM, IDR and Unicamp.
- Sample preparing, storage and sending to MAA and France by EMBRAPA.
- Finalisation of Aflatoxins analyses by EMBRAPA.
- Finalisation of Fumonisins, zearalenone and ochratoxin A analyses by MAA.

Results

The samples were collected in poultry feed factory in Parana state, Brazil, in 2005 and 2006, including the principal harvest and "*safrinha*" ones.

The samples were collected aiming to evaluate four studies:

- Quality of the grains received in the factory from different farms, with and without drying.
- Efficiency of cleaning and drying in the poultry feed factory.
- Quality control of the grains and feed storage in silo.
- Quality control of the grains and feed storage in the poultry farm.

The samples were milled (mesh), homogenised, packed under vacuum and freezing stored. The sub-samples were analysed concern moisture, water activity, and mycotoxins content (aflatoxins at Embrapa Food Technology; fumonisins zearalenon and ochratoxin A by MAA). The Table 1 summarised the mycotoxins results obtained in collected samples.

Table 1: The aflatoxins contamination in different studies

Study Number	Study description/Sample-type	Pop	Batches	Pts	Rep	Samples number/ AFL cont. samples
I	Poultry feed plant: Impact of corn supplier-type Corn grain	1	14	1	1	40/13
II	Poultry feed plant: Impact of cleaning & drying Corn grain	1	3	5	3	45/19
III	Poultry feed plant: Impact of storage in silo Ground corn	1	3	4	1	12/7
IV	Poultry farm: Impact of on-farm storage and handling Feed	2	6	3	1	36/3
TOTAL						**133/42**

1st study: Only 13 samples were contaminated by aflatoxin B_1 from 40 collected samples, the maximum concentration obtained was 13,5 µg/Kg

2nd study: 3 replicates
Group A (at reception and before first cleaning).
Group B (After first cleaning/before drying).
Group C (Before second cleaning/after drying).
Group D (After second cleaning).
Group E (sub-product of first cleaning).

On the study 2: In the first replicate, as well as the third one, the greatest contamination was observed in the sub-product of first cleaning. On the second replicate it was not found contamination by aflatoxins. Drying processing was efficient since the moisture and the water activity were reduced under the critical limits.

3rd study: Seven samples were contaminated by aflatoxin B_1 in 12 collected samples, the maximum concentration obtained was 9 µg/Kg. On the stored effects could not be observed aflatoxins contamination.

4th study: Only 3 samples were contaminated by aflatoxin B_1 in 36 collected samples, the maximum concentration obtained was 2.52 µg/Kg. In the ending point (poultry feed), the moisture and the water activity were significantly higher than in the storage.
Concerning of zearalenone and ochratoxin A contamination, only samples from 4th study were analysed. The range of contamination was nd – 383 µg/Kg. No samples were contaminated by ochratoxin A.

Concerning of Fumonisins contamination all collected samples were positive for B_1 and for B_2 only 3 samples weren't contaminated. The range of contamination for B_1 was 0.11 – 9.64 mg/Kg and for B_2 nd – 6.84 mg/Kg.

Considering detection limits:
nd < 3,6 µg/kg Zearalenone
nd < 0,12 µg/kg ochratoxin A
nd < 0,100 mg/Kg Fumonisins B_1 and B_2
nd < 0,12 µg/kg Aflatoxins

The obtained data were statistically analysed in collaboration with CIRAD (partner 1) and University of Campinas.

An abstract was submitted and approved for poster presentation at the IUPAC Mycotoxin congress that will be held at Turkey, in May 2007.

A scientific paper is under preparation for submission to a scientific journal.

Dissemination activities

- Meeting with Brazilian mycotoxin legislator sector. Brazilian appointments to the CODEX - Brasilia, Brasil, November 2006. (Otniel Freitas-Silva and Maria de Lourdes Mendes de Souza).

- Several meetings were held with the Brazilian Regulation sector for mycotoxins (ANVISA, Healthy Ministry, MAA and Academy)

- Meeting with Brazilian Technician Group of Contaminantes in Foods with legislator sector (ANVISA, Ministry of Health, MAA and Academy). This group is a formal team working on discussion paper on maximum levels of mycotoxins in food/CODEX *Alimentarius* Brazil, Brasilia, 15th August 2006 (Maria de Lourdes Mendes de Souza).

- Meeting with Brazilian Technician Group of Contaminantes in Foods with legislator sector (ANVISA, Ministry of Health, MAA and Academy). This group is a formal team working on discussion paper on maximum levels of mycotoxins in food/CODEX *Alimentarius* Brazil Brasilia, Brazil, 16th and 17th November 2006 (Otniel Freitas-Silva).

- Meeting with Brazilian Technician Group of Contaminantes in Foods with legislator sector (ANVISA, Ministry of Health, MAA and Academy). This group is a formal team working on discussion paper on maximum levels of mycotoxins in food/CODEX *Alimentarius* Brazil, Brasilia, 17th and 18th December 2006 (Otniel Freitas-Silva.).

- A meeting was planned with Brazilian Technician Group of Contaminantes in Foods with legislator sector (ANVISA, Ministry of Health, MAA and Academy). This group is a formal team working on discussion paper on maximum levels of mycotoxins in food/CODEX *Alimentarius* Brazil, Brasilia, 7th and 8th February 2007 (Otniel Freitas-Silva).

- A meeting was planned with Brazilian Technician Group of Contaminantes in Foods with legislator sector (ANVISA, Ministry of Health, MAA and Academy). This group is a formal team working on discussion paper on maximum levels of mycotoxins in food/CODEX *Alimentarius* Brazil, Brasilia, 15th and 16th March 2007 (Otniel Freitas-Silva).

Dissemination by Video and TV program

- A field day was planned on TV – 29th June 2007 – *Food safety and maize quality to broiler feed*. This program it will be produced by EMBRAPA. It is one of 41 TV programs selected and accepted. The structure of it will be composed of two parts to become most attractive and dynamic. On the first block the theoretical approach it will be given, with the technological aspects and applications. On the second one an interview with actors of the chain, including the Brazilian Mycotox project team. Now we are doing the elaboration of scripts and selecting areas to take images.

- The program *Food safety and maize quality to broiler feed* will be presented on June 29th by TV Rural, a Brazilian cable channel.

Participation in training course, congresses and Mycotox meetings

- First meeting of the Mycotox project, Montevideo, Uruguay, 17-19 February: Tania B. S. Corrêa and Otniel Freitas-Silva.

- WP 1 meeting: 24-25 September 2003, Havana, Cuba: Tania B. S. Corrêa and Otniel Freitas-Silva. Enaal "Encontro nacional de analistas de alimentos", Rio de Janeiro, RJ, Brazil, 22-25 June 2003.

- "IV Congreso Latino Americano de Micotoxicologia", 24-26 September 2003, Havana, Cuba: Tania B. S. Corrêa and Otniel Freitas-Silva. Presentation of one oral communication (see Publications).

- Second meeting of the Mycotox project, Montevideo, Uruguay, 04-07 October 2004: Otniel Freitas-Silva.

- Meetings with the Mycotox Brazilian team: Eugenia Vargas, O. Freitas-Silva, M. de L.M de Souza, T.B.S Correa, C. Brabet e B. Sekiyama (2003 to 2006).

- "IV Congresso Latino Americano de Micotoxicologia", 24-26 September, Havana, Cuba: Tania B. S. Corrêa and Otniel Freitas-Silva.

- Training course "HACCP principles application and mycotoxins prevention", 22-23 September, Havana, Cuba. This course was ministred by Dra. Maya Piñeiro, FAO: Tania B. S. Corrêa and Otniel Freitas-Silva.

- Progress meeting of WP 4&5, 20-22 August 2003, Buenos Aires, Argentina: Otniel Freitas-Silva.

- WP 4&5 Brazilian team meeting with the industry Frango Canção and UEM: Maringá, PR, Brazil, in order to define the partnership with the private sector: Otniel Freitas-Silva (2003 to 2006).

- Training Course on Mycotoxin Analyses in Food by LC/MS/MS, Applied Biosystem,

Florianópolis, Brazil, June 2006, Maria de Lourdes Mendes de Souza.

- Mycotoxins analyses training, Laboratory of Mycotoxicology, INCQS/FIOCRUZ, Ministry of Health, 5-14 June 2006, Andre Montello Pires.

- Participation in the XIII World Congress of Food Science and Technology, Food is Life", IUFOST, 2006. Meeting with Nadine Zakhia (general coordinator), Nantes, France, 17-21 September 2006.

- III Annual MYCOTOX Project meeting. Villa Carlos Paz, Cordoba, Argentina, 13-14 March 2006. Otniel Freitas-Silva.

- Participation in Myco-Globe conference. Villa Carlos Paz, Cordoba, Argentina, 15-17 March, 2006. Otniel Freitas-Silva.

- V Latin American Congress of Mycotoxicology - V CLAM, XII Mycotoxin Brasilian meeting - ENM; IV Mercosur Symposium on Qualitative Storage of Grains - IV SAG-MERCOSUL, 18-21 June 2006 – Florianopolis, Brazil – (Maria de Lourdes Mendes de Souza, Otniel Freitas-Silva and Antonio Xavier de Farias).

- WP 1 final meeting: Belo Horizonte, Brasil. 23 and 24 November 2006. (Otniel Freitas-Silva and Maria de Lourdes Mendes de Souza).

Publications

- Corrêa T.B.S., Vargas E.A., Cea J., Vega M., Resnik S., Souza M.L.M., Freitas-Silva O., Zakhia N. Sistema e gerenciamento da calidade para o control de micotoxinas nas cadeias da produção e processamento de cereais dos países do cono sul. In: IV Congreso Latino Americano de Micotoxicologia, 24-26 September, Havana, Cuba, 2003.

- Brabet C., Salay E., Freitas-Silva O., Alves A.F., Machinski Jr. M., Vargas E.A., Zakhia-Rozis N., 2005. Gestão integrada de micotoxinas na cadeia produtiva do milho destinado à alimentação de frangos de corte no Brasil. Cadernos de Ciência & Tecnologia, Brasília, 22 (2), 439-451.

- Brabet C., Salay E., Freitas-Silva O., Alves A.F., Machinski Jr M., Vargas E.A., Zakhia-Rozis N., 2005. Maîtrise des mycotoxines dans la filière maïs au Brésil. Cahiers Agricultures, 14 (1), 164-168.

- Vargas E.A., Castro L., Corrêa T.B.S., Freitas-Silva O., Brabet C., Cea J., Vega M.A.H., 2004. Desenvolvimento e padronização de ferramentas analiticas efetivas para determinação de micotoxinas em cereais e subprodutos. In: XI Encontro Nacional de Micotoxinas, LAN/ESALQ-USP, 30th June-2nd July 2004, Piracicaba, Brazil.

- Brabet C., Freitas-Silva O., De Souza M. de L.M., Correa T.B.S., 2003. Literature review: Mycotoxin ocurrence in Brazilian corn and wheat. Deliverable 2, INCO-DEV

Mycotox Project "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries", Campinas, SP, Brazil, 60 p.

- Sekiyama B.L., Brabet C.J., Lemes R.O., Dalpasquale V.A., Freitas-Silva O., Vargas E.A., França R.C.A., Machinski Jr. M., 2005. Desenvolvimento dos pontos críticos de controle para prevenir a contaminação por micotoxinas ao longo da cadeia produtiva do milho. In: XIV Congresso Brasileiro de Toxicologia, 9-12 de outubro de 2005, Recife, Pernambuco, Brazil.

- Souza M.L.M., Vasconcelos M.G., Teixeira A.S., Farias A.X., Freitas-Silva O., Costa S.S., 2006. Desenvolvimento e validação de método para análise de zearalenona em milho por CLAE. In: V Congreso Latino-americano de Micotoxicologia, 18-21 junho 2006, Florianópolis, Brazil. [http://www.labmico.ufsc.br/micotoxlatinam5].

- Vargas E.A., Castro L., Dos Santos E.A., França R.C.A., Cea J.M., Moriyama C., Vega M.H., Freitas-Silva O., Souza M.L.M., 2006. Interlaboratory control among INCO-DEV MYCOTOX project laboratories. In: V Congreso Latino-americano de Micotoxicologia, 18-21 Junho 2006, Florianópolis, Brazil.
ttp://www.labmico.ufsc.br/micotoxlatinam5].

- Souza M.L.M., Farias A.X., Freitas-Silva O., Montello A.P., Cunha F.Q., Brabet C., Dalpasquale V.A., Machinski Jr M., Sekiyama B.L., Costa S.S., 2006. Avaliação da qualidade do milho utilizado no processamento de rações quanto a contaminação por aflatoxinas In: V Congreso Latino-americano de Micotoxicologia, 18-21 Junho 2006, Florianópolis, Brazil. [http://www.labmico.ufsc.br/micotoxlatinam5].

- Souza M.L.M., Freitas-Silva, O., Brabet C., Dalpasquale V., Machinski Jr M., Castro L., Vargas E.A., Nagler M., Costa S.S., 2007. Minimizing risks by mycotoxins in Maize and Poultry feed using the HACCP PLAN. To be presented at the XII International IUPAC Symposium in Mycotoxins and Phycotoxins, May 2007, Turkey.

EMBRAPA TEAM:

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Partner 05 - MAA

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

The Work Package 1 (WP 1) "Development and standardization of effective analytical tools (sampling, sample preparation & analysis) for mycotoxin determination in wheat and maize" is part of INCO-DEV MYCOTOX project 2003-2006 (ICA4-CT-2002-10043) "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries". The implementation of the WP 1 was under MAA-LACQSA (Laboratory for Quality Control and Food Safety) coordination (since 2004) and aimed to implement the interlaboratory control between the laboratories involved in the project, from Brazil, Uruguay, Chile and Argentina. The project partners involved in mycotoxin analysis were:

- Eugênia Azevedo Vargas, Eliene Alves dos Santos, Luciana de Castro and Regina Coeli Alves França – MAA-LACQSA-MG. Brazil - Fumonisin, zearalenone and ochratoxin A analysis in maize and poultry meal and implementation of WP 1 activities;
- Otniel Freitas-Silva, Maria de Lourdes Mendes Souza and Antônio Xavier de Farias - EMBRAPA/CTAA. Brazil - Aflatoxins analysis in maize and poultry meal;
- Jacqueline Cea – Laboratorio Tecnologico del Uruguay – LATU, Uruguay;
- Mario Herrera Vega – Universidad de Concepcion – UDEC, Chile.

OBJECTIVES

The WP 1 principally intended to implement the interlaboratory control between the laboratories involved in the project, from Brazil, Uruguay, Chile and Argentina.
The objectives of the proficiency test were to:

- evaluate the performance of the laboratories and the main difficulties encountered in performing the analytical procedure for determination of aflatoxins, zearalenone, ochratoxin A and fumonisins in maize and wheat;
- contribute to the harmonisation of analytical procedures of the project partner laboratories;
- contribute to the laboratory's proficiency in aflatoxins, zearalenone, ochratoxin A and fumonisin analysis.

The objectives of the participation in the WP 4&5 were to:

- evaluate the contamination of maize and poultry feed by fumonisins B_1 and B_2, zearalenone and ocratoxin A
- define CFD and sampling plan for maize and poultry feed.

WP 1 ACTIVITIES

1. Elaboration of protocols for harmonization of sampling and mycotoxin analytical procedures

Four protocols were elaborated: Interlaboratory control; Intralaboratory control; Instructions for reporting analytical results; General guidance for sampling.
These protocols describe the different steps and the methodology for implementing and harmonizing the intra- and interlaboratory works, as well as the recommendations for the sample collection and result reporting.

2. Interlaboratory comparison and proficiency testing on FAPAS samples

In order to evaluate the performance of the analytical chromatographic methods currently used by the WP 1 partners for mycotoxin determination in corn and wheat, a first round of interlaboratory works was performed using FAPAS materials of known mycotoxin contamination (Table 1), and following the protocol of the interlaboratory control elaborated by MAA.

Table 1: FAPAS materials used by the WP 1 partners

Matrix	FAPAS reference number	Mycotoxin	FAPAS assigned value (µg/kg)
	T0446	Aflatoxin B_1	6.78
		Aflatoxin B_2	1.66
		Total aflatoxins	8.66
	T2208	Fumonisin B_1	879.1
		Fumonisin B_2	305.9
	T2209	Zearalenone	228
	T2211	Fumonisin B_1	650
		Fumonisin B_2	230
	T0453	Aflatoxin B_1	6.56
		Aflatoxin B_2	4.45
		Aflatoxin G_1	2.46
		Aflatoxin G_2	1.65
Wheat flour	T2210	Deoxynivalenol	463

3. Production of reference materials

Four homogeneous maize materials were prepared by MAA-LACQSA: blank for zearalenone, blank for aflatoxins and one naturally contaminated for aflatoxins. In summary, the preparation of these samples involved: milling, homogeneization, analysis to verify the homogeneity of the bulk material, packing and analysis to verify the homogeneity of the packaged material as well as to estimate their contamination value (Horwitz, 1995. Thompson and Wood, 1993, ISO/IEC 43, 1997, Thompson, 2000, FAPAS, 2002, Vargas *et al*., 2001 and ISO Guide 30 to 35). All batches of test material were stored under – 18°C and protected from light prior to and after packaging. Also, surplus test materials were purchased from FAPAS. The identification of each material is given in Table 2.

Table 2: Reference maize samples used during the proficiency testing

Mycotoxin	Reference result (µg/kg)	Identification
Aflatoxin (AF)	<0.3 B_1	04BR157
	3.4 B_1	05AT001
	18.6 B_1 and 0.7 B_2	04NT033
Zearalenone (ZON)	< 52.0	04BR157

4. Interlaboratory comparison on reference materials

4.1. Organisation of the Proficiency Test

The participating laboratories received refrigerated parcel containing the items, as described in Table 3. All materials were dispached to the laboratories, together with the following documents: Letter, Test Material Receipt Form, Additional instructions to the participants, Results reporting sheets for analytical data and Analytical Work Questionnaire.

Table 3: Material dispatched at each proficiency test round

Round	1st afla	2nd afla	1st zea
Samples	Two coded maize samples (Amostra D and Amostra E) One blank maize sample for spiking (Branco)	One coded maize samples (Amostra H) One blank maize sample for spiking (Branco)	One coded maize samples (Amostra I) One blank maize sample for spiking (Branco)
Dispatch Data	February, 22nd 2005	April, 5th 2005	
Deadline	March 18th, 2005	May 09th, 2005	
Laboratories	UDEC, EMBRAPA, LATU and MAA-LACQSA		

Round	3rd afla	2nd zea	3rd zea
Material	One coded maize sample (Amostra J) One blank maize sample for spiking (Branco)	One coded maize sample (Amostra K) One blank maize sample for spiking (Branco)	One coded maize sample (Amostra L) One blank maize sample for spiking (Branco)
Analysis date	When received	When received	August, 15th to 19th
Deadline	August 19th, 2005	August 19th, 2005	August 26th, 2005
Laboratories	UDEC, EMBRAPA, LATU and MAA-LACQSA		

Round	1st OTA	2nd OTA	3rd OTA
Material	One coded cereal sample (Amostra M)	One coded cereal sample (Amostra O)	One coded cereal sample (Amostra Q)
Analysis date	When received	August, 15th to 19th	September, 19th to 25th
Deadline	August 19th, 2005	August 26th, 2005	September30th, 2005
Laboratories	UDEC, LATU and MAA-LACQSA		

Round	1st DON	2nd DON	3rd DON
Material	One coded wheat flour sample (Amostra N)	One coded maize sample (Amostra P)	One coded maize sample (Amostra R)
Analysis date	When received	August, 15th to 19th	September, 19th to 25th
Deadline	August 19th, 2005	August 26th, 2005	September 30th, 2005
Laboratories	UDEC, LATU, UBA and UNLU		

4.2. Evaluation of the analytical methods used by the Laboratories

Questionnaires on analytical methodologies in use by partners in standardised form detailing the techniques in use and characteristics of the methods were applied.

4.3. Analysis

Aflatoxins and zearalenone

The laboratories were required to analyse the test samples together with the samples collected within the project and with the blank sample spiked with ~20 μg/kg total aflatoxins or ~300 g/kg zearalenone, considering the dates defined at Table 3 and following the additional instructions to the participants. Also, the laboratory was requested to run his own internal quality control procedures, using artificially contaminated wheat or maize samples at the same level of the spiked.

Ochratoxin A and Deoxynivalenol

The laboratories were required to analyse the test samples together with the samples collected within the project and with their own blank samples spiked with ~5 μg/kg ochratoxin A or ~200 μg/kg deoxynivalenol, considering dates defined at Table 3 and following the additional instructions to the participants. Also, the laboratory was requested to run his own internal quality control procedures, using artificially contaminated wheat samples at the same level of the spiked.

4.4. Evaluation of the results

The results were evaluated by MAA-LACQSA and displayed in control graphs using z-score function being calculated by the following equation:

$$z = \frac{x - \bar{x}}{\sigma}$$

In which:

x is the contamination value determined by the Laboratory;

$\bar{x}$ is the value that best represents the true measure of the mycotoxin in the sample (as per homogeneity tests for MAA-LACQSA reference materials or declared by FAPAS™;

σ is the standard deviation of the value that best represents the true measure of the mycotoxin, being the standard deviation (σ) calculated as b $\bar{x}$ where:

b= % RSD_R /100

For concentrations of the analyte <120 μg/kg, relative standard deviation (RSD_R) is obtained from modified Horwitz's equation, where RSD_R = 22%.

The z-score interpretation is made as described below:

Ranges	The Laboratory result is considered:
If $\|z\| \leq 2$	"Satisfactory"
If $2 < \|z\| \leq 3$	"Questionable"
If $\|z\| > 3$	"Unsatisfactory"

4.5. Recommendations

In case the results are deemed as unacceptable, the concerned laboratory should make the necessary modifications and adjustments in the methods, taking into account the method performance criteria for each mycotoxin.

The unacceptable results should be treated as non-conformity. The laboratories are strongly recommended to write a report containing the analysis of the causes and corrective actions should be proposed.

Special attention should be given to:

a) Correct use of calibrated pipettes;
b) Chromatographic condition including the calibration curve (including number of points);
c) Importance of chromatographic confirmation techniques;
d) Injected/applied volume and concentration of extract and standard.

5. Implementation of internal quality control (blind and not blind) as recovery test at each batch using homogeneous samples produced by MAA-LACQSA

The laboratories received homogeneous samples produced by MAA-LACQSA (blank and naturally contaminated samples) to be used as internal quality control, at each batch, as blind and not blind (Table 4).

Table 4: MAA-LACQSA reference samples distributed to each laboratory

Laboratory	Nº of project samples*	Nº of samples by batch**	Nº of batches	Nº of reference samples received
UDEC	48	10	5	5 – 04BR157 (blank for aflatoxins) 5 – 04BR157 (blank for zearalenone) 5 – 04NT033 (blind for aflatoxins) 5 – 05AT001 (not blind for aflatoxins)
LATU	30	10	3	3 - 04BR157 (blank for aflatoxins) 3 - 04BR157 (blank for zearalenone) 3 – 04NT033 (blind for aflatoxins) 3 – 05AT001 (not blind for aflatoxins)
EMBRAPA	230	10	23	15 - 04BR157 (blank for aflatoxins) 15 - 04BR157 (blind for aflatoxins) 15 – 04NT033 (blind for aflatoxins) 15 - 05AT001 (not blind for aflatoxins)

*as decided during Montevideo meeting 2004, **estimated value

6. Summary of methods

The summary of methods used by the laboratories was elaborated based on the questionnaires answered during the proficiency testing. This allowed to elaborate an analytical database. The questionnaires were annexed to the MAA previous annual reports.

WP 4&5 ACTIVITIES

1. Meetings

- Participation in meeting to discuss about WP 4&5 activities (Maringá/Paraná State, 09-11 December 2003).

- Participation in meeting to discuss about sample preparation and mycotoxin analysis (Rio de Janeiro, 01st February 2005).

- Participation in meeting to discuss about sampling plan for maize and poultry feed and training of technicians to implement the sampling (Maringá/Paraná State, 20-23 February 2005).

- Participation in meeting to discuss analysis results (Maringá/Paraná State, 31st July-2nd August 2006).

2. Sampling procedures

It was established the CFD for poultry feed and defined the sampling procedures for maize and poultry feed.

3. Sample analysis

It was analysed 136 samples: 100 maize and 36 poultry feed samples for fumonisins B_1 and B_2, 12 poultry feed samples for ochratoxin A and 24 poultry feed samples for zearalenone, totalling 318 determinations. The results were annexed to the fourth 2006 annual report of MAA.

RESULTS ACHIEVED

1 The four protocols were elaborated and used by the laboratories during the implementation of the project;
2 The laboratories used the FAPAS samples, with known concentration to adjust their methods to analyse the project samples;
3 It was produced four homogeneous maize materials, used at interlaboratory rounds;
4 Proficiency testing reports were elaborated for the 1st, 2nd and 3rd rounds of aflatoxins, zearalenone, ochratoxin A and deoxynivalenol;
5 The summary of the analytical work questionnaire was elaborated;
6 It was presented 7 works in 4 congress;
7 It was analysed 136 samples.

PROBLEMS ENCOUNTERED

Delays occurred during the material reception by the laboratories due to carrier delays and problems with customs. Delays occur also during the reporting results by the

laboratories, which extended the duration of interlaboratory studies.

PUBLICATIONS AND PAPERS

- Corrêa T.B.S., Vargas E.A., Cea J., Vega M., Resnik S., Souza M.L.M., Freitas-Silva O., Zakhia N. Sistema e gerenciamento de la calidad para el control de micotoxinas en las cadenas de producción y procesamiento de cereales de los países del cono sur. *In: IV Congreso Latinoamericano de Micotoxicología*, 24-26 de Septiembre del 2003, Havana, Cuba. (oral presentation).

- Vargas E.A., Castro L., Corrêa T.B.S., Freitas-Silva O., Brabet C., Cea J., Vega M.A.H. Desenvolvimento e Padronização de Ferramentas Analíticas Efetivas para Determinação de Micotoxinas em Cereais e Subprodutos. *In: Encontro Nacional de Micotoxinas*, 2004, Piracicaba, SP (poster).

- França R.C., Dos Santos E.A., De Castro L., Vargas E.A. INCO-DEV MYCOTOX PROJECT "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries" (ICA4-CT-2002-10043): **Part I – Reference material preparation,** *In: Mycoglobe Conference, Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Villa Carlos Paz, Córdoba, Argentina, 14-16 de Marzo del 2006 (poster).

- De Castro L., Dos Santos E.A., França R.C., Cea J., Moriyama C., Vega M.A.H., Freitas-Silva O., Souza M.L.M., Vargas E.A. INCO-DEV MYCOTOX PROJECT "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries" (ICA4-CT-2002-10043): **Part II - Interlaboratory Control,** *In: Mycoglobe Conference, Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Villa Carlos Paz, Córdoba, Argentina, 14-16 de Marzo del 2006 (poster).

- Vargas E.A. Current Situation on Standardization and Validation of Analytical Methods for Mycotoxins in South América, *In: Mycoglobe Conference, Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Villa Carlos Paz, Córdoba, Argentina, 14-16 de Marzo del 2006 (oral presentation).

- De Castro L., Dos Santos E.A., França R.C., Cea J., Moriyama C., Vega M.A.H., Freitas-Silva O., Souza M.L.M., Vargas E.A. Interlaboratory control among INCO-DEV MYCOTOX project laboratories. *In: V Congreso Latinoamericano de Micotoxicología*, Florianópolis, 18-21 de Junio del 2006 (poster).

- De Castro L., Dos Santos E.A., França R.C., Cea J., Vega M.A.H., Freitas-Silva O., Souza M.L.M., Vargas E.A. Interlaboratory Control Involving Participant Laboratories within the INCO-DEV MYCOTOX PROJECT 2003-2006 "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and

Processing Chains in Latin America South Cone Countries", *In: IUPAC 2007 – XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins*, 21-25 May 2007, Istanbul, Turkey (resume approved to be presented as poster).

PARTICIPATION IN MYCOTOX MEETINGS AND CONGRESS

1 24-26 September 2003, Havana, Cuba

WP 1 meeting organised during the IV Congreso Latino Americano de Micotoxicologia: The four protocols elaborated by the MAA - Interlaboratory control and Intralaboratory control, Instructions for Reporting results and General Guidance for sampling - were presented and discussed.

2 Mycotoxin National Meeting (ENAM), Piracicaba/SP

Participation of WP 1 partners. Presentation of 1 oral communication (see publication).

3 WP 1, WP 4 and WP 5 meetings:

The agenda of WP 1, the CFD´s and sampling plans were presented and discussed during the meeting (9-11 December 2003, 1st February 2005 and 20-23 February 2005).

4 Second Annual Meeting of Mycotox Project

Montevideo/Uruguay (4-7 October 2004).

5 Mycoglobe Conference

Villa Carlos Paz, Córdoba, Argentina (14-16 March 2006).

6 V Congresso Latino Americano de Micotoxicología (V CLAM), XII Encontro Nacional de Micotoxinas (ENM'2006), IV Simpósio de Armazenagem Qualitativa de Grãos do Mercosul (IV SAG-MERCOSUL)

Florianópolis, (18-21 de junho de 2006).

CONCLUSION

The use of reference samples and the participation in interlaboratory controls enabled the laboratories to evaluate their analytical methods performance, to optimize and to improve the quality and the reliability of the mycotoxin analytical procedures used in the mycotoxin analysis of the project samples.

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Partner 06 – INIAGR-PV

Contract number: ICA4-CT-2002-10043

THIRD ANNUAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

INIA Uruguay (partner 6) was involved in work packages 4&5&6.

OBJECTIVES

The objective of these WPs of the project was to adapt the HACCP method throughout the whole wheat chain to control the mycotoxin DON, using a "case study" mill at the industrial level. The project was concentrated on wheat and on the toxin DON because there were two mayor outbreaks of *Fusarium* head blight during two consecutive years, 2001 and 2002. After 2001, 57% of the Uruguayan wheat had DON concentrations above 2000 ppb and 61% of the flour had concentrations above 1 ppm which is the law enforced tolerance level in the country.
The whole idea was to reduce DON to an acceptable level, minimizing the number of analysis and concentrating in certain steps of the chain that are key for reducing the toxin or avoiding high levels of it. The HACCP plan as output might apply to other similar mills or apply to other mills with some modification.

ACTIVITIES

- Constitution of a multidisciplinary HACCP team.

- Selection of the wheat/DON commodity system.

- Elaboration of the CFD.

- Partnership with the private sector (Río Molino mill).

The project included specific surveillance studies, control measure studies, validation and an overall HACCP plan to control the problem.

- Surveillance studies.

1) Carry out a surveillance of mycotoxins in flour.
2) Carry out a consumers survey to learn about Uruguayan purchase habits and their willingness to pay for quality.
3) Carry out a farmers survey to find out their perception on mycotoxins and quality.
4) Carry out a survey at the mill level to find out willingness to implement HACCP or any other kind of quality assurance system

- Control measure studies

5) Trends in DON production from physiological maturity to harvest time.

- Validation of control measures

6) Influence of the crop rotations, with species that are *Fusarium* hosts and non-*Fusarium* hosts as previous crops, in DON concentration at harvest.

- Elaboration of the HACCP plan, a manual of Good Practices and the architecture of the Food Quality Management System (FQMS)

RESULTS ACHIEVED

- A multidisciplinary HACCP team was assembled including an agronomist, a socio-economist, a phytopatologist, a mycotoxin analyst, and a representative of PROCISUR. HACCP support was given by the leader of WP 4&5 and Dr Martin Nagler.

- The Río Molino mill accepted to get involved in the project activities.

- The Commodity Flow Diagram was elaborated for the wheat/wheat flour/DON system. The Critical Control Points (CCP) were identified.

- Mycotoxin surveillance studies

Before the 2001 outbreak, there were few written articles about mycotoxin contamination. The bibliography search done for the project (D2 - surveillance data) resulted that there were not many written reports on mycotoxin hazards in the country. There was a very limited amount of samples analyzed on corn and subproducts. During 1993-1995, around 70 samples were analyzed for aflatoxins (afla) and zearalenone (zea) resulting in 1 and 7% positive in food and 25 and 17% in feed, respectively for those two toxins. During 1995-96, 64 samples of corn were analyzed for fumonisins giving 37% positive on food and 100% in feed. During 1993-95, a survey was carried out by LATU to quantify mycotoxins in wheat from more than 100 samples; 24% were positive for aflas, 5% for zea (over 100 ppb) and 55% for deoxynivalenol (DON), but only 3% with DON over 1000 ppb.

As we couldn´t find any written articles on the mycotoxins contamination during the years of the outbreaks, MYCOTOX collected analysis results from official Institutions and private laboratories. Out of the 1591 DON results on flour given to us by these entities, 61% of the flour had contamination levels over 1000 ppb. In 2001/02, the average DON contamination yielded 1765 ppb, while the median was 1600 with a deviation of 1218 ppb. In 2002/03, the average DON contamination yielded 1217 ppb, while the median was 1100 with a deviation of 930 ppb.

Since the start of MYCOTOX project, Uruguay had three years of no *Fusarium* Head Blight in the field. A survey of mycotoxins in flour was carried out in 2006, with the objective of seeking principally DON after a three year period of no Fusarium head blight in the field. Moreover, zearalenone (Zea), aflatoxins B_1, B_2, G_1 and G_2 (Aflas), ochratoxin (Ota) and total alcaloids were also analyzed on approximately one third of the samples. Eighty four samples were bought in different stores of the Departments of Montevideo and Colonia, they represented 35 different brands that were produced by 14 different mills (14 out of the 20 mills in the country). All of the 84 samples were analyzed for DON, 38% (32 samples) of these were analyzed for all the other toxins as well. Flour was sent to LATU (partner 12) where the toxins were analysed using standardized methods and international protocols. Moreover, within this project all these toxins were standardized throughout the laboratories participating in the project, except for total alkaloids which was a toxin that only Uruguay analyzed.

The results from this survey were really clear; none of the samples were beyond the quantification level for all the toxins analyzed. For DON the quantification level was set in 120 µg/kg, and all 84 samples had concentration below this level. For each of the analyzed aflas, the detection limit of the method is 0.7 µg/kg and all samples were below detection levels. For zea, the detection limit of the method is 60 µg/kg and all samples were below detection levels. For Ota, detection levels is 2 µg/kg and all samples were again below this level. For ergot alkaloids, although each of the 6 different alkaloids analyzed have different quantification limits, only two samples at the most for each individual alkaloid had limits that were higher than the quantification level, the highest sample was 19 µg/kg of ergocornine mesilate alcaloid.

- Consumer survey on Uruguayan purchase habits

Willingness to pay for certified quality by final consumers was studied in a survey.
The survey was conducted to randomly selected consumers in Montevideo. Sixty samples were collected with 56 valid forms processed.

The main objective of the questionnaire was to know more about the purchase habits of Uruguayans when it comes to products with wheat flour. Up to this research, no public or private body evaluated the final consumer as an entity on food safety issues on mycotoxins in wheat. The main point was to determine if there was a price premium and if consumers were willing to pay in the case of mycotoxin-free wheat flour products, and if so how much off the current price that is.

Reasons behind this question rely on the slow response of government authorities to provide safe food to major segments of the Uruguayan population. It may be valid to determine if consumers are sensitive to food safety on wheat related products in order to quantify a possible private certification of food safety, and depending on the premium they can pay for it, if it is technically achievable.

About 70% of the population interviewed were women, with major concentration of income for the whole household is in the range between $2500 to $7500 (104 to 312 USD). Ninety percent of the sampled population buys at groceries or large supermarkets (Hypermarkets) and only a small percentage in local food stores.

More than half of the population interviewed consumes French bread on a daily basis (51.8%), 10.7% every two days and 7.1% every 3 days. Other types of bread are more spaced in time. The bulk of bakery value added products (croissants) are consumed once per week as the largest percentage indicates (39.3%). Cookies if industrial origin (not made at the bakers) are also consumed very frequently since daily consumption accounts for 32.1% of the total consumption. Industrial bread is not so frequent.

Pasta consumption in the form of dry pasta is evenly spaced with the bulk of the usage every 7 days, but with another peak of consumption every 2 to 4 days. Fresh pasta is consumed mainly every week or so in the reported households. Among the poorest homes, the consumption of dry pasta is proportionally largest than in the richest.

Uruguayans believe wheat based products are healthy on a 64% of the cases. Since *Fusarium* outbreaks promoted an extensive media coverage of the problem, it was important to know if the consumer perceived the problem where he purchased the food that may be contaminated. Radio interviews and TV broadcast on the problem also helped to create public awareness on the problem. 66% of the interviewed recalled information made available through the radio, TV of newspapers.

When asked who should take care of the control of finished products, consumers have different opinions. Around 57% indicates that the Ministry of Public Health should be in charge of controlling food safety of food products with wheat. LATU (Laboratorio Tecnológico del Uruguay) was the second ranked with a 21,4%. As of today local governments are in charge of this issue by the decree that sets a limit in DON content. A large percentage of the consumers interviewed expressed they know what a certified product is.

When asked if, as consumers, they were willing to pay more in the case of certified quality for wheat based products, a large majority expressed they will. On average, more than 80% of the people indicated they will be willing to pay more if certification could assure organoleptic or safety issues. As of being mycotoxin free, about 90% of the interviewed expressed their willingness to pay for certification on this issue. On average a consumer from our survey is willing to pay 31% more than the current price for a certified French bread as being safe (mycotoxin free). For high value added products, percentage drops to an average of 22 to 24% as in the case of fresh pasta and croissants.

Finally who should be in charge of controlling or certifying these products. 53% of the consumers think that certification should come from LATU (Laboratorio Tecnológico del Uruguay) and only 35% from the Ministry of Public Health.

- Farmers perception of quality

Wheat quality in Uruguay has been an issue of long and bitter debate between farmers and millers. So strong was the discussion that up to this day opinions are well divided on the concept of quality and how to implement objective parameters to measure it.
A survey was done by MYCOTOX which included the opinion of the farmer on what was quality on the wheat crop.

Table No 1. Wheat quality concept for farmers

Wheat quality for farmers	
Quality indicator	Times mentioned
Specific weight	21
Gluten content	9
Protein content	8
Falling number	8
Baking quality	7
% *Fusarium*	6
Good color	5

Phytosanitary behaviour	3
Good grain size	3
Low broken kennels	2
Clean wheat	2
Farinographic parameters W	1
Flour extraction	1
Others	3

Key parameters in terms of quality were specific weight, followed by gluten content and protein content (related concepts all the three). *Fusarium* goes to the 6th place in the ranking of wheat quality. All the three parameters listed in the top of the ranking are related to the same agronomic concept: the protein content of the wheat and its quality.

Can Uruguayan farmers produce good industrial quality wheat? The answer is yes. Why they don't do that? Because they don't see the millers paying the quality. 50% of the farmers say that the buyer does not recognize the quality of the product, 19% say they recognize the quality but there is no price premiums for it and 56% indicated discrepancies between their delivered wheat (or perception of quality) and the buyers receipt.

It seems clear that quality signalization (including *Fusarium* and mycotoxin contamination) stand as a major conflicting point between actors of the supply chain. It may be the case in which public intervention is needed, clear signalization is a key aspect in order to determine price premiums for standard compliance.

- Survey on millers perception of quality of Uruguayan wheat, GMP and HACCP at mill level

A survey on the millers' perception of quality on wheat, usage of GMP and HACCP, was sent to all the members of the Uruguayan Mills Association (Comisión Gremial de Molinos). Currently there are 13 mills listed as members of the association, but only 6 mills acceded to give back information on their operations. The rest of the mills ignored the call for information or decided not to provide information since they considered certain parts of the questionnaire "sensitive to their interests".

Information provided represents a partial analysis of the Uruguayan milling industry who is highly concentrated but remains heterogeneous. The information obtained represents 244.300 tons of effective milling, which represents about 65% of the total domestic milling.

A first question was based on the origin of the wheat they mill. Sixty five percent of the mills reported they purchase directly their wheat and indicate quality requirements of the grain to be purchased every year. This quality signalization is made in written or by phone in order to inform their suppliers about the quality demanded.

Quality demanded by each mill depends greatly on the current grain stocks and the average quality of the harvest. Some of the quality parameters listed were:

Table No 2. Quality requirements indicated by the milling industry.

Quality parameter
W (dough strength) limits on the variety purchased
Gluten %
Humidity of the gluten
Protein
W value
Falling number

An important issue was to determine if the mills had any specific and long standing relation with a specific supplier.

More than 80% of the mills don't have any specific contract with their provider of grain, depending largely on the spot market to obtain the grain. Many of the interviewed people expressed that since they are firms established in the market for many years and they often purchase to the same clients, an informal relationship is established and therefore no formal contracts are needed. Even if it not mandatory, 67% of the mills use the official decree to establish premiums and discounts on the grain purchased every year. None of the mills expressed the existence of incentives beyond the ones established in the decree for the farmers that assure a specific quality level.

As of the varieties of wheat purchased by the mills every year, 50% knows occasionally what variety it is, 33% generally knows what variety they are buying and 17% always know.

An interesting finding was that largest mills often fail to know the origin of the wheat their purchase. The largest operators, who account for 56% of the total milling can barely trace the origin of 30% of the wheat they purchase.

Upon arrival, the main factor to determine quality is gluten content for 50% of the millers interviewed, and as second factor for 33% of them. W is the following parameter being the main quality attribute for 16.7% of the mills.

As of mycotoxin contamination problems, 66% of the mills indicated that they do routinely check the presence of mycotoxins on the grain purchased while 33% does not. The most common mycotoxin detected was DON, in 100% of the cases, while Afla was detected by 20% of the mills as well as Zea in 20% of the cases also.

Although some mills use a GMP approach, none has a HACCP plan in place. None of them has ISO certification on quality.

- Testing and validation of control measures

Influence of the crop rotations and harvest time on DON concentration
DON content of two population was studied; a) FHB high risk (FHB-HR) population,

wheat fields sown over wheat or corn stubble b) FHB low risk (FHB-LR) population, wheat fields sown over non-host crops of *Fusarium* such as forage and sunflower. Each population was represented by 5 fields or batches, each batch or subpopulation had three harvesting moments; physiological maturity (T1), 6-8 days after (T2) and 12-16 days after (T3) physiological maturity, with three repetitions each. Mallets were harvest at three different point in each field and the place was marked so the same place was harvested at T2 and T3. Humidity content was taken at harvest, samples were dried, DON analysis was done at LATU (partner 12) laboratory.

Results showed statistical differences in DON content for the FHB-HR and FHB-LR populations and for DON between T1, T2 and T3. FHB-LR population ended up with 34.1 % less DON in average than the FHB-HR population. DON content declined as harvest was postponed, T1 had 8866 ppm, T2 7893 while T3 had 6885 ppb of DON, similar results were reported in literature (Scott *et al*, 1983). The effect of crop rotation as a GAP on DON content was validated; there was significantly less DON on those wheat fields sown on non-susceptible *Fusarium* crops (forage crops and sunflower) than on those fields sown on wheat or corn stubble. The average for the low risk population was 6258 ppm of DON, which would be manageable at the mill, as opposite to 9504 ppm for the high risk population which would be very hard to handle at the mill.

- Integration of technical and socio-economic data and elaboration of the HACCP plan

The idea of the HACCP plan to control DON mycotoxin by reducing it to an acceptable level, was quite a goal to pursue. Searching for a way to achieve DON levels lower than 1ppm in flour, law enforced tolerance level in the country, was not an easy task. A HACCP plan was worked out for Río Molino mill as a case study, but preventive measures such as GAPs are essential, and needed as pre-requisites specially due to the fact that *Fusarium* is a field fungi that grows in the field and DON also develops in the field prior to harvest. A series of written reports such as; Deliverable 9 (socio-economic studies), 15 (HACCP plan), D16 (GAPs) and the final socio-economic report, give a detailed version of the problems encountered in each step of the chain and some recommendations on how to reduce the contamination. The CCPs suggested were at grain reception, mixing grain silos and mixing flour, and their critical limits should be validated in a FHB year, unfortunately there was no FHB favorable year during the duration of the MYCOTOX project.

Table No 3. Hazard analysis form

CFC Step	Hazard & Source	T*	C*	R*	Possible Control Measure(s)	Type of control	Is the step a CCP?
1. Seed selection	Fungus (*Fusarium*) infecting the seed	B	H	L	Select varieties that are not highly susceptible to FHB and purchase healthy seeds or use seed dressings	GAP	NO
2 Soil preparation	Fungus (*Fusarium*) in stubble on the surface	B	H	M	Plough, bury stubble, any practice that accelerates decomposition, exceptionally burn.	GAP	NO
3. Sowing practice	Fungus (*Fusarium*) in stubble if cero tillage Concentration of flowering date	B	H	H	If using cero tillage, choose fields that had non-susceptible host (avoid wheat/barley and corn stubbles). Spread out sowing date for the same variety or use varieties with different cycle, so as to not concentrate flowering periods in the field and escape favorable conditions for the disease	GAP	NO
4. Husbandry and disease control	-	B	H	L	Doesn´t alter FHB infection	GAP	NO
5. Fungicide application	*Fusarium* infecion if climate is condusive	B	H	H	Apply recommended fungicides at the beginning of flowering based on climate pronostics or DON-CAST model	GAP	NO
6. Harvest	*Fusarium* and mycotoxin contaminated grains	B/C	H	M	Harvest at approximately 14% humidity content. If infection present than "open wind" and regulate sieves on combine harvester so as to eliminate the most infected grain	GAP	NO
7. On farm storage	*Fusarium* and mycotoxin contamination pos-harvest	B/C	H	L	Harvest dry grain and monitor humidity on storage. Not very common	Good storage practices (GSP)	NO
8. Transport	*Fusarium* and mycotoxin contamination pos-harvest	B/C	H	L	Use clean trucks. Avoid humid grain if transport is far, cover truck to avoid rain	GSP	NO
9. Reception at traders	Fungus and mycotoxins contmaination	B/C	H	H	Segregation is usually done only by commercial classification grades 1, 2 and 3, try segregating within this classification by modified %FDK ** truck by truck. Training staff is necessary. Differentiate payment by quality.	**CCP1**	YES
10. Drying	Fungus and mycotoxins contmaination	B/C	H	H	Drying wheat is not commonly required. Certain traders have this option, if needed, usually using wood burning dryers	GMP	NO
11. Pre-cleaning	Fungus and mycotoxins contmaination	B/C	H	H	If modified %FDK is high then we should pay less and pre-clean wheat using a gravity table	GMP	NO
12. Storage at traders	Fungus and mycotoxins	B/C	H	M/L	Segregate based on modified %FDK, trasile (empty a silo, pass the content of a silo to another silo) to maintain temperature and humidity content.	GSP	NO
13. Transport	*Fusarium* and mycotoxin	B/C	H	L	Use clean trucks. Avoid humid grain if transport is far, cover truck to avoid rain	GSP	NO
14. Mill reception	Fungus and mycotoxins	B/C	H	H	Analyze *Fusarium* affected grain by modified %FDK in truck by truck samples. Segregate in high/medium/low categories. If modified %FDK is high then we should pay less and pre-clean wheat using a gravity table.	**CCP2**	YES
15. Silos and Storage	Fungus and mycotoxins	B/C	H	M/L	Segregate based on modified %FDK, trasile (empty a silo, pass the content of a silo to another silo) to maintain temperature and humidity content.	GSP	NO
16. Cleaning	Fungus and mycotoxins	B/C	H	M/L	Minimize Fusarium and mytoxin levels	Good manufacturing practices (GMP)	NO
17. Conditioning	Fungus and mycotoxin	B/C	H	L	Do no leave for more then required, fungus might start growing, control temperature	GMP	NO
18. Mixture of silos	Fungus and mycotoxins	B/C	H	M/L	Analyze DON when trasile to know DON content of each silo and use mixture of silos for desired mycotoxin level	**CCP3**	YES
19. Milling process	Mycotoxin	C	H	L	Extraction of part of toxin in seed covers	GMP	NO
20. Flour storage	Mycotoxin	C	H	L	Control temperature, there are 15 flour silos so mixing can be done to dilute mycotoxin levels	**GMP**	NO
21. Flour mixing	mycotoxin	C	H	L	Mix flour silo by weight to yield a flour with < 1 DON ppm	**CCP4**	YES
22. Additives	Mycotoxin	C	H	L	Doesn't alter the toxins level	GMP	NO
23. Packing	Mycotoxin	C	H	L	Avoid humidity and dirt	GMP	NO
24. Flour storage	Mycotoxin	C	H	L	Store in a dry and clean place	GMP	NO
25.Transport	Mycotoxin	C	H	L	Avoid humidity and dirt, use clean trucks specially for bulk transport	GMP	NO

***T = Type = Chemical, Biological, or Physical**
C = Class. = Deadly, Serious, Harmful
R = Risk = High, Medium, Low risk of occurring

PUBLICATIONS AND PAPERS

- Actor organization for QAS along agro supply chains: the case of micotoxins reduction in Sourthern Cone graines. *In*: *92nd Seminar of the European Association of Agro Economists (EAAE) on Quality Management and Quality Assurance in Food Chain,* 2-4 March 2005, Göttingen, Germany (oral presentation).

- Adoption constrains of QAS implementation in Argentina and Uruguay wheat supply Chain. *In: V International Pensa Conference an Agri food Chains/Networks Economics and Management*, July 2005, Riberao Preto, Brazil (oral presentation).

- Policies for QAS implementation in export chains: Mycotoxin management for MERCOSUR wheat actors. *In: 7th International Conference on Management in AgriFood Chains and Networks*, 31st May-2nd June 2006, Ede, The Netherlands (oral presentation).

- Impacts from norms in export chains: Mycotoxin management for MERCOSUR wheat actors. *In: 16th Annual World Forum; Symposium and Case Conference,* International Food and Agribusiness Management Association (IAMA), 10-13 June 2006, Buenos Aires, Argentina (oral presentation).

- Stewart, S. 2006. Experience from a decision support system approach to reduce DON contamination in Uruguay. *In: Advances in research on toxicogenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context.* 15-17 March 2006, Carlos Paz, Cordoba, Argentina (oral presentation).

- J. Cea, S. Stewart, G. Gutiérrez. 2007. A HACCP Plan Along The Wheat Chain To Prevent DON In Wheat Flour In Uruguay. Submitted and approved as poster to be presented at the *12th International IUPAC Symposium on Mycotoxins and Phycotoxins,* May 2007, Turkey.

CONCLUSION

The project was a great experience for scientists that didn´t have experience with HACCP, working with people from different disciplines and from different countries was also very enjoyable. We were able to study some specific steps with more detail and draw an overall picture of the DON situation throughout the wheat chain. Some recommendations were made to reduce the toxin to acceptable levels (Deliverables 15, D16 and D18). The key to the problem is the integrated management of the disease in the field. Once the toxin is in the grain there is no efficient way of reducing it, only diluting it. The CCPs suggested and their critical limits should be validated in a FHB year, unfortunately there was no FHB favorable year during the duration of the MYCOTOX project.

Partner 07 – INIACL-CRIR

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

Participation in WP 4&5

Activity 1. Compilation of literature and existing data in Chile

A literature review and data compilation was done at the project start, on the occurrence of mycotoxins in Chile. This helped in setting the priority combination as wheat grain/deoxynivalenol DON and choosing the pilot site of Valdivia (South of Chile) for project WP 4&5 activities.

Activity 2. Establishment of a multidisciplinary HACCP team

A multidisciplinary team was constituted, including one agronomist, one analyst, one socio-economist, one HACCP specialist and one phytopathologist, some of them coming from UDEC (partner 11). Contacts were officialised to involve the national PDP programme wheat farmers, the seed producer (von Baer Company) as well as the Collico mill within the MYCOTOX project.

Activity 3. Elaboration of the Commodity Flow Diagram (CFD)

The Commodity Flow Diagram was elaborated for the selected commodity system wheat/DON, the chain steps and actor organisation were identified, as well as data on the production, volumes, transportation, processing at the mill, grain and flour storage, and market requirements.

Activity 4. Implementation of a surveillance programme in Chile

A surveillance programme was designed and implemented for identifying the Critical Control Points in the wheat chain, where sampling was essential for monitoring DON contamination. Wheat samples were collected from different harvests, respectively 60 samples for 2003, 90 samples for 2004 and 60 samples for 2005, in the South Valdívia region, either directly from producers or at reception by the Collico mill. The samples were then analysed for DON contamination (analyses performed by UDEC, partner 11) and mycological identification at our laboratory. This latter revealed the presence of *Fusarium* fungi but absence (or very low levels) of the DON mycotoxin. This absence of DON was confirmed in the next harvests. This interesting finding was analysed in terms of impact of agro-climatic factors which were favourable in the South of Chile for avoiding wheat contamination, i.e. moderate temperatures (an average of 19°C) and relative humidities, especially during wheat spike flowering. Another favourable factor for DON absence was the used agricultural practices, such as the use of certified seeds by wheat farmers, the burning of stubble, the soil preparation for the seed bed, the absence of corn in the crop rotation. All these practices did not favour the development of *Fusarium* Head Blight and the production of corresponding mycotoxins.

The absence of DON in wheat from South Chile was on one hand, an advantage for this region, and by extension to the whole country, as the wheat and derived wheat flour were safe products for consumers; however, for the project's research purpose, it was difficult to find DON-contaminated wheat batches. This became the main limitation to implement the next steps of the planned activities, i.e. testing and validation of control measures and construction of the HACCP plan. The surveillance studies were then extended to the North Chile (the Curicó area) where 28 wheat samples were collected and analysed. They showed higher DON levels than in the South, but still reasonable

values for safe consumption (ranging between 47 and 90 ppb of DON). Some of these samples (showing the highest DON values) were also analysed using HPTLC/MS at the Hohenheim University, in the frame of a collaboration with the Food Chemistry Institute (laboratory of Dr. Gertrud Morlock). Presence of DON was confirmed. This finding stressed the impact of climatic conditions, as the North Chile temperatures and relative humidities were more favourable to the development of *Fusarium* Head Blight.

The mycoflora identified on the samples collected in the South Chile was the same for 2004 and 2005 harvests. The main species identified were *Alternaria*, *Cladosporium* and a few *Fusarium* (mainly *F. poae*, *F. graminearum*, *F. verticilloides* and *F. avenaceum*). Some *Penicillium* and *Aspergillus* were detected at the 2005 harvest characterized by unusual humidity during the summer period. A test was developed for detection of mycotoxin production by the *Fusarium* isolates under controlled conditions. The hazard analysis had also identified high contamination with the fungus *Alternaria* even if no detectable levels of *Alternaria* toxins were found. In addition, the ochratoxin A (OTA) contents found in the blood samples analysed within Work Package 2 made us suspect the wheat flour as contaminated with OTA, especially the stored flours. 30 wheat flour samples were collected from different mills in Chile and analysed. The distribution of positive samples showed the highest percentage (56%) for the Northern region of Chile, even the OTA levels were not so high (maximum level of 2 ppb). This showed that the methodology applied within the MYCOTOX project for DON should be extended to OTA in the future.

In absence of DON in wheat, there was no need for testing and validating control measures. The HACCP plan was then constructed as a compilation of recommendations and guidelines, dealing mainly with agricultural practices and weather forecasts, which should be considered as essential for mycotoxin prevention in Chile. The obtained results were used to demonstrate to wheat farmers the importance of the Good Agricultural Practices that are used in the national agriculture and that explain the good health of the harvested grains.

Activity 5. Socio-economic studies on the selected wheat/DON commodity system

At the start of project, the wheat supply chain in South Chile was studied, in terms of stakeholder organisation, production volumes and areas, market requirements, prices, traded quantities and incentives for mycotoxin-free quality, perception of the mycotoxin problem by the different stakeholders. Surveys were conducted with wheat producers, mills and bakeries, through questionnaires. The supply chain structure and interactions were then understood, and the costs for implementation of Quality Management Systems were assessed, regarding the constraints of the local context. The New Institutional Economics approach was chosen for expanding the analysis from a single level to a whole chain system.

The studies focused later on the mill and consumer level, mainly the perception of quality and needed incentives for implementation of Quality Management Systems. Indeed, the wheat supply chain had a very changing scenario in which the concepts of quality and quality assurance systems were evolving. The mill industry was then a good reflection of this situation. A pre-graduated student (José Mouat) started a thesis in 2006

which topic concerned the assessment of quality concept for the milling industry in Chile. A survey was conducted with 34 mills throughout Chile which allowed to find different situations regarding the quality assurance capacity, and the perspectives of the industry regarding this topic. The first results indicated a clear tendency to the quality improvement of the mill process, even some of them have ISO 9001: 2000 in place or being implemented.

Only 68% of the mills differentiated wheat according to quality characteristics and stored them into different silos. The mills that did not differentiate quality said it was because their small milling capacity. Only 50% of the surveyed mills produced special flours, and all of them produced and sold by-products. Only 38% of the surveyed mills, mainly small-size mills, were directly supplied with wheat from the farmers. The five biggest surveyed mills bought wheat, according to their needs, only from brokers. The mill industry was not used to formalize contracts, and only two mills reported having contract with some producers. The main reasons mentioned by the surveyed mills were: (a) enough wheat supply in the market, (b) price and quality uncertainty, (c) the size of the mill did not justify contract elaboration.

Most mills (65%) established premiums and penalties (prize discounts) for different quality aspects. In this sense, the most important parameters for premiums were hectoliter weight, gluten index, humidity and impurities. The parameters that generated penalties were: impurities, humidity and hectoliter weight. For the milling industry, the hectoliter weight was easily measurable and represented how much flour might be obtained from the grain. Although some authors disagreed with this relationship (Graybosch *et al*, 1996, Hazen *et al*., 1997, Bergman *et al*., 1998, Mladenov *et al*., 2001), the Collico Mill (involved in the Chilean HACCP team of the MYCOTOX project) established the weight of 1000 kernels as the most important parameter to evaluate the grain quality, they considered it for giving prize premiums, since they concluded that it correlated the best with the flour quality.

The milling industry was generally satisfied with the quality of Chilean wheat, judging it as good to regular. The main reasons why the wheat was considered as regular was the grain homogeneity and standardization. When asked to grade the grain according to the different quality parameters, the humidity and gluten content appeared to have the highest ranking and. Regarding safety standards, 10 mills (out of 34) performed mycotoxin analysis, 1 mill had an accredited HACCP system and 2 were in the process of HACCP implementation, while 10 were sensitive to Good Practices and 3 were certified for ISO 9001:2000. This was a good indicator on the feasible introduction of quality assurance systems into the Chilean milling industry.

According to this study, the milling industry was shown to evolve and progressively integrate quality management systems, by incorporating quality norms and standards. This was in line with the objective of the MYCOTOX project and methodology. However, there still was a lack of communication between stakeholders and a need for official interventions from authorities, especially for setting regulations and putting in place a policy of incentives to the producers, such as premium prices for mycotoxin-free grain.

All technical and socio-economic data generated from the project were fully integrated and assembled for elaborating the HACCP Plan (deliverable 15), the Good Practices (deliverable 16, this one jointly elaborated with Argentina and Uruguay) and the Implementation of the Food Quality Management System (deliverable 18). Those documents will be essential for further dissemination at the national and regional level, for helping decisions and policies in Chile and the MERCOSUR.

Participation in WP 6

Dissemination activities were performed either for promoting the project itself and the obtained results with wheat stakeholders and academic/research institutions, or for meeting official authorities (Ministry for Agriculture, Ministry for Health) and regulatory bodies for recommendations further implementation of Food Quality Management System for mycotoxin control in Chile.

Several visits were done to the Valdivia pilot site in Chile and meetings were organised with the wheat chain actors involved in the project (Semillas von Baer, Collico mill, PDP farmers and wheat producers).

Various dissemination workshops, trainings and meetings were organised with cereal stakeholders (in South and North Chile), Ministries for Health and Agriculture, in order to promote the project's activities and output, sensitize the official authorities on the mycotoxin hazard in cereal chains and stress the need for specific regulations. Brochures were then distributed for this purpose.

Participation in the international seminar organised by the Chilean government on Food Quality and Safety, April 2006, Chillán, Chile.

Participation at the workshop organised with the national PDP programme farmers and wheat producers, June 2006, Temuco, Chile.

Participation in the workshop on cereal storage "Granos Almacenados: Situación de Mercado y Almacenaje" organised by a group of private companies (Bayer Crop Science, Degesch de Chile Ltda and Sin Plagas Ltda), August 2006, Temuco, Chile.

Participation in scientific events and project's meetings

- Participation in the first annual meeting of the project (17-19 February 2003) and the second annual meeting (4-7 October 2004) held in Montevideo (Uruguay).

- Participation in the specific meetings for WP 4&5&6 held in Buenos Aires, Argentina on 20-22 August 2003 and 15-17 August 2006.

- Participation in the specific meetings for socio-economics held in Campinas (Brazil) on1-2 December 2003 and in Mar de Plata, (Argentina) on 4-5 November 2004, and in the informal meetings which were held among the socio-economists involved in the project during participation in regional scientific events and conferences (27-29 July

2005 in Ribeirão Preto (Brazil), 15-17 March 2006 in Villa Carlos Paz (Argentina) and 10-13 June 2006 in Buenos Aires (Argentina).

- Participation in the third annual meeting of the project (13-14 March 2006) held in Villa Carlos Paz, Argentina.

- Meetings with the MYCOTOX general coordinator, Dr Nadine Zakhia, during her visits to Chile (in December 2005 and December 2006) and with Dr Guy Henry (CIRAD scientist outposted to Argentina).

- Meetings with the University of Concepción (partner 11) for discussing activities and results. A strong collaboration was done between INIA Chile (partner 7) and UDEC (partner 11) within the WP 4&5 activities of the project in Chile.

- Participation in the 55th Congress of the Chilean Agronomical Society, 19 October 2004, Valdivia, Chile.

- Participation in the 56th Congreso Agronómico de Chile, 12 de Octubre del 2005, Chillán, Chile

- Participation in the V International PENSA Conference on Agri-food Chains/Networks Economics and Management, 27-29 July 2005, Ribeirão Preto, Brazil.

- Participation in the 16th Annual World Forum; Symposium and Case Conference, International Food and Agribusiness Management Association (IAMA), 10-13 June 2006, Buenos Aires, Argentina

- Participation in the EC Mycoglobe conference, 15-17 March 2006, Villa Carlos Paz, Argentina.

Publications and papers

- Henry G., Salay E., Engler A., **2003**. Integration of socio-economic and food science and technology research in quality management of food supply chains: Mycotoxin control system of grains in the Southern Cone. Invited paper presented at the *V Simposio Latino Americano de Ciencias de Alimentos*, 3-6 November 2003, Campinas-SP, Brazil.

- Madariaga R., Engler A., Vega M., Villegas R., Saelzer R., Ríos G., **2004**. Flora fungosa de los granos de trigo cosechado en el sur de Chile. *In: 55th Congress of the Chilean Agronomical Society*, 19 October 2004, Valdivia, Chile (oral presentation).

- Henry G., Iglesias D., Engler A., Salay E., Gutiérrez G., **2004**. Organización de actores alrededor de la gestión de calidad en cadenas agroalimentarias. *In: XIII Seminario Latinoamericano y del Caribe de Ciencia y Tecnología de Alimentos*, 12-15 de Octubre del 2004, Montevideo, Uruguay.

- Engler A., Henry G., Iglesias D., Alves A., Gutiérrez G., Salay E., **2005**. Actor organization for QAS along agro supply chains: the case of mycotoxin reduction in Southern Cone grains. *In*: *92nd Seminar of the European Association of Agro-Economists (EAAE) on Quality Management and Quality Assurance in Food Chain,* 2-4 March 2005, Göttingen, Germany (oral presentation).

- Muñoz K.S., Vega M., Ríos G., Madariaga R., **2005**. Ochratoxin A in cereals. Study of two procedures of analysis. *In: 27 Mykotoxin-Workshop Ifado,* 13-15 de Junio del 2005, Dormund, Germany (poster).

- Muñoz K.S., Vega M., Ríos G., Madariaga R., **2005**. Determination of ochratoxin A in human blood. Preliminary study to estimate risk exposure in Chile. *In: 27 Mykotoxin-Workshop Ifado,* 13-15 de Junio del 2005, Dormund, Germany (poster).

- Engler A., Gutiérrez G., Henry G., Iglesias D., **2005**. Adoption constraints of QAS implementation in Argentina and Uruguay wheat supply chains. *In*: *V International PENSA Conference on Agri-food Chains/Networks Economics and Management,* 27-29 July 2005, Ribeirão Preto, Brazil (oral presentation).

- Madariaga R., Bustamante S., Engler A., Vega M., **2005**. Flora fungosa de los granos de trigo cosechado en el sur de Chile. I. *Fusarium*. *In: 56 Congreso Agronómico de Chile,* 12 de Octubre del 2005, Chillán, Chile (oral presentation).

- Madariaga R.B., **2005**. Hablemos de micotoxinas. *Internal Seminar*, 21st November 2005, Instituto de Investigaciones Agropecuarias INIA, Chile (dissemination).

- Henry G., Engler A., Iglesias D., Gutierrez G., **2006**. Socio-economic constraints and opportunities affecting the implementation of mycotoxin control measures in Southern Cone grain supply chains. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina (oral presentation).

- Madariaga R., Bustamante S., **2006**. Fungal flora on wheat grains harvested on Southern Chile. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina (poster).

- Iglesias D., Henry G., Engler A., Gutierrez G., **2006**. Policies for QAS implementation in export chains: mycotoxin management for Mercosur wheat actors. *In: 7th International Conference on Management in AgriFood Chains and Networks*, 31st May-2nd June 2006, Ede, The Netherlands (oral presentation).

- Iglesias D., Henry G., Engler A., Gutierrez G., **2006**. Adoption of QAS and impact from norms in export chains: mycotoxin management for Mercosur wheat actors. *In: 16th Annual World Forum; Symposium and Case Conference,* International Food and

Agribusiness Management Association (IAMA), 10-13 June 2006, Buenos Aires, Argentina (oral presentation).

- Vega M., Madariaga R., Saelzer R., Villegas R., Ríos G., Muñoz K., Carrillo D., Bastias C., Torres O., **2006**. Deoxinivalenol en Chile. Estudio de 3 años. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil (poster).

- Vega M., Madariaga R., Ernesto J., Muñoz K., Villegas R., Sepúlveda C., Torres O., **2006**. Estudio de hongos y fumonisina B_1 y B_2 presentes en maíz para ensilaje y su relación con la altura de corte en el campo. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil (poster).

- Madariaga R., Bustamante S., **2006**. Fungal flora on wheat grains harvested on Southern Chile. *In: Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring Food and Feed Safety in a Myco-Globe Context*, Conference of the EC MYCO-GLOBE Project, 15-17 March 2006, Villa Carlos Paz, Córdoba, Argentina (poster).

- Madariaga R., June **2006**. Ausencia de micotoxinas y de hongos micotoxigénicos en la cadena de trigo. Presentation at the meeting organised with farmers and wheat producers, Temuco, Chile (dissemination).

- Vega M., Madariaga R., Saelzer R., Villegas R., Ríos G., Muñoz K., Carrillo D., Bastias C., Torres O., **2006**. Deoxinivalenol en Chile. Estudio de 3 años. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil (poster).

- Vega M., Madariaga R., Ernesto J., Muñoz K., Villegas R., Sepúlveda C., Torres O., **2006**. Estudio de hongos y fumonisina B_1 y B_2 presentes en maíz para ensilaje y su relación con la altura de corte en el campo. *In: V Congreso Latinoamericano de Micotoxicología*, 18-21 de Junio del 2006, Florianópolis, Brazil (poster).

- Madariaga R., August **2006**. Micotoxinas y problemas de hongos. *In: Granos Almacenados: Situación de Mercado y Almacenaje*. Presentation at this seminar organised by a group of private companies, Temuco, Chile (dissemination).

- Vega M., Madariaga R., Aranda M., Morlock G. Application of HPTLC/MS: confirmation of deoxynivalenol presence in Chilean wheat. *Peer-reviewed scientific paper under revision for publication*.

Training

- A pre-graduate student (José Mouat) in Agricultural Engineering, from University of Concepción, was co-supervised by INIA Chile and CIRAD (partner 1) on the socio-economic surveys with mills and consumers.

Partner 08 – INTECA TA

Contract number: ICA4-CT-2002-10043

FNAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

INTA participation in the MYCOTOX project: The development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America south Cone Countries was in Work Packages 4, 5 and 6 in which INTA also had the leadership.

Existing data on natural occurrence of mycotoxins in Argentina was collected from the available sources: published papers and reports from regulatory authorities. These data plus the expert opinion of leader mycologists from Argentina supported the combination of DON - wheat as the mycotoxin - food combination of choice to study. The main reasons for this selection were the consensus on the importance that the commodity wheat has on the national food supply and the high levels reported of DON in Argentinean wheat that could enter the human food supply in the local market.

The activities, described in detail below, for the application of a Food Quality Management System were performed on a particular cereal supply chain. For this task the wheat flour chain of Molinos Don Antonio of General Pico-La Pampa, a medium size flour mill was selected.

For the purpose of the HACCP Plan implementation, a HACCP team led by INTA was established with a socio-economist, agronomists and sector specialists from INTA General Pico-La Pampa, and with the technical staff from the private counterpart. During the development of the project, and given the scarcity of data of the levels of DON in Argentinean wheat under controlled conditions, a series of studies were conducted to supplement the information with the collaboration of an INTA team from INTA-Pergamino, Buenos Aires. INTA Socio-ecomic studies were conducted by the socioeconomic INTA team led by Daniel Iglesias with the collaboration of Guy Henry (CIRAD). For analytical aspects, the HACCP team was supported by the Argentinean Universities UBA (partner 9) and UNLu (partner 10).

INTA has promoted by different media the activities of the project, and of the mycotoxin hazard in cereal chains, as there is a partial comprehension from food producers and processors of the impact of HACCP and FQMS management system to control mycotoxin contamination levels.

INTA Participants in the HACCP-FQMS activities

Team member	Institution	Skills/Knowledge
M. Phil. Marcelo Masana	INTA, CIA, INTA Castelar	HACCP specialist
Dr Daniel Iglesias	NTA, Anguil, Santa Rosa - La Pampa	Agricultural economist
Ing. Agr. Ruben Bogino	INTA, Extensionnist, Gral Pico - La Pampa	Agronomist, Extensionnist
Ing. Agr. Juan Torrado	INTA, Extensionnist, Gral Pico - La Pampa	Agronomist, Extensionnist

INTA Participation in Activities according to Work Packages and Deliverables

WP 4 - D6 – Commodity Flow Diagram (CFD) verified (INTA) and WP 5 – D9 – Socio-economics studies – Chain Actor's Surveys (INTA)

A series of surveys were carried out on the wheat supply chain of Don Antonio Mill in order to gather information on agronomical and socio-economics aspects of the chain. Twenty farmers, 1 rural establishment with GAP, 3 assembly points, 8 bakeries, a noodle factory and the Don Antonio Mill.

Through the surveys a characterization of suppliers was performed

Also a cost-benefit evaluation for the implementation of Food Quality management system was performed.

Two papers were presented on this work (see Publications).

WP 5 – D13- Control Measures: Wheat Variety Studies (INTA in collaboration with CIM-UNLU (partner 10) and UBA (partner 9)

As part of the work conducted to determine feasible control measures for the mycotoxin contamination of wheat, several studies on the resistance/susceptibility to mycotoxin contamination in different wheat varieties were carried out. A summary of those studies is given in the following tables.

Table 1. Summary of Mycotoxin Studies and Number of Samples Generated (2)

Point	Replicate	Samples
A = at INTA-Bs. As. Experimental fields/03-04	2	126
A = at INTA-Pico Experimental field/04-05	1	15
A = at INTA- Lincoln Experimental field/04-05	2	32
A = at mill gate/04-05	3	150
	Total	323

Table 2. Summary of Mycotoxin Studies and Number of Samples Generated (1)

Study	Study Description	Type of Study	Batch	Point	Replicate	Samples
0	Effect of wheat varieties/Location	Control Measure	9 per variety - 9 exp. Fields	A = at INTA-Bs. As. Experimental fields/03-04	2	126
I	Effect of wheat varieties	Control Measure	1 per variety	A = at INTA-Pico Experimental field/04-05	1	15
II	Effect of wheat varieties	Control Measure	1 per variety	A = at INTA-Lincoln Experimental field/04-05	2	32
IV	Wheat at mill gate	Hazard Analysis	Variable by location	A = at mill gate/04-05	3	150
					Total	323

Analyses carried out on wheat varieties harvested in 2003-4 at INTA experimental fields showed that *Alternaria alternata*, *Fusarium graminearum*, *Fusarium poae* and *Fusarium semitectum* were predominant fungal species identified as endogenous flora.

Tricotheces type A detected were HT-2 and T-2 triol toxins and type B were deoxy nivalenol, nivalenol, and 3-acetyldeoxynivalenol. Based on 120 samples the incidences were 21.7% for 3-ADON, 22.5% for HT-2, 23.3% for T-2 triol and 85% For DON. Mean levels of positive samples were between 7 and 2.788 µg/kg. A NIV positive was also reported for the first time in Argentina. A scientific paper was submitted to Mycopathologia (Gonzalez *et al*, 2006).

Results of the analyses for mycotoxin contamination of wheat varieties harvested in 2004-5 at INTA experimental fields were:

Variety	µg/kg
n = 1	DON CGL
Buck Guapo	**66**
Buck Guapo	**10**
Buck Guapo	**10**
Buck Sureño	**10**
Buck Mataco	**10**
Buck Guatimozin	**10**
Prointa Puntal	**10**
Klein Sagitario	**0**
Klein Capricornio	**0**
Klein Escorpion	**0**
Buck Arriero	**0**
B10	**0**
Buck Panadero	**0**
Buck Pnadero	**0**
ACA 223	**0**
Coop. Liquen	**0**

General Pico Experimental Fields Trial 2004-5

Variety	µg/kg
n = 2	DON CGL
Relmo Tigereta	**837**
Buck guapo	**553**
Nidera Baguette 11-	**182**
Nidera Baguette 10	**157**
Buck Mataco	**148**
Aca 304	**95**
Klein Martillo	**77**
Buck Pingo	**44**
Nidera Baguette 13-	**36**
Klein Chaja	**13**
Don Mario Onix	**11**
Pronta-Gaucho	**11**
Prointa Molinero	**8**
Klein Escorpión	**5**
Relmo Chirrinche	**5**
Aca 801	**0**

Lincoln Experimental Fields Trial 2004-5

WP 5- Hazard Study Sample Analyses (INTA - Don Antonio Mill - CIM-UNLU and UBA)

As part of the Hazard Analysis in the Don Antonio Mill, samples from local farmers in the DON Antonio Mill region wheat samples were collected from General Pico, Arata, Ingeniero Luiggi, Castex, Villa Mirasol, Winnifreda and Embajador Martini at the mill´s gate and analyzed at the CIM-UNLU laboratories. Analyses performed were: Deoxinivalenol (DON), Nivalenol (NIV), AcetilDON (ADON) Zearalenona (ZEA), Toxina T2 (T2), Toxina HT2 (HT2), T2 tetraol (T2-4OH), T2 triol (T2-3OH), Neosolaniol (NEO), and Diacetoxiscirpenol (DAS)

Significantly the only toxin found was DON, for which, a summary of the results obtained can be seen in the following table showing percentage of positives, maximum, minimum and medium values:

Culture zone	n	% Positive	Maximum	Average	Minimum
			µg/kg		
Arata	7	29%	**265**	**52**	**0**
Eduardo Castex	11	73%	**28**	**7**	**0**
Embajador Martini	12	42%	**311**	**57**	**0**
Ingeniero Luiggi	11	55%	**37**	**5**	**0**
Villa Mirasol	7	43%	**134**	**34**	**0**
Winifreda	2	50%	**3**	**2**	**0**

A detailed document is available in the restricted acces area of the MYCOTOX website.

Results from the WP 5 - Hazard Study (INTA - Don Antonio Mill - CIM-UNLU and UBA) from the analyses of wheat samples taken at the mill´s gate will be further published.

WP 4&5 Training of INTA personnel

Attendance at the *FAO Workshop: Taller FAO/CENSA/INHA para la aplicación de los principios de HACCP en la prevención y control de mycotoxinas.* La Havana, Cuba. 22-23 de Septiembre del 2003. Participant: Marcelo Masana.

WP 4&5&6. Presentation of deliverables at MYCOTOX Meetings (INTA)

- *Specific MYCOTOX Meeting to WP 4&5&6,* Buenos Aires, Argentina,15-17 August 2006. Participants from INTA: Marcelo Masana and Daniel Iglesias.

- *III MYCOTOX Annual Meeting.* Villa Carlos Paz, Córdoba, Argentina. 13-14 March 2006. Participants from INTA: Marcelo Masana and Daniel Iglesias.

- *II MYCOTOX Annual Meeting.* Montevideo, Uruguay. 4-7 October 2004. Participant from INTA: Marcelo Masana.

- *MYCOTOX Socio-econimist Meeting.* Villa Carlos Paz, Córdoba, Argentina. 14 March 2006 to discuss GAP´s, CFD, Benefits/costs, Policy recommendations. Participant from INTA: Daniel Iglesias.

- *Specific WP 4&5 Meeting.* Buenos Aires, Argentina. 20-22 August 2003. Participants from INTA: Marcelo Masana, Ricardo Rodríguez, Norma Pensel.

- *I MYCOTOX Annual Meeting.* Montevideo, Uruguay. 17-19 February 2003. Participants from INTA: Marcelo Masana, Ricardo Rodríguez.

WP 6. Coordination activities (INTA)

WP6. Coordination Meeting in Buenos Aires 2006 (INTA)

A coordination meeting was held in Buenos Aires from 15 to 17 August 2006 organized by INTA (responsible: Marcelo Masana). Leaders of WP 4&5 from Argentina, Uruguay, Chile and Brazil, and a socioeconomist leader of each country and CIRAD attended the encounter. During the meeting a review of the advances from each participant was carried out, and, with the assistance of Dr. Martin Nagler from the NRI (UK), the main objective of the meeting was achieved by establishing the tasks for completing deliverables covering HACCP, GAP and FQMS. The output of the meeting was an agreed schedule and working format for the deliverables D15-16-18 and D 19.

WP 6. Coordination follow-up (INTA)

The output of deliverables D15 HACCP, D16 GAP was followed by emailing progress draft of those documents to all participants. D19 activities were also followed through email communication in this case in coordination with PROCISUR. Final documents by country were gathered.

WP 5 – D13 - Control Measures: Wheat Variety Studies (INTA-CIM-UNLU and UBA)

The development of the HAACP study and plan gave a series of publications in different congresses proceedings and papers.

- González H.H.L., Moltó G.A., Pacin A., Resnik S.L., Zelaya M.J., Masana M. and Martinez E. Trichothecenes type A and B and related mycoflora in commercial wheat cultivars harvested in 9 locations in Buenos Aires province, Argentina. Submitted to Mycopathologia.

- Frusteri Lucila M., Molto Gustavo A., Pacin Ana, González Héctor H.L., Resnik Silvia L., Masana Marcelo O. 2006. Tricotecenos tipo A y B y micoflora contaminante asociada en el trigo cosechado en la provincia de Buenos Aires. *In: Congreso Internacional de Ciencia y Tecnología de los Alimentos*, 15-17 de Noviembre del 2006, Córdoba, Argentina.

- Pacin A., Gonzales H.L., Resnik S.L., Molto G.A., Masana M.O. Microflora contaminante y presencia d tricotecenos tipo A y B en trigo cosechado en la provincia de Buenos Aires. Proccedings of the Congress: Ciencia y Tecnología de Alimentos CYTAL y 1er Simposio Internacional de Nuevas Tecnologías, 18-20 de Mayo del 2005, Mar del Plata, Argentina.

WP 5 – D9 - Socioeconomic Studies [INTA - CIRAD (partner 1) - INIA Uruguay (partner 6) - INIA Chile (partner 7) and Unicamp Brasil]

A series of socio-economics studies were produced for the characterization of the supply chains in each country through a tem of socio-economist with the participation of Daniel Iglesias from Argentina.

- Socio-economic Workshop (UE-CIRAD-NRI-PROCISUR, 1 y 2 de Diciembre del 2003, Campinas, Brasil). Disertation: Argentine Wheat Agrifood Chain: Actors Organization/Characterization (Daniel Iglesias)

- Organización de actores alrededor de la gestión de calidad en cadenas agroalimentarias 2004. Henry G., Iglesias D., Engler A., Salay E., Gutiérrez G. *In: XIII Seminario Latinoamericano y del Caribe de Ciencia y Tecnología de Alimentos*, 12-15 de Octubre del 2004, Montevideo, Uruguay.

- Actor organization for a QAS along supply-chain: the case of mycotoxins reduction in Southern Cone grain. *In: 92nd EAAE Seminar Quality Management and Quality Assurance in Food Chains*, 2-4 March 2005, Göttingen, Germany.

- Adoption constraints of QAS implementation in Argentina and Uruguay wheat supply chains. Engler A., Henry G., Iglesias D.H., Gutierrez G. *In: V International PENSA Conference on Agri-food Chains/Networks Economics and Management,* 27-29 Jully 2005, Ribeirao Preto, Brazil.

- Socio-economics constrains and opportunities affecting the implementation of mycotoxin control measures in the Southern Cone grain supply chains. Engler A., Henry G., Iglesias D.H., Gutierrez G. *In: Advances in research on toxigenic fungi and mycotoxins in South America ensuring food and feed safety in a Myco-Globe context*, 15-17 March 2006, Villa Carlos Paz, Argentina.

- Policies for QAS implementation in export chains: mycotoxin management for Mercosur wheat actors. Iglesias D., Henry G., Engler A. and Gutierrez G. *In: 7th International Conference on Management in Agrifood chains and Networks,* 31st May-2nd June 2006, Ede, Netherlands. Wageningen.

- Adoption of QAS and impacts from norms in export chains: mycotoxin management for MERCOSUR wheat actors. Iglesias D., Henry G., Gutierrez G. and Engler A. *In: 16th Annual Forum and Symposium IAMA,* 10-13 June 2006, Buenos Aires, Argentina

WP 5 - D15 - Hazard Analysis Critical Control Point Document (INTA)

A HACCP plan was prepared taking into account the present situation of the Don Antonio mill wheat flour chain

WP 4 - D16: Good Agronomical Practices Document [INTA, INIA Uruguay (partner 6), INIA Chile (partner 7)]

A document describing recommended Good Agronomical Practices for the prevention of the production of mycotoxin in wheat for Uruguay, Argentina and Chile was jointly prepared by INTA, INIA Uruguay (partner 6) and INIA Chile (partner 7).

WP 6 - D18: Food Quality Management System Document (INTA)

A document was prepared giving basis for the management of quality and safety, especially referring to mycotoxin prevention, of the mill wheat flour chain.

All documents related to the deliverables D15, D16 and D18 are available on the project website (restricted access area).

WP 6 – D19 (INTA - Don Antonio Mill). Dissemination of the MYCOTOX project and the Mycotoxin problem in the Cereal Chain among chain stakeholders in the General Pico region and nationwide (INTA)

- Conference: Desarrollo de un sistema de gestión con base HACCP para cadenas de cereales seleccionados. Primer Congreso Argentino y Primer Congreso Mercosur de BPM - POES - HACCP. 27-28 de Noviembre del 2003 Sede: Universidad Nacional de Río Cuarto

- Oral presentation: La prevención de riesgos en las cadenas agroalimentarias. 2005. Conferencia Regional FAO/OMS sobre Inocuidad de los Alimentos para las Américas y el Caribe. San José de Costa Rica. 5-9 December 2005.

- Workshop: Primera Reunión de Trabajo Instituto Tecnología de Alimentos - Programa Nacional de Cereales: Proyectos de Investigación en Control de Micotoxinas en Cereales". Centro de Capacitación de la EEA Pergamino, Centro Regional Buenos Aires Norte, INTA, 6 de Mayo del 2003.

- Workshop for the Awareness of the Mycotoxin problem in the Cereal Chain. Dissemination conferences by Ana Pacín (partner 10), Silvia Resnik (partner 9), Nadine Zakhia (partner 1) and Marcelo Masana, Gral Pico, La Pampa. 1st December 2005.

- Local media Press Conference with interview to Dr Nadine Zakhia-Rozis (CIRAD), Ana Pacín, Silvia Resnik, Gral Pico. 1st December 2005.

- Local media Press Conference with interview to Dr. Martin Nagler (NRI). Gral Pico. 27–28 September 2004.

- INTA Informa Nº233 (May 2003) and INTA Informa Internacional Nº1 (June 2003). Desarrollo de un sistema de gestión de la calidad de los alimentos para el control de las micotoxinas en la cadena de producción y procesamiento de cereales en el Cono Sur de Latinoamenrica.

Articles in newspapers

- Suplemento "La Tranqurera" de diarios de la Provincia de Buenos Aires. 22-28 May 2003.Pretenden controlar las micotoxinas en maíz y trigo.

- Diario "La Nueva Era de Tandil" Pretenden controlar las micotoxinas en maíz y trigo. 24 de Mayo del 2003.

- Diario "Democracia de Junín". Científicos del MERCOSUR controlarán micotoxinas en trigo y maíz. 28 de Mayo del 2003.

- La Reforma Newspaper, Gral Pico (Daily newspaper of the Gral. Pico region). Five newspaper articles for dissemination of the Mycotoxin problem in the Cereal Chain and the MYCOTOX project with Don Antonio Mill from Gral Pico. [March 2004 (2), June 2004 (1), September 2004 (1) and November 2004 (1)].

- Informe Rural Pampeano (Monthly Journal for La Pampa). One newspaper article (March 2004) and two press conferences (May and December 2005).

- La Arena Newspaper (newspaper of the Gral Pico region). One newspaper article (March 2004).

- Multicanal TV channel (Local TV broadcast). Two TV press conferences (March 2004) and five TV interviews (December 2005).

- FM La Isla (local FM radio broadcast), Two Radio Interviews (2004&2005).

- LU37 Radio Gral Pico (local AM radio broadcast). Two Radio Press Conferences and ten radio informative (2004&2005&2006).

- TV Closed Circuit Regional Cable. Three TV Interviews (2004&2005&2006).

- LU33 Radio Emisora Pampeana de Santa Rosa. TV program “Acortando Distancias” on MYCOTOX poject and mycotoxin problems.

Forthcoming actions and consequences of the MYCOTOX project within INTA

Indirect Output of MYCOTOX

A new strategy for INTA, described in the Plan Estratégico del INTA (INTA Strategic Plan), is being developed through research projects focusing in the solution of specific problems of the agricultural and food production. Within them, mycotoxin contamination in the cereal chains has now being incorporated as an important issue (INTA, DB-PNCER, 2005) benefiting from the development of the MYCOTOX project. INTA personnel participating in MYCOTOX have integrated to it the hazard analysis approach of MYCOTOX to specific projects (INTA-PNCER 3353).

Specifically for the mycotoxin problem in wheat the INTA, DB-PNCER. 2005 document has set medium and long-term research goals. In the medium term (5 years) the efforts will be to:

- Analyze and characterize the impact of the interaction agricultural practices-varieties on mycotoxin production by Fusarium and to identify micro satellites markers linked to FHB resistance.

- Improve the knowledge of agricultural practices to suppress the development of plant diseases associated to no tillage practice.

In the long term (10 years) the research focuses on:

- Incorporate genes expressing resistance to FHB along with genes for specific quality aspects.

- Develop wheat germoplasm with low mycotoxin production capacity under severe FHB episodes.

It is worth noting that in the INTA, DB-PNCER document the mycotoxin problem is a significant aspect discussed inside a strategy framework for quality improving and differentiating of the wheat chain.

WP 5 – D13 - Control Measures

Results from the WP 5 - Hazard Study (INTA, Don Antonio Mill, CIM-UNLU and UBA) from the analyses of wheat samples taken at the mill´s gate will be further published.

WP 6-D19-Dissemination

Dissemination activities regarding the importance of HACCP management systems to prevent mycotoxin contamination are planned to continue beyond the project end within the frame of the new INTA projects (INTA-PNCER 3353).

References

- INTA, DB-PNCER. 2005. Documento base. Programa Nacional Cereales. Eyhérabide G.H., Giorda L.M., Livore A.B., Nisi J. and Tomaso J.C. Editing Committee. Pergamino, 2 de Septiembre del 2005.

- INTA-PNCER 3353. Identificar situaciones de riesgo, desarrollar y validar métodos de prevención de la contaminación pre y postcosecha

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Partner 09 – UBAIR DCO

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

Participation in Work Package 1

CIRAD, through the coordinator of the project Dr Nadine Zakhia, provided FAPAS samples that were used for fitting the analytical methodology of DON in wheat and fumonisins in corn.
The methodologies for the diverse mycotoxins and the results obtained on FAPAS analysis were sent to the coordination of the WP 1.

Participation in Work Package 2

This work was made with the University of Luján (partner 10). The methodologies for OTA determination and confirmation in blood serum from human and pig samples were validated (CIM-UNLu and UBA).

Blood samples were respectively collected from the General Rodriguez area (236 samples) and Mar del Plata area (199 samples). These samples were analysed for OTA determination according to the method validated and implemented in the laboratory. The mean value for OTA was 0.625 ng/ml in Mar del Plata and 1.417 ng/ml in General Rodriguez. The percentages of positive samples for OTA were respectively 28.6% in General Rodriguez and 25.4% in Mar del Plata. During the project the work performed in WP 2, either in Argentina or Chile, was pioneer in the South Cone countries. It will provide baseline studies for helping the health-related stakeholders in decision-making and action planning to overcome the risk of ochratoxin A exposure for human.

In relation with the sources of OTA intake in Argentina, the occurrence of ochratoxin A in wines in the Chilean and Argentinean markets showed that this one is not the principal source of exposure. On the other hand, only a partial study was done in the Province of Entre Rios to identify the probability of OTA in the cereals and soybean cultivated in this region. The results allowed us to think that *Alternaria* toxins were the most probably mycotoxins that were possible to find in Entre Rios on sorghum, wheat, maize or oats at harvest. In a study of major scope in soybean, there was verified that OTA's occurrence was not probable in this leguminous at harvest in Argentina.

It is necessary to continue with the identification of the source of OA's exposure. In relation to the dietary intake it was verified in Argentina that bakery products accounted for 82% of daily intake of the population of the two studied regions in Argentina, whereas poultry and noodles respectively accounted for 78% and 77% of weekly consumption.

Since in Chile was verified OTA's presence in wheat flour as one of the sources of population exposure, the future studies in Argentina should consider the information obtained in this project to identify OTA source of Argentinean population exposure.

Leadership and participation in Work Package 3

It was coordinated the work in the WP 3 with the CIRAD (partner 1), with Concepción's University (partner 11) and with CIM - Lujan's University (partner 10).

- Grain contamination by *Fusarium* toxins such as deoxynivalenol (DON) in wheat and fumonisins in maize often shows high degree of variability. The distribution of the contaminants is not uniform inside the grains. Besides, few grains may be contaminated and some of them might contain high levels of mycotoxins. According to the demonstrated implication of those mycotoxins in human and animal toxicosis, it is essential to ensure a precise and accurate determination of their levels in grains.

The design of an efficient sampling plan depends on the knowledge of the contamination distribution, either inside the grains or between grains in bulk silo storage. This function of distribution is specific for each type of mycotoxin and for each commodity or food matrix.

The work done focused on statistically analyzing the contamination occurring with DON in wheat and derived milling fractions and with fumonisins in maize. This allowed defining of the statistical function associated with the contamination distribution and variability for the considered mycotoxins. Schematic procedures for wheat and corn sampling were then tested and optimised in Argentina and the sampling, sample preparation and analysis methodology were previously discussed, shared and harmonized among all partners before initiation and implementation.

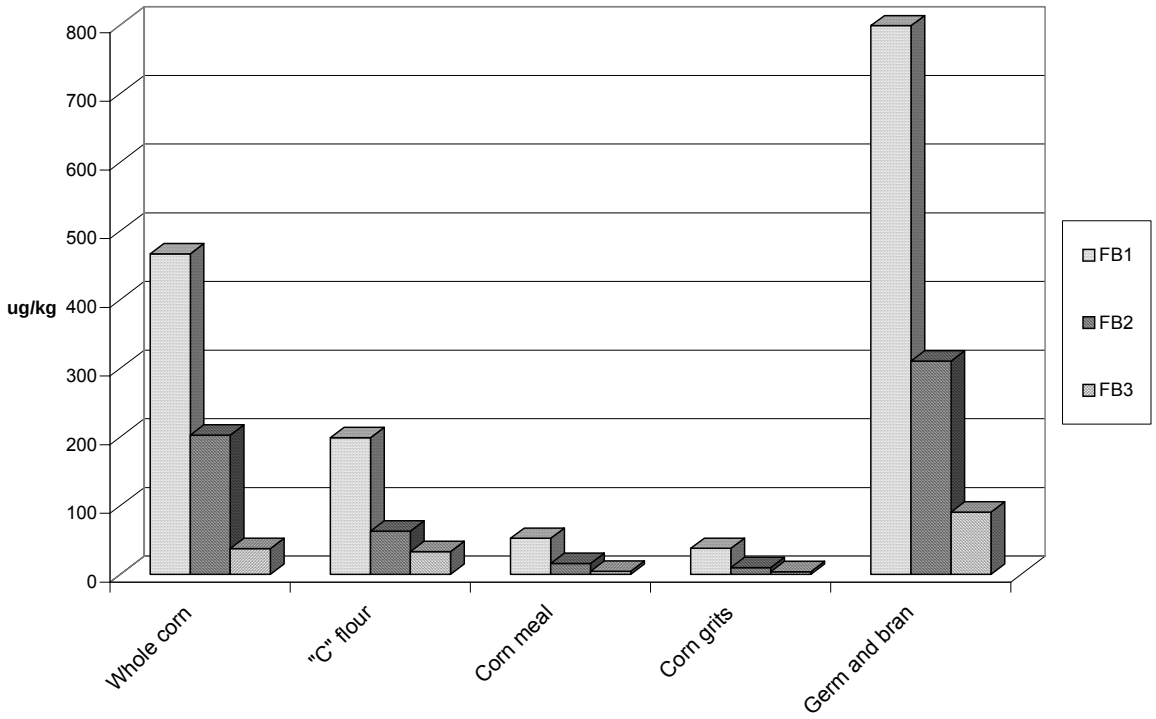
- The partners 1 & 11 jointly studied the influence of grain structural properties and processing steps on the distribution of mycelium and DON in wheat and derived milling fractions in a lab-scale dry milling to identify the critical points for each type of wheat grain processing and proposing adapted control measures.

- More than 120 milling plants make *wheat dry milling* in Argentina, with an estimated capacity of 2.000.000 ton/year. The estimated average wheat processed after 2001 was 1.000.000 ton/year. Only one industrial wheat wet milling plant is found in Argentina that produces 20 -25 mil ton starch and 5 - 6 mil ton gluten. The study was made in one milling plant from Santa Fe province (near 1.5% of the country dry milling capacity) and the wet milling plant.

DON Analysis in wheat and derived fractions were divided in two steps to optimise resources. Extraction and clean-up of analytical samples, first step, were performed in CIM-UnLu and derivatization and gas chromatography quantification in UBA (second step). The high-precision analytical method used to determine DON was evaluated by calculating the HORRAT ratio, derived in the small analytical variability found. The variability associated with the procedure of testing DON in a 3 kg sample of Argentinean wheat is in good agreement with the variability measured in 20 kg sample of U.S. wheat. To our knowledge this was the first report on the variability of the analytical method used to determine DON in bran, wheat flour, and gluten and on the distribution of DON contamination in these products. This variability and distributional information will allow estimation of errors in the evaluation of DON concentration in lots of these products and the design of sampling plans, selection of sample size or number of samples needed, to reduce the total variability.

- In Argentina it was possible to find 60 *maize dry milling* plants. With the capacity of 350.000 ton/year, average maize processed after 2001 was 250.000 ton/year. The study was made in the only one situated on Entre Rios Province (78,781 km^2) with a capacity of 24 ton/day.
The activities performed within WP 3 (jointly with the University of Luján – partner 10) began in the cleaning step. Maize cleaning using different sieve sizes always resulted in the small particles containing higher levels of mycotoxins than the bulk. This applied when monitoring aflatoxins, zearalenone, DON, and fumonisins on corn. Different losses where found with the sieves and they were quantified in relation with the size to allow economic evaluation of this cleaning step.

It was possible to observe the fumonisins content (B_1, B_2 and B_3) distribution among whole grain, corn flour, corn meal, corn grits, germ and bran after the dry milling in the following figure.



- *Wet maize milling* was studied first at pilot scale. In 2005, the wet maize milling was followed at industrial scale. An industrial Plant, 20% of the Argentinean capacity (1 million ton/year) was sampled. Maize samples were analyzed for aflatoxins, DON, ZEA, OTA and fumonisins.

21 wet milling processes of 120 ton each were done on maize (Total range fumonisins: 253 - 21500 µg/Kg) and sampling was made on maize and their milled fractions, and analyzed; they showed to be negative for DON, zearalenone, ochratoxin A and the aflatoxins, but positive for fumonisins. The fumonisins (B_1 and B_2) content was

determined in all fractions and in the waters of the process (starch, wash water, gluten meal, steep water, germ, fibre or gluten feed). The reductions were lower than those found in wet milling at pilot scale, probable due to the thorough washing process at laboratory scale. However, the reduction in contamination was of great importance, in the order of 90% of fumonisins. Not only were absent from the flour, but also fumonisins could not be found in the bran or steep water. The theory is that fumonisins might be binding more strongly to the maize matrix, which might form derivatives. Fumonisins derivates have been cited in the literature. This opens the way towards a new investigation such as the application of an *in vitro* digestion model to assess the bioavailability of fumonisins from wet milled maize fractions.

Studies on wet milling of maize have shown that both starch and gluten products were low in fumonisins content as wet milling of wheat related to DON contamination. This suggested that a corrective action for maize that is too highly contaminated with fumonisins, or wheat with DON, for human or animal consumption could be use in starch and gluten production.

- As contribution to WP 3 and both WP 4&5, a method was evaluated to reduce the number of samples in surveillance studies, in order to supply more economical tools for the implementation of an efficient Food Quality Management System along the chain stakeholders to ensure high quality maize and wheat production regarding mycotoxins contamination. The samples were analyzed from the most contaminated previous places found to the least contaminated ones. The offer for reduction of the number of analysis consisted in when nine consecutive analyzed samples were negative for a mycotoxins, no further analysis was made.

- Certain products such as wheat flour are susceptible to be contaminated with deoxynivalenol as the fungus able to produce it (*Fusarium graminearum*) is frequently isolated from the whole kernel. This mycotoxin is frequently found in foods, especially in those elaborated with cereals and it has been demonstrated that different products of massive consumption in Argentina like bakery products and beer are frequently contaminated by DON. Due to the culinary customs and considering that in Argentina appreciable amounts of wheat based foods are consumed, it is important to establish how processes are able to diminish the contamination by DON in those foods. The effect of frying in the reduction of DON contamination in a high consumption food in Argentina as is empanadas was studied together with partner 10. “Empanadas” are prepared with different filling types within the cover (turnover pie cover) and they are baked, or fried in vegetable oil or animal fat (pork or cow). Turnover pie covers are raw dough flattened and disc shaped (main component is wheat flour). They appear in packages of the thermoformed type closed with a flexible film but with no type of modified atmosphere. Each package contains several separated units with polyethylene films.

A reduction of DON contamination was observed during the home-made frying process. This DON reduction seems to depend on the frying temperature. A major DON reduction was obtained when the fried covers reached the home-made colour at the minor of the tested temperatures.

Participation in Work Package 4, 5 & 6

Support to the activities of WP 4, 5 & 6 integrating as expert on mycotoxins the HACCP team and collaborating through the analysis of wheat samples collected from the field. This helped the Argentinean HACCP team for controlling mycotoxins contamination in wheat, and validating the choice of the critical control points (CCP) and potential control measures. In particular was evaluated the resistance of the local varieties to DON contamination in La Pampa Province (with partners 8 and 10) The high incidence of *Alternaria alternata* isolates should be a matter of concern and the natural occurrence of *Alternaria* toxins in Argentinean wheat is to be studied in the future.

In addition, we participated in the dissemination meetings and conferences organised by INTA (partner 8) with the wheat supply chain stakeholders (producers, enterprises, extension agents) in the General Pico pilot site of the project.

Training or Posgraduate students

The following students participated in the activities of the project under supervision and/or presented their thesis:

1 Advisee: Margarita Samar. Director: Dra.Silvia L. Resnik
Place: Departamento de Química Orgánica. Facultad de Ciencias Exactas y Naturales, U.B.A. CIM-UNLu. Tesis presentada en Departamento de Industrias Facultad de Ciencias Exactas y Naturales.UBA. 2003. Tesis 3560 – http://www.opac.bl.fcen.uba.ar/. Grade: Outstanding (A).

2 Advisee: Leticia Broggi
"Study of natural fungi and mycotoxin contamination in cereals from Entre Ríos Province. Probability of contamination through industrial processes involved in the elaboration". Director: Dra. Silvia L. Resnik. Place: Department of Industry. Departamento de Química Orgánica Facultad de Ciencias Exactas y Naturales, U.B.A. Universidad Nacional de Entre Ríos. CIM-UNLu. Tesis presentada en Departamento de Industrias Facultad de Ciencias Exactas y Naturales.UBA, 2003. Tesis 3646 – http://www.opac.bl.fcen.uba.ar/. Grade: Outstanding (A)

3 Estela Motta, Faculty of Natural Sciences, University of Buenos Aires, Argentina. Participation in WP 3 and WP 2. PhD student under the joint supervision of partners 9 and 10. Topic: “Degradación de fumonisinas en la molienda húmeda de maíz y evaluación de la exposición por ocratoxina A”.

4 Gustavo Funes, Faculty of Natural Sciences, University of Buenos Aires, Argentina (supervision partner 9). Participation in WP 3. PhD student. Topic: “Fumonisins derivates in corn wet milling fractions”.

5 Lucila Frusteri, Faculty of Natural Sciences, University of Buenos Aires, Argentina (supervision partner 9). Participation in WP 7. Magister student Topic: Agrochemical

effects on DON and fungal contamination on wheat and soybean.

Publications in peer-reviewed scientific journals

- Distribution of Deoxynivalenol in wheat, wheat flour, bran, and gluten, and variability associated with the test procedure. Samar M., Ferro Fontán C., Resnik S., Pacin A. and Castillo M. Journal of AOAC International 86 (3), 551-556 (2003). ISSN: 1060-3271.

- Occurrence of ochratoxin A in wines in the Argentinean and Chilean markets. Pacin A., Resnik S., Vega M., Saelzer R., Ciancio Bovier E., Ríos G., Martínez N., ARKIVOC, (xii) 214-223, 2005. ISSN 1424-6376 http://www.arkat-usa.org.

- Deoxynivalenol reduction during the frying process of turnover pie covers. Samar M.M., Resnik S.L., González H.H.L., Pacin A.M. and Castillo M.D. Food Control (2007), 18 (10), 1295-1299.

- *Alternaria alternata* prevalence in cereal grains and soybean seeds from Entre Ríos, Argentina. Broggi L.E., González H.H.L., Resnik S.L. and Pacin A.M. Revista Iberoamericana de Micología, (2007), 24, 47-51.

- Natural occurrence of aflatoxins, deoxynivalenol, fumonisins and zearalenone in maize from Entre Ríos Province, Argentina. Broggi L.E., Pacin A.M., Gasparovic A., Sacchi C., Rothermel A., Gallay A. and Resnik S. Accepted for publication in Mycotoxin Research, 2007.

- Survey of Argentinean human plasma for ochratoxin A. Pacin A.M., Ciancio Bovier E.V., Motta E., Resnik S.L., Villa D. & Olsen M. Submitted for publication in Food Additives and Contaminants.

Oral presentations in conferences and congresses

- Mycotoxins in our countries. Resnik Silvia y Pacin Ana. Conferencia Magistral. *In: IV Congreso Latinoamericano de Micotoxicología.* Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003. Conferencia.

- Sistema de gerenciamiento de la calidad para el control de micotoxinas en las cadenas de producción y procesamiento de cereales de los países del Cono Sur. Correa TBS, Vargas E.A., Cea J., Vega M., Resnik S.L., Souza M.L.M0, Freitas-Silva O., Zakhia N. *In: IV Congreso Latinoamericano de Micotoxicología.* Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003.

- Reduction of fumonisins during the cleanliness of the maize. Round Table Redonda on Mycotoxins Decontamination Methods in Food. Pacin A., Taglieri D., Cano G. y Resnik S. *In: IV Congreso Latinoamericano de Micotoxicología.* Seminario Anual Animal. La Habana, Cuba, 24-26 September 2003.

- Managing of the mycotoxins contamination after the crop and during the

industrialization. Resnik Silvia. AATA's congress, Mar del Plata, 18-20 May 2005.

- Food processing to reduce the entry of mycotoxins to the food and feed chains. Disertante: Resnik Silvia. Conference: Advances in research on toxigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006, Villa Carlos Paz, Córdoba, Argentina.

- Evaluation of outbreaks of trichotechenes in Argentina and Uruguay. Resnik S.L., González H.H.L., Pacin A. Disertante: Silvia Resnik. V Congreso Latinoamericano de Micotoxicología, XII Encuentro Nacional de Micotoxinas - IV Simposio en Almacenaje Cualitativa de Granos del MERCOSUR. Florianópolis, Brasil, 19th June 2006.

- Quality of Stored Grains versus Water Activity and Fungi. Disertante: Silvia Resnik. V Congreso Latinoamericano de Micotoxicología, XII Encuentro Nacional de Micotoxinas - IV Simposio en Almacenaje Cualitativa de Granos del MERCOSUR. Florianópolis, Brasil, 18-21 June 2006

- Practices for mycotoxins reduction in the production and industrialization of the food chain. Disertante: Silvia Resnik. Congreso Internacional de Ciencia y Tecnología de los Alimentos. Córdoba, Argentina, 15-17 November 2006.

Book Chapters

- Regulaciones nacionales e internacionales, perspectivas de la producción de cereales y alimentos a base de cereales en la provincia de Córdoba (National and international regulations, perspectives of the production of cereals and food based on cereals in the province of Cordoba). Pacin A., Resnik S. En: Micotoxinas: Impacto en la Producción y Salud Humana y Animal. (Mycotoxins: Impart on production and human and animal health) Ed. Héctor R. Rubinstein. ISBN: 987-530-068-3. Chapter 1, pp. 29-47 (2006).

- Identificación y cuantificación de micotoxinas en maíz cosechado en la provincia de Córdoba y en productos de molienda (Identification and quantification of mycotoxins in maize harvested in Cordoba province and in maize milling products). Resnik S., Pacin A., Funes G. En: Micotoxinas: Impacto en la Producción y Salud Humana y Animal (Mycotoxins: Impact on production and human and animal health). Ed. Héctor R. Rubinstein. ISBN: 987-530-068-3. Chapter 8, pp. 199-220 (2006).

- Detección de OTA en muestras biológicas provenientes de humanos (OTA's detection in human biological samples). Pacin A., Resnik S., Ciancio Bovier E. En: Micotoxinas: Impacto en la Producción y Salud Humana y Animal (Mycotoxins: Impact on production and human and animal health) Ed. Héctor R. Rubinstein. ISBN: 987-530-068-3. Chapter 10, pp. 255-269 (2006).

- Toxinas T-2 y HT-2 (T-2 and HT-2 toxins). Silvia Resnik and Ana Pacin. *In: Micotoxinas en alimentos.* Jose Miguel Soriano del Castillo (ed.), capítulo 15, pp. 293-312, Editorial Díaz de Santos, Madrid, España, (2007).

- Acido ciclopiazónico (Ciclopiazonic Acid). Ana Pacin y Silvia Resnik. *In: Micotoxinas en alimentos.* Jose Miguel Soriano del Castillo (ed.), capítulo 18, pp. 335-356, Editorial Díaz de Santos, Madrid, España, (2007).

Posters presented in congresses

- Aflatoxinas en las fracciones obtenidas durante la limpieza del maíz (Aflatoxins in the fractions obtained during corn cleaning). Resnik Silvia L., Taglieri Daniela, Cano Gabriela y Pacin Ana María. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003.

- Estudio preliminar sobre la contaminación por ocratoxina A en vinos argentinos (Preliminary study on ochratoxin A contamination in Argentine wines). Pacin Ana María, Resnik Silvia L., Ciancio Emilia, Cano Gabriela y Taglieri Daniela. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003.

- Incidencia de la contaminación por aflatoxinas en maíz argentino, período 1995-2002 (Aflatoxins incidence in Argentine corn, period 1995-2002). Pacin Ana María, Cano Gabriela, Resnik Silvia L., Villa Daniel, Taglieri Daniela, y Ciancio Emilia. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003.

- Distribución de fumonisinas en el maíz y en las fracciones obtenidas durante la limpieza de maíz (Distribution of fumonisinas in the corn and in the fractions obtained during the cleaning of corn). Resnik Silvia L., Villa Daniel y Pacin Ana María. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003.

- Incidencia de la contaminación por AF en maíz (Aflatoxins ocurrence in maize). Pacin Ana M., Cano Gabriela, Resnik Silvia L., Villa Daniel, Taglieri Daniela. Jornadas Bonaerenses de Ciencia y Tecnología. La Plata, Pcia de Buenos Aires, 17 de Diciembre del 2003.

- Contaminación por ocratoxina A en vinos argentinos (Ochratoxin A ocurrence in Argentine wines). Pacin Ana M., Resnik Silvia L., Ciancio Emilia, Cano Gabriela, Taglieri Daniela. Jornadas Bonaerenses de Ciencia y Tecnología. La Plata, Pcia. de Buenos Aires, 17 de Diciembre del 2003.

- Reducción de micotoxinas en maíz. Limpieza (Reduction of mycotoxins in maize. Cleaning). Resnik Silvia L., Taglieri Daniela, Cano Gabriela, Ciancio Emilia, Pacin Ana M. Jornadas Bonaerenses de Ciencia y Tecnología. La Plata, Pcia de Buenos Aires, 17 de Diciembre del 2003.

- Muestreo de fumonisinas en maíz. Función de distribución (Maize sampling for fumonisins quantification. Distribution function). Resnik Silvia L., Villa Daniel, Pacin Ana M. Jornadas Bonaerenses de Ciencia y Tecnología. La Plata, Pcia de Buenos Aires, 17

de Diciembre del 2003.

- Micoflora contaminante en soja cosechada en la principal zona de producción de la República Argentina (Mycoflora in soybean harvested in the principal production zone of Argentina). Zelaya M.J., González H.H.L., Resnik S.L. y Martínez M.J. X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías. Mar del Plata, Argentina, 18-20 de Mayo del 2005, (Published in the proceedings, vol. V, 2006, pp. 1788-1794).

- Micoflora contaminante y presencia de tricotecenos tipo A y B en trigo cosechado en la provincia de Buenos Aires (Mycoflora and trichothecenes type A and B contamination in wheat harvested in Buenos Aires province). Pacin A.M, González H.H.L., Resnik S.L., Moltó G.A. y Masana M. X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías. Mar del Plata, Argentina, 18-20 de Mayo del 2005, (Published in the proceedings, vol. III, 2006, pp. 936-942).

- Contaminación por fumonisinas en fracciones obtenidas en la molienda húmeda de maíz (Fumonisins contamination in wet milling corn fractions). Funes G.J., Taglieri D., Cano G., Pacin A. y Resnik S.L. X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías. Mar del Plata, Argentina, 18-20 de Mayo del 2005, (Published in the proceedings, vol. III, 2006, pp. 929-935).

- Estimación de la ingesta de alimentos en 210 donantes de sangre en la ciudad de Mar del Plata (Estimation of the food intake in 210 blood donors in the city of Mar del Plata). Motta E., Ciancio Bovier E., Pacin A., Resnik S.L. y Villa D. X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías. Mar del Plata, Argentina, 18-20 de Mayo del 2005, (Published in the proceedings, vol. III, 2006, pp. 1197-1203).

- Micoflora contaminante y ocurrencia natural de micotoxinas en el maíz almacenado y los subproductos del proceso de industrialización por molienda seca (Mycoflora and natural occurrence of micotoxins in stored maize and the by-products of the dry milling process). Broggi Leticia E., Pacin Ana M., Gónzalez Héctor H.L., Resnik Silvia L., Cano Gabriela y Taglieri Daniela. II Jornadas de difusión de proyectos de investigación – extensión - UNER, INEX 2005, Concordia, Junio 2005.

- Aislamiento e identificación de la micoflora contaminante en cereales y oleaginosas: Un caso de estudio de en Soja cosechada en la República Argentina (Isolation and identification of the mycoflora in cereals and oilseeds: A case of study of in Soybean harvested in the Republic Argentina). Bienal de Ciencia y Tecnología 2005 de la Provincia de Buenos Aires, 8-10 de Noviembre, ciudad de La Plata.

- Food Intake Estimation In 236 Blood Donors In General Rodríguez, Buenos Province, Argentina. Castillo M.D., Ciancio Bovier E.V., Pacin A.M., Resnik S.L., Villa D. Conference: Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006. Villa Carlos Paz, Córdoba, Argentina.

- Ochratoxin A In Human Plasma In Buenos Aires Province, Argentina. Motta E., Ciancio Bovier E.V., Pacin A.M., Resnik S.L., Villa D. Conference: Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006. Villa Carlos Paz, Cordoba, Argentina.

- Contamination By Aflatoxins, Zearalenone And Deoxynivalenol In Corn And The Fractions Obtained In The Wet Milling Process. Castillo M.D., Pacin A.M., Molto G.A., Resnik S.L. Conference: Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006, Villa Carlos Paz, Córdoba, Argentina.

- Insecticide effect on the mycoflora of soybean RR isolated from the Pampean region in Argentina. Frusteri L.M., Gonzalez H.H.L., Zelaya M.J., Resnik S.L., Pacin A.M., Martinez M.J. Conference: Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006. Villa Carlos Paz, Córdoba, Argentina.

- Contaminant mycoflora of soybean RR seeds harvested in different production areas in Argentina. Zelaya M.J., Gonzalez H.H.L., Resnik S.L., Martinez M.J. Conference: Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006. Villa Carlos Paz, Córdoba, Argentina.

- Micoflora contaminante y ocurrencia natural de micotoxinas en avena cosechada en la provincia de Entre Ríos, Argentina (Mycoflora and micotoxins natural occurrence in oats harvested in Entre Rios province, Argentina). Sacchi C.A., Broggi L.E., Resnik S.L., González H.H.L. y Pacin A.M. V Congreso Latino Americano De Micotoxicología - V Clam – XII Encuentro Nacional De Micotoxinas - Enm 2006 – IV Simposium De Almacenaje Cualitativo De Granos Del Mercosur - IV Sag-Mercosul. Florianópolis, SC, Brasil, 18-21 de Junio del 2006.

- Tricotecenos tipo A y B y micoflora contaminante asociada en el trigo cosechado en la provincia de Buenos Aires (Trichotechenes type A and B and mycoflora contaminant associated in the wheat harvested in Buenos Aires province). Frusteri L.M., Molto G.A., Pacin A., González H.H.L., Resnik S.L. y Masana M.O. Congreso Internacional de Ciencia y Tecnología de los Alimentos. Córdoba, 15-17 de Noviembre del 2006. pp. 232-233.

Dissemination conferences

Mycotoxins, a present enemy in the food. Resnik Silvia and Pacin Ana. The IVth Students' National Congress of Biochemistry and Biotecnology. National university of the Litoral. 1st October 2005, Santa Fé.

What are the Mycotoxins? Modifications of the natural mycotoxins contamination for processing effect. Resnik Silvia. General Pico. 1st December 2005, La Pampa.

Participation in scientific events and project meetings

- First annual meeting of MYCOTOX project, 17-19 February 2003, Montevideo, Uruguay.

- Progress meeting of MYCOTOX, 20-22 August 2003, Buenos Aires, Argentina.

- Workshop on soybean quality. 9 September 2004. INTA Marcos Juárez.

- Meeting workshop on Mycotoxins. 29 of September of 2004. Salón Carillo, SECYT, Buenos Aires, Argentina. Organized by National Agency of Scientific and Technological Promotion.

- Bio-safety, surveillance and segregation of grain and seeds modified and not modified genetically. 22 and 23 September 2004, Auditorio de la Bolsa de Cereales de Buenos Aires, City of Buenos Aires, Argentina.

- Second Annual Meeting of Mycotox Project. "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries", ref ICA4-CT-2002-10043. 4-7 de Octubre del 2004, Montevideo, Uruguay.

- X Argentine Congress of Science and Technology of Food and 1st International Simposium of New Technologies. Mar del Plata, Argentine, on May 18-20, 2005.

- Mycotoxins and Pesticides workshop. National Strategic programs. "Quality and food Security, Contaminants in food". Buenos Aires, Argentina, on 7 October 2005.

- Third Annual Meeting of Mycotox Project. "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries", ref ICA4-CT-2002-10043. 13-14 de Marzo del 2006, Villa Carlos Paz, Còrdoba, Argentina.

- Myco-Globe conference. Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 marzo, 2006. Villa Carlos Paz, Córdoba. Argentina.

- V Congreso Latino Americano De Micotoxicología - V Clam – XII Encuentro Nacional De Micotoxinas - Enm 2006 – IV Simposium De Almacenaje Cualitativo De Granos Del Mercosur - IV Sag-Mercosul. Florianópolis, SC, Brasil, 18 al 21 de Junio de 2006.

- Meeting with Dr Nadine Zakhia (general coordinator), Lujan, Argentina, 1st December 2006.

Training Courses

- "Mycotoxins in Foods". Asociación Argentina de Tecnólogos en Alimentos. Fundación

de Investigaciones Científicas Teresa Benedicto de la Cruz. 15 de Junio del 2006. Professors: Dra. Ana M. Pacin and Dra. Silvia Resnik. Assistants: Tec. Gabriela Cano, Tec. Daniela Taglieri and Bioq. Manuel Zelaya.

Future publications

- Trichothecene type A and B and related mycoflora in wheat harvested in Buenos Aires Province, Argentina. Gonzalez H.H.L., Molto G., Pacin A., Resnik S., Zelaya M., Masana M.

- Fumonisins behaviour on laboratory-scale corn wet milling process. Funes Gustavo J., Bello M.O., Resnik S.L., Pacin A.M., Cano Gabriela.

- Effect of industrial wet milling process on the distribution of aflatoxins, deoxynivalenol, fumonisin b1 and b2, and zearalenone in corn fractions. Gustavo Funes, Marcelo D. Castillo, Gustavo A. Molto, Ana M. Pacin, Silvia L. Resnik.

- Effect of maize cleaning by sieving before storage. Resnik S., Pacin A.

- Mycoflora and mycotoxin natural occurrence in oats from Entre Rios and Buenos Aires provinces, Argentina. Sacchi C., González H.H.L., Broggi L.E., Pacin A., Resnik S.L., Cano G. & Taglieri D.

Partner 10 - University of Lujan

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

Leadership of WP 2 activities

Risk assessment of human exposure to Ochratoxin A

Objetive: Evaluate exposure to OTA in Latin American South Cone region

The activities related to this WP can be divided into the following stages:

1. Preparation stage:

1 a. Selection and standardization of OTA methodology for blood samples. Survey of Canadian human blood plasma for Ochratoxin A. (P.M. Scott, S.R. Kanhere, B.P.-Y. Lau, D.A. Lewis, S. Hayward, J.J. Ryan and T. Kuiper-Goodman. *Food Additives and Contaminants*, 15: 555-562, 1998).

1 b. Methodology Standardization for dietary inquiry

1 c. Standardization of the analytical methodology. The following actions were undertaken:

- Finetuning of the analytical methodology for OTA determination in wine (*Pacin A., Resnik S., Vega M., Saelzer R., Ciancio Bovier E., Ríos G., Martinez N. "Occurrence of ochratoxin A in wines in the Chilean and Argentinean markets" ARKIVOC (2005) XII 214-223).*
- Finetuning of the methodology for OTA in swine serum. The slaughter house nearest to the University was contacted and 120 samples were obtained. Due to the speed of blood hemolysis in swine it was impossible to reach valid conclusions.
- Finally, samples in human plasma were obtained from a pool of a laboratory of Luján to finetune the methodology. With these samples detection and quantification limits for OTA were established as 0.012 ng ml^{-1} and 0.019 ng ml^{-1} respectively.

1 d. Recoveries for human plasma were established. The greatest difficulty was that, due to the fact that the pool of samples used was all contaminated, it was not possible to establish the recovery with minimum values.

Recoveries of spiked samples with OTA standard (Sigma Chemical Co., St. Louis, MO, USA) were 85% for contamination level of 2.4 ng ml^{-1}; 95% for 1.5 ng ml^{-1} and 96% for 0.8 ng ml^{-1}. The mean recovery was 89.8 %.

Conclusions of the first stage:

- Plasma were analysed for the presence of Ochratoxin A according to the method of Scott *et al.* (1998) with some modifications as reported below.
- Confirmation of OTA by methyl ester.
- Thanks to the experience gained in the slaughter house, a MSC thesis was projected with two Argentine companies dedicated to pork meat retailing.

- The normal evaluation procedure comprises three concentrations covering the range of linearity (low, mid and high). As we needed large sample size to prepare triplicate samples at each OTA level, it was asked to a diagnosis laboratory to collect blood remaining samples to perform these recovery studies. The pools obtained were always OTA contaminated, so it was impossible to work at low level because of the error coming from the initial contamination.

2. Procedure stage:

Samples

The first step of the blood donation procedure in the hospitals was to ask donors to fill in a questionnaire which allowed a physician to set apart some of them (eg. low weight people, elderly people, the ones who suffered from hepatitis). Moreover, it was compulsory in Argentina to make HIV and Chagas virus analysis on the blood before including it in the blood bank. The numbers of samples were 205 for Mar del Plata and 275 for General Rodríguez. However after excluding samples from donors with HIV and Chagas virus, the numbers of samples were 199 plasma samples from Mar del Plata and 236 from General Rodríguez

Procedure Ochratoxin A extraction and clean-up

The plasma were analysed for the presence of Ochratoxin A according to the method of Scott et al. (1998) with some modifications as reported below. One ml of plasma was mixed with 0.25 ml saturated sodium chloride solution and 5ml methanol with a vortex mixer, for 15 seconds in a centrifuge tube and centrifuged at an average relative centrifugal force of 500 x g for 15 min. The supernatant was transferred to another tube and mixed with 5ml 0.015M o-phosphoric before adding to a Bakerbond® C-18 cartridge (art.7020), preconditioned with 10ml MeOH followed by 6ml methanol:(0.015 M)o-phosphoric acid (1:1, v/v). Solvents passed through the column by gravity at a flow rate of 1-2 drops sec^{-1}. Following the supernatant addition, the column was washed with 5ml 0.015 M o-H_3PO_4 followed by 5 ml methanol: (0.015 M) o-phosphoric acid (1:1, v/v). Two ml MeOH were added to the cartridge and stand for 3 min before elution. The evaporated extract was dissolved in 3 x 2.5 ml p*hosphate-buffered saline* solution (PBS, a mixture of 0.26 g monoacid sodium phosphate, 1.14 g diacid sodium phosphate was dissolved separately and then added 7.02 g sodium chloride, 0.201 g potassium chloride and 0.5 g sodium azide adjusted to pH: 7.4 and diluted to 1 l with bi-distilled water) - MeOH (85:15) and added to an Ochraprep® column. All solvents were passed through the column by gravity flow. The column was washed with 5ml PBS solution - MeOH (85:15) followed by 10 ml distilled water.

OTA was eluted in two back flushing steps. Firstly, with 3 ml MeOH and secondly, with 1.5 ml MeOH into a silanized vial. The eluate were pooled and evaporated to dryness under vacuum, at 30ºC.

The evaporated extract was dissolved in 200 μl mobile phase. An injection of 100 μl of sample extract was analysed by HPLC as described below.

The HPLC system used was Agilent®1100 series that included a degasser (G1322A), an auto sampler (G1313A), a fluorescence detector (G1321A), quaternary pump (G1311A) and a thermostatted column compartment (G1316A). The utilized column was a C18 reverse phase (4 mm i.d. x 125 mm containing 5 µm particle size, Hypersil BDS, Hypersil® with a guard column of the same phase (Hypersil BDS C18 4 mm i. d. x 4 mm, 5 µm). The mobile phase was acetonitrile: water: acetic acid (49.5:67:1, v/v/v). The flow rate was 1 ml min^{-1}. Fluorescence excitation and emission wavelengths were set at 330 nm and 470 nm, respectively. Retention times of OTA were in the range of 2-3 min.

Confirmation of OTA by methyl ester

The confirmation of OTA presence was done through the formation of Ochratoxin A methyl ester. Slight modifications of Grosso et al. (2003) procedure were made. Briefly, a quantitative portion of the methanolic elution phase from the immunological column was evaporated to dryness and re-suspended into 200 µl of a 12% methanolic solution of boron trifluoride (Baker C701-07). After 15 min heating at 60ºC, the derivative was analysed by HPLC with the same chromatographic conditions as for OTA. Confirmation of OTA as methyl ester was performed on all contaminated samples. Retention time of the OTA methyl ester was approximately 16.3 min. Detection and quantification limits expressed as OTA were calculated signal-to-noise ratios of approximately 3:1 and 5:1 respectively (0.017 ng ml^{-1} and 0.028 ng ml^{-1}).

Dietary intake

The enquiries of frequency food intake were done in both localities in the same moment as the blood samples were taken.

As some plasma samples were discarded, there were more enquiries than levels of contamination by OTA in plasma.

A descriptive statistical analysis was undertaken to identify the food intake by locality, gender and age group.

A model for the input of data of the enquiries was designed in such a way as to identify frequency of intake (percentage of intake) and the amount of food consumed (grams consumed).

Conclusion of the procedure stage:

- From the analytical methodology point of view it is important to note that the frequency with which false positives of OTA were observed made it absolutely necessary to confirm, as in this case, with methyl ester.

Table 1.

False Positive samples Both cities				False positive
	n	Positives	Negatives	Percentage
General Rodriguez	236	148	88	28,64
Mar del Plata	199	128	71	25,42

The analysis revealed that 63.8% from Mar del Plata and 62.3% from General Rodríguez of human plasma sampled were positive for OTA, as a result, a winsorized

mean of 0.11 ng ml^{-1} and 0.43 ng ml^{-1} respectively, was found (*Survey of Argentinean human plasma for Ochratoxin A. Pacin A.M., Ciancio Bovier E.V., Motta E., Resnik S.L., Villa D. & Olsen M. Food Additives and Contaminants*).

- Concerning the food intake enquiry, it was not possible to obtain a correlation between the level of plasma contamination and the food consumed for each person, by means of logistical correlations. This has proved a challenge for statistics as it is evident that contamination of plasma is the result of contaminated food intake.

- Nevertheless, both information (percentage of intake and the amount of food consumed) are shown in the following tables:

Table 2. Mar del Plata data, percentage of food consumed

Food	Daily		Weekly		Monthly		Sometimes		Never	
	No.	%	No.	%	No.	%	No.	%	No.	%
Teas	192	94.1	5	2.5	0	0.0	0	0.0	7	3.4
Bakery products	174	84.5	25	12.1	2	1.0	4	1.9	1	0.5
Sugar	160	78.0	3	1.5	0	0.0	4	2.0	38	18.5
Uncooked vegetables	135	65.9	57	27.8	3	1.5	1	0.5	9	4.4
Cookies	134	65.0	35	17.0	2	1.0	15	7.3	20	9.7
Fresh fruits	129	62.9	55	26.8	7	3.4	5	2.4	9	4.4
Canned fruits	107	52.2	49	23.9	10	4.9	19	9.3	20	9.8
Soft drinks (diet)	105	512	42	20.5	7	3.4	11	5.4	40	19.5
Beef	98	47.6	97	47.1	7	3.4	2	1.0	2	1.0
Milk and sub products	82	39.8	62	30.1	23	11.2	13	6.3	26	12.6
Cooked vegetables	74	35.9	86	41.7	14	6.8	2	1.0	30	14.6
Jam Marmalade	71	34.6	53	25.9	6	2.9	19	9.3	56	27.3

No. = Number of surveyed people

Table 3. Mar del Plata data, percentage of food consumed

Food	Daily		Weekly		Monthly		Sometimes		Never	
	No.	%	No.	%	No.	%	No.	%	No.	%
Teas	192	94.1	5	2.5	0	0.0	0	0.0	7	3.4
Bakery products	174	84.5	25	12.1	2	1.0	4	1.9	1	0.5
Sugar	160	78.0	3	1.5	0	0.0	4	2.0	38	18.5
Uncooked vegetables	135	65.9	57	27.8	3	1.5	1	0.5	9	4.4
Cookies	134	65.0	35	17.0	2	1.0	15	7.3	20	9.7
Fresh fruits	129	62.9	55	26.8	7	3.4	5	2.4	9	4.4
Canned fruits	107	52.2	49	23.9	10	4.9	19	9.3	20	9.8
Soft drinks (diet)	105	51.2	42	20.5	7	3.4	11	5.4	40	19.5
Beef	98	47.6	97	47.1	7	3.4	2	1.0	2	1.0
Milk and sub products	82	39.8	62	30.1	23	11.2	13	6.3	26	12.6
Cooked vegetables	74	35.9	86	41.7	14	6.8	2	1.0	30	14.6

Jam Marmalade	71	34.6	53	25.9	6	2.9	19	9.3	56	27.3

No. = Number of surveyed people

Table 4. Mar del Plata data, percentage of food consumed

Food	Daily		Weekly		Monthly		Sometimes		Never	
	No.	%	No.	%	No.	%	No.	%	No.	%
Fruit juice	67	33.3	29	14.4	5	2.5	12	6.0	88	43.8
All kind of cheese	58	28.2	120	58.3	8	3.9	11	5.3	9	4.4
Coffee	53	26.2	35	17.3	9	4.5	34	16.8	71	35.1
Candies	43	21.1	49	24.0	10	4.9	46	22.5	56	27.5
Wines	42	20.6	40	19.6	4	2.0	11	5.4	107	52.5
Soybeans	32	15.6	38	18.5	19	9.3	14	6.8	102	49.8
Dulce de leche	29	14.2	45	22.1	21	10.3	38	18.6	71	34.8
Sweeteners	27	13.2	4	2.0	0	0.0	0	0.0	173	84.8
Mixed cereals	24	11.8	29	14.2	11	5.4	21	10.3	119	58.3
Beers	20	9.8	72	35.1	10	4.9	21	10.2	82	40.0
Cold cut (boiled)	19	9.2	95	46.1	38	18.4	32	15.5	22	10.7
Cold cut (unboiled)	17	8.3	92	45.1	34	16.7	27	13.2	34	16.7
Poultry	17	8.3	150	72.8	18	8.7	8	3.9	13	6.3
Salad snacks	16	7.9	76	37.6	30	14.9	40	19.8	40	19.8
Noodles	15	7.3	170	82.5	7	3.4	4	1.9	10	4.9

No. = Number of surveyed people

Table 5. Mar del Plata data, percentage of food consumed

Food	Daily		Weekly		Monthly		Sometimes		Never	
	No.	%	No.	%	No.	%	No.	%	No.	%
Chocolate	12	5.9	53	25.9	16	7.8	28	13.7	96	46.8
Eggs	11	5.4	157	77.3	10	4.9	16	7.9	9	4.4
Rice and sub products	10	4.9	151	73.3	16	7.8	13	6.3	16	7.8
Pastries	8	3.9	51	25.1	48	23.6	63	31.0	33	16.3
Dried fruits	7	3.4	18	8.8	11	5.4	15	7.3	154	75.1
All nuts	6	3.0	10	5.0	10	5.0	43	21.4	132	65.7
Soft drinks	5	2.4	3	1.5	1	0.5	5	2.4	191	93.2
Oats and sub products	5	2.4	8	3.9	10	4.9	8	3.9	175	85.0
Beans	4	2.0	41	20.2	32	15.8	41	20.2	85	41.9
Soups	4	1.9	14	6.8	12	5.8	11	5.3	165	80.1
Peanuts pasta	1	0.5	19	9.4	25	12.4	32	15.8	125	61.9
Alcoholic beverages	1	0.5	6	3.0	0	0.0	1	0.5	195	96.1
Corn meals. Corn products	1	0.5	57	27.8	36	17.6	44	21.5	67	32.7
Other meats	1	0.5	64	31.1	66	32.0	37	18.0	38	18.4
Pork	0	0.0	21	10.2	37	18.0	67	32.5	81	39.3
Pig kidney	0	0.0	0	0.0	3	1.5	16	8.0	181	90.5

No. = Number of surveyed people

Table 6.

Mar del Plata Food	Amounts of food consumed Average mg or ml	St δ	Mar del Plata Food	Amounts of food consumed Average mg or ml	St δ
Soft drinks (diet)	7814.75	9810.3	Dulce de leche	449.13	813.3
Bakery products	7355.76	4246.4	Soybean cereals	397.97	788.2
Beef	4627.21	4229.8	Salad snacks	372.66	755.7
Teas	4224.96	2161.2	Other meats	365.19	783.4
Fresh fruits	3654.36	3214.9	Cold cut (boiled)	362.90	641.8
Uncooked vegetables	3425.73	2451.8	Soft drinks	332.62	2302.2
Juices fruit	3179.62	5206.0	Cold cut (unboiled)	330.99	559.8
Milk and sub products	3054.85	3836.4	Corn meal. Corn products	264.62	532.9
Wines	2643.42	4500.1	Coffee	206.68	490.6
Cookies	2513.88	2041.2	Pastries	202.92	435.1
Canned fruits	2443.76	2635.1	Beans	193.18	754.9
Cooked vegetables	2299.10	2567.8	Pork	144.59	300.6
Beers	1958.45	3770.3	Dried fruits	134.22	514.3
Poultry	1300.02	1982.0	All nuts	117.43	489.7
All kind of cheese	1155.24	1589.0	Oats	111.50	509.4
Noodles	969.88	1007.5	Chocolate	97.15	272.1
Jam Marmalade	935.48	1157.2	Alcohol drinks	82.16	752.8
Candies	769.81	1243.4	Peanuts pasta	65.22	214.7
Rice and sub products	764.45	760.0	Soups	57.16	224.4
Eggs	516.44	561.4	Pig kidney	2.99	11.2
Mixed cereals	484.09	1173.2			

Some Mar del Plata figures:
Daily 84 % bakery products
Weekly 73% poultry 83% and noodles
Coffee daily 26%, weekly 17%
Pig kidney never 90%

Table 7. General Rodriguez data on 236 surveyed people (n = Number of surveyed people)

Food Category	***Percentage of food consumed***						
	n	*daily*	n	*weekly*	*monthly*	*sometimes*	*never*
Infusions (mate, any kind of tea, coffee)	232	98.3	2	0.8	0	0.8	0
Bakery products	193	81.8	25	10.6	0	2.5	5.1
Beef, viscera and related products	168	71.2	67	28.4	04	0	0
Cookies and crackers	127	53.8	72	30.5	2.5	1.7	11.4
Milk and Diary Products	94	39.8	85	36.0	6.8	8.9	8.5
Fresh Fruits	84	35.6	100	42.4	6.8	5.1	10.2
Carbonated Soft Drinks	73	30.9	72	30.5	2.1	4.2	32.2
Citrus Fruits	69	29.2	100	42.4	4.7	6.4	17.4
Candie	64	27.1	61	25.8	3.4	8.1	35.6
Jams, jellies, marmalades	57	24.2	67	28.4	8.9	4.7	33.9
Raw Vegetables	53	22.5	158	66.9	3.8	1.7	5.1
Cooked vegetables	51	21.6	166	70.3	3.4	1.3	3.4
Cheese, cream cheese and grated cheese	50	21.2	144	61.0	8.5	3	6.4
Citric Juices, Apple juice	41	17.4	68	28.8	6.4	3.8	43.6

Table 8. General Rodriguez data

Food Category	Percentage of food consumed					
	daily	n	weekly	monthly	sometimes	never
Any Kind of Wine	15.3	67	28.4	5.5	6.4	44.5
Dried Noodles	14.8	188	77.1	3.8	0.8	3.4
Dulce de leche	14.0	83	35.2	13.1	8.9	28.8
Light soft drinks	12.7	22	9.3	0.4	0.8	76.7
Eggs	8.1	164	69.5	10.2	1.7	10.6
Peanut butter	6.8	59	25.0	6.4	14.0	47.9
Cold Meat	6.8	119	50.4	16.1	7.2	19.5
Rice and related products	6.4	180	76.3	7.2	1.7	8.5
Ready-to-eat cereals, cereal mixes	5.9	26	11.0	3.4	7.2	72.5
Raw meat	5.9	85	36.0	11.0	9.3	37.7
Pastry Products	5.9	67	28.4	20.3	18.6	26.7

Table 9. General Rodriguez data

Food Category	Percentage of food consumed					
	daily	n	weekly	monthly	sometimes	never
Packed soups	4.2	22	9.3	5.5	4.2	76.7
Chicken Meat, viscera and related products	3.8	185	78.4	11.9	1.7	4.2
Salty Snacks	3.0	75	31.8	11.0	13.6	40.7
Any kind of beer	2.5	85	36.0	8.5	11.9	41.1
Oat and oat-based products	2.1	5	2.1	1.3	3.4	91.1
Soybean and Soy-based products	1.3	18	7.6	5.1	4.7	81.4
Beans, lentils, chickpeas	0.8	63	26.7	22.0	12.7	37.7
Pork meat, viscera and related products	0.4	20	8.5	18.2	29.7	43.2
Raisins, apricot, plums, peaches	0.4	3	4.2	4.2	7.2	90.3
High Grade Alcoholic Beverages	0.4	14	5.9	3.8	3.8	86.0
Dried Fruits	0.4	10	1.3	3.8	3.8	83.9
Other meats	0.0	26	11.0	24.2	25.0	39.8
Polenta, Corn, Cornflakes	0.0	67	28.4	25.0	11.9	34.7

Table 10. General Rodriguez data

Food Category	Average food consumed g or ml	St Deviation
Carbonated Soft Drinks	7138.1	12321.0
Bakery products	6129.8	4066.3
Beef viscera and related products	6015.0	4113.1
Infusions (mate, any kind of tea, coffee)	4204.1	4189.7
Milk and Diary Products	3879.7	4471.2
Any kind of wine	3125.4	6707.8
Citric Juices, Apple juice	2970.2	5496.1
Light soft drinks	2756.5	7461.7
Any kind of beer	2578.2	5367.1
Cookies and crackers	2438.3	2209.9
Fresh Fruits	2223.5	2727.3
Cooked vegetables	2048.7	2325.1
Raw Vegetables	1787.9	2300.5
Citrus fruits	1591.2	2233.2
Chicken Meat, viscera and related products	1308.4	1631.2
Dried Noodles	1231.4	1718.4
Candies	1133.6	1668.9
Cheese, cream cheese and grated cheese	1038.4	1556.7

Table 11. General Rodriguez data

Food Category	*Average food consumed g or ml*	*St deviation*
Rice and related products	944.3	1259.0
Jams, jellies, marmalades	890.0	1299.6
Eggs	612.4	1024.9
Dulce de leche	611.7	1117.9
Other meats	494.9	597.1
Pork meat, viscera and related products	482.3	634.0
Pastry products	441.8	868.0
Cold Meat	435.4	637.6
Peanut butter	417.2	1037.5
Polenta, Corn, cornflakes	355.8	409.3
Raw meat	329.8	548.0
Ready- to -eat cereals, cereal mixes	307.5	915.4
Bens, lentils, chickpeas	214.9	303.2
High Grade Alcoholic Beverages	200.3	795.4
Salty Snacks	170.6	318.0
Packed soups	99.5	307.8
Oat and oat-based products	94.2	483.0
Soybean and soy based-products	87.6	302.7
Dried Fruits	70.6	250.0
Raisins, apricot, plums, peaches	43.1	234.4

Some General Rodriguez figures:
Daily 81.8 % bakery products
Weekly 78.4% poultry 77.1% and noodles
Never 91.1% oats, 90.3% raisin, 83.9 % dried fruits, 81.4 % soybean, 72.5 % ready to eat cereals, 43.2 % pork meat and viscera

- The enquiry as such allowed for the verification of the intake of foods identified in previous enquiries in Argentina.

Table 12.

	Percentage of consumption			
	Daily	Weekly		Never
City	Bakery products	Poultry	Noodles	Pork meat and viscera
General Rodriguez	81.8	78.4	77.1	43.2
Mar del Plata	84	73	83	90

Participation in WP 1 Development and standardisation of effective analytical tools for mycotoxin determination in cereals and by-products.

Implementation of interlaboratory works between all partners for comparison, harmonisation and standardisation of the analytical methods they are currently using, in terms of sampling procedures, sample preparation and techniques (mainly TLC, GC and HPLC) for mycotoxin determination. Mycotoxins to be determined were Aflatoxins B_1, B_2,

G_1 and G_2; Fumonisins B_1 and B_2; DON, Zearalenone, upon reference FAPAS test materials (when available) and naturally and spiked wheat and maize samples.

We collaborated to undertake the objectives and work of this WP, intervening in the interlaboratory work by means of the analysis of the FAPAS samples (DON in wheat).
Results were obtained as solicited by the leader of the WP 1, they were sent by electronic and were given in at the meeting in Carlos Paz, in March 2006.
Even though it was not planned, the method for Ochratoxin A was also validated.

Outputs
Standardised and validated analytical chromatographic methods applicable by all partner laboratories for mycotoxin determination in wheat and maize

Interlaboratory works between partner laboratories for comparing, harmonising and standardising the analytical chromatographic methods currently used.
Validation and standardisation of the most adequate analytical tools for monitoring mycotoxin contamination and supplying further input to WP3 (milling) and WP 4 and WP 5 (HACCP, control measures)

Participation in WP 3. Evaluation of milling procedures as potential CCPs

Objectives
To evaluate distribution and variability of Deoxynivalenol (DON) (in wheat) and Fumonisins (in maize) in fractions obtained through wet and dry milling processes in the Southern Cone.

We collaborated to undertake the objectives and work of this WP 3, intervening in the mycotoxin analysis of the samples. Samples were received, conditioned, extractions for analysis with HPLC, GC and TLC (as confirmation) were undertaken and determinations by HPLC (Fumonisins) and TLC (DON).

Wet maize milling was studied first at pilot scale. In 2005, the wet maize milling was followed at industrial scale. An industrial Plant, 20% of the Argentinean capacity (1 million ton/year) was sampled. Maize samples were analyzed for aflatoxins, DON, ZEA, OTA and fumonisins. 21 wet milling processes of 120 ton each were done on maize (Total range fumonisins: 253 - 21500 µg/Kg) and sampling was made on maize and their milled fractions, and analyzed; they showed to be negative for DON, zearalenone, ochratoxin A and the aflatoxins, but positive for fumonisins. The fumonisins (B_1 and B_2) content was determined in all fractions and in the waters of the process (starch, wash water, gluten meal, steep water, germ, fibre or gluten feed). The reductions were lower than those found in wet milling at pilot scale, probable due to the thorough washing process at laboratory scale. However, the reduction in contamination was of great importance, in the order of 90% of fumonisins. Not only were absent from the flour, but also fumonisins could not be found in the bran or steep water. The theory is that fumonisins might be binding more strongly to the maize matrix, which might form derivatives.

Studies on wet milling of maize have shown that both starch and gluten products were low in fumonisins content as wet milling of wheat related to DON contamination. This suggested that a corrective action for maize that is too highly contaminated with fumonisins, or wheat with DON, for human or animal consumption could be use in starch and gluten production.

Maize cleaning using different sieve sizes always resulted in the small particles containing higher levels of mycotoxins than the bulk. This applied when monitoring aflatoxins, zearalenone, DON and fumonisins in corn. Different losses were found with the sieves and they were quantified in relation to the size to allow economic evaluation of this cleaning step.

Certain products such as wheat flour are susceptible to be contaminated with deoxynivalenol as the fungus able to produce it (*Fusarium graminearum*) is frequently isolated from the whole kernel. This mycotoxin is frequently found in foods, especially in those elaborated with cereals and it has been demonstrated that different products of massive consumption in Argentina like bakery products and beer are frequently contaminated by DON. Due to the culinary customs and considering that in Argentina appreciable amounts of wheat based foods are consumed, it is important to establish how processes are able to diminish the contamination by DON in those foods. The effect of frying in the reduction of DON contamination in a high consumption food in Argentina as is empanadas was studied together with partner 10. “Empanadas” are prepared with different filling types within the cover (turnover pie cover) and they are baked, or fried in vegetable oil or animal fat (pork or cow). Turnover pie covers are raw dough flattened and disc shaped (main component is wheat flour). They appear in packages of the thermoformed type closed with a flexible film but with no type of modified atmosphere. Each package contains several separated units with polyethylene films. A reduction of DON contamination was observed during the home-made frying process. This DON reduction seems to depend on the frying temperature. A major DON reduction was obtained when the fried covers reached the home-made colour at the minor of the tested temperatures.

Outputs

1 Standardized procedures for wheat and maize sampling during milling processes in the South Cone region.
2 Better knowledge of (DON) and Fumonisin variability in wheat and maize
3 Identification of the impact of different milling processes on the distribution of mycotoxin contamination in the different cereal fractions.

Participation in WP 4&5&6

We collaborated to undertake the objectives and work of the WP 4&5&6, collaborating with INTA, intervening in the mycotoxin analysis of the samples.

1 Reception of wheat samples, collected in the field
2 Conditioning
3 Extractions for analysis with HPLC, GC and TLC (as confirmation)
4 Determinations by HPLC (Fumonisins, Zearalenona and Aflatoxins) and TLC

(DON).
We collaborated also in WP 6 through dissemination conferences and presentation to farmers of a leaflet, in the La Pampa zone, and in the conference about mycotoxin risks (General Pico La Pampa, 1 December 2005).
We also collaborated in the evaluation of the results, discussing about and wrote posters and papers.

Training or Post graduate students

The following students participated in the activities of the project under supervision and/or presented their thesis:

Estela Motta, Faculty of Natural Sciences, University of Buenos Aires, Argentina. Participation in WP 3 and WP 2. PhD student under the joint supervision of partners 9 and 10. Topic: “Degradación de fumonisinas en la molienda húmeda de maíz y evaluación de la exposición por ocratoxina A”.

Emilia Ciancio Bovier, University of Lujan. Participation in WP 2. Topic: Ochratoxin A determination in blood and correlation to the donor diet in Argentina.

Collaboration with: Margarita Samar. Director: Dra.Silvia L. Resnik
which was develop at Departamento de Química Orgánica. Facultad de Ciencias Exactas y Naturales, U.B.A. Centro de Investigación en Micotoxinas-UNLu. Tesis presentada en Departamento de Industrias Facultad de Ciencias Exactas y Naturales UBA 2003.Tesis 3560 – http://www.opac.bl.fcen.uba.ar/ Grade: Outstanding (A).

Collaboration with: Leticia Broggi. Director: Dra. Silvia L. Resnik
"Study of natural fungi and mycotoxin contamination in cereals from Entre Ríos Province. Probability of contamination through industrial processes involved in the elaboration", which was develop at: Department of Industry. Departamento de Química Orgánica Facultad de Ciencias Exactas y Naturales, U.B.A. Universidad Nacional de Entre Ríos. Centro de Investigación en Micotoxinas-UNLu. Tesis presentada en Departamento de Industrias Facultad de Ciencias Exactas y Naturales.UBA, 2003. Tesis 3646 – http://www.opac.bl.fcen.uba.ar/ Grade: Outstanding (A).

Bioquímica Patricia Silvina Knass Tesista de Magíster en Tecnología de los Alimentos: Identificación de los puntos críticos para el control de Aflatoxinas, y Ocratoxinas en dos sistemas de producción de Porcinos en Argentina. Universidad Nacional de Misiones (a new research deriving from MYCOTOX project).

Microbiologist Lelis Meichtri, training in mycotoxins analysis. Universidad Nacional de Río Cuarto, Laboratorio Lazo, 30 y 31 de agosto 2006, Fundación ICTB de la Cruz, Luján.

Publications in peer-reviewed scientific journals

- Distribution of Deoxynivalenol in wheat, wheat flour, bran, and gluten, and variability associated with the test procedure. Samar M., Ferro Fontán C., Resnik S., Pacin A. and Castillo M. Journal of AOAC International 86(3); 551-556 (2003). ISSN: 1060-3271.

- Occurrence of ochratoxin A in wines in the Argentinean and Chilean markets. Pacin A., Resnik S., Vega M., Saelzer R., Ciancio Bovier E., Ríos G., Martínez N. ARKIVOC, (xii) 214-223, 2005. ISSN 1424-6376 http://www.arkat-usa.org.

- Deoxynivalenol reduction during the frying process of turnover pie covers. Samar M.M., Resnik S.L., González H.H.L., Pacin A.M. and Castillo M.D. Food Control (2007), 18 (10), 1295-1299.

- *Alternaria alternata* prevalence in cereal grains and soybean seeds from Entre Ríos, Argentina. Broggi L.E., González H.H.L., Resnik S.L. and Pacin A.M. Revista Iberoamericana de Micología, 2007, 24, 47-51.

- Natural occurrence of aflatoxins, deoxynivalenol, fumonisins and zearalenone in maize from Entre Ríos Province, Argentina
Broggi L.E., Pacin A.M., Gasparovic A., Sacchi C., Rothermel A., Gallay A. and Resnik S. Accepted for publication in Mycotoxin Research, 2007.

- Survey of Argentinean human plasma for ochratoxin A.
Pacin A.M., Ciancio Bovier E.V., Motta E., Resnik S.L, Villa D. & Olsen M. Submitted to Food Additives and Contaminants).

Oral presentations in conferences and congresses

- Mycotoxins in our countries. Resnik Silvia y Pacin Ana. Conferencia Magistral. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003. Conferencia.

- Reduction of fumonisins during the cleanliness of the maize. Round Table Redonda on Mycotoxins Decontamination Methods in Food. Pacin A., Taglieri D., Cano G. y Resnik S. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 -26 September 2003.

- Evaluación del riesgo a la ingesta de maíz contaminado por micotoxinas. Dra. Ana Pacin, Panelista. Panel sobre micotoxinas, con los siguientes co-disertantes: Dra. Sofía Chulze, Ing. Agr. Ana Di Giulio, Ing. Agr. Daniel Presello, Ing. Agr. Juan Rodríguez, Dra. Adriana Torres VIII Congreso Nacional de Maíz organizado por AIANBA (Asociación de Ingenieros Agrónomos de la zona Norte de la Provincia de Buenos Aires), Bolsa de Comercio de Rosario, Rosario, Santa Fe, 16-18 de Noviembre del 2005.

- Ochratoxin A occurrence and significance in human blood samples in South America. Ana Pacin, Conferencia. Advances in research on togigenic fungi and mycotoxins in

South America ensuring food and feed safety in a myco-globe context, Argentina 2006, Villa Carlos Paz-Córdoba, Argentina, 15-17 March 2006.

- Evaluación de los brotes epidémicos por tricotecenos en Argentina y Ururguay. (Evaluation of outbreaks of trichotechenes in Argentina and Uruguay). Resnik S.L., González H.H.L., Pacin A. Disertante: Silvia Resnik. V Congreso Latinoamericano de Micotoxicología, XII Encuentro Nacional de Micotoxinas - IV Simposio en Almacenaje Cualitativa de Granos del MERCOSUR. Florianópolis, Brasil, 19th June 2006.

Book Chapters

- Regulaciones nacionales e internacionales, perspectivas de la producción de cereales y alimentos a base de cereales en la provincia de Córdoba (National and international regulations, perspectives of the production of cereals and food based on cereals in the province of Cordoba). Pacin A., Resnik S. En: Micotoxinas: Impacto en la Producción y Salud Humana y Animal. (Mycotoxins: Impart on production and human and animal health) Ed. Héctor R. Rubinstein. ISBN 987-530-068-3. Chapter 1, pages 29-47 (2006).

- Identificación y cuantificación de micotoxinas en maíz cosechado en la provincia de Córdoba y en productos de molienda (Identification and quantification of mycotoxins in maize harvested in Cordoba province and in maize milling products). Resnik S., Pacin A., Funes G. En: Micotoxinas: Impacto en la Producción y Salud Humana y Animal (Mycotoxins: Impact on production and human and animal health). Ed. Héctor R. Rubinstein. ISBN 987-530-068-3. Chapter 8, pages 199-220 (2006).

- Detección de OTA en muestras biológicas provenientes de humanos (OTA's detection in human biological samples). Pacin A., Resnik S., Ciancio Bovier E. En: Micotoxinas: Impacto en la Producción y Salud Humana y Animal (Mycotoxins: Impact on production and human and animal health) Ed. Héctor R. Rubinstein. ISBN 987-530-068-3. Chapter 10, pages 255-269 (2006).

- Toxinas T-2 y HT-2. Silvia Resnik and Ana Pacin. Micotoxinas en alimentos (Mycotoxins in food.). Jose Miguel Soriano del Castillo (ed.), capítulo 15, pp. 293-312, Editorial Díaz de Santos, Madrid, España, (2007).

- Acido ciclopiazónico. Ana Pacin y Silvia Resnik. Micotoxinas en alimentos (Mycotoxins in food). Jose Miguel Soriano del Castillo (ed.), capítulo 18, pp. 335-356, Editorial Díaz de Santos, Madrid, España, (2007).

Posters presented in congresses

- Aflatoxinas en las fracciones obtenidas durante la limpieza del maíz (Aflatoxins in the fractions obtained during corn cleaning). Resnik Silvia L., Taglieri Daniela Cano Gabriela y Pacin Ana María. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003.

- Estudio preliminar sobre la contaminación por ocratoxina A en vinos argentinos

(Preliminary study on ochratoxin A contamination in Argentine wines). Pacin Ana María, Resnik Silvia L., Ciancio Emilia, Cano Gabriela y Taglieri Daniela. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003.

- Incidencia de la contaminación por aflatoxinas en maíz argentino, período 1995-2002 (Aflatoxins incidence in Argentine corn, period 1995-2002). Pacin Ana María, Cano Gabriela, Resnik Silvia L., Villa Daniel, Taglieri Daniela, y Ciancio Emilia. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003.

- Distribución de fumonisinas en el maíz y en las fracciones obtenidas durante la limpieza de maíz (Distribution of fumonisinas in the corn and in the fractions obtained during the cleaning of corn). Resnik Silvia L., Villa Daniel y Pacin Ana María. IV Congreso Latinoamericano de Micotoxicología. Seminario Anual Animal. La Habana, Cuba, 24 y 26 de Septiembre del 2003.

- Incidencia de la contaminación por AF en maíz (Aflatoxins ocurrence in maize). Pacin Ana M., Cano Gabriela, Resnik Silvia L., Villa Daniel, Taglieri Daniela. Jornadas Bonaerenses de Ciencia y Tecnología. La Plata, Pcia. de Buenos Aires, 17 de Diciembre de 2003.

- Contaminación por ocratoxina A en vinos argentinos (Ochratoxin A ocurrence in Argentine wines). Pacin Ana M, Resnik Silvia L, Ciancio Emilia, Cano Gabriela, Taglieri Daniela. Jornadas Bonaerenses de Ciencia y Tecnología. La Plata, Pcia. de Buenos Aires, 17 de Diciembre de 2003.

- Reducción de micotoxinas en maíz. Limpieza (Reduction of mycotoxins in maize. Cleaning). Resnik Silvia L, Taglieri Daniela, Cano Gabriela, Ciancio Emilia, Pacin Ana M. Jornadas Bonaerenses de Ciencia y Tecnología. La Plata, Pcia. de Buenos Aires, 17 de Diciembre de 2003.

- Muestreo de fumonisinas en maíz. Función de distribución (Maize sampling for fumonisins quantification. Distribution function). Resnik Silvia L, Villa Daniel, Pacin Ana M. Jornadas Bonaerenses de Ciencia y Tecnología. La Plata, Pcia. de Buenos Aires, 17 de Diciembre de 2003.

- Micoflora contaminante y presencia de tricotecenos tipo A y B en trigo cosechado en la provincia de Buenos Aires (Mycoflora and trichothecenes type A and B contamination in wheat harvested in Buenos Aires province). Pacin A.M., González H.H.L., Resnik S.L., Moltó G.A. y Masana M. X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías. Mar del Plata, Argentina, 18-20 de Mayo de 2005 (published in the proceedings, vol. III, 2006, pp. 936-942).

- Contaminación por fumonisinas en fracciones obtenidas en la molienda húmeda de maíz (Fumonisins contamination in wet milling corn fractions). Funes G.J., Taglieri D., Cano G., Pacin A. y Resnik S.L. X Congreso Argentino de Ciencia y Tecnología de

Alimentos y I Simposio Internacional de Nuevas Tecnologías. Mar del Plata, Argentina, 18-20 de Mayo de 2005 (published in the proceedings, vol. III, 2006, pp. 929-935).

- Estimación de la ingesta de alimentos en 210 donantes de sangre en la ciudad de Mar del Plata (Estimation of the food intake in 210 blood donors in the city of Mar del Plata). Motta E., Ciancio Bovier E., Pacin A., Resnik S.L. y Villa D. X Congreso Argentino de Ciencia y Tecnología de Alimentos y I Simposio Internacional de Nuevas Tecnologías. Mar del Plata, Argentina, 18-20 de Mayo de 2005 (published in the proceedings, vol. III, 2006, pp. 1197-1203).

- Micoflora contaminante y ocurrencia natural de micotoxinas en el maíz almacenado y los subproductos del proceso de industrialización por molienda seca (Mycoflora and natural occurrence of micotoxins in stored maize and the by-products of the dry milling process). Broggi Leticia E., Pacin Ana M., Gónzalez Héctor H.L., Resnik Silvia L., Cano Gabriela y Taglieri Daniela. II Jornadas de difusión de proyectos de investigación – extensión - UNER, INEX 2005, Concordia, Junio 2005.

- Aislamiento e identificación de la micoflora contaminante en cereales y oleaginosas: Un caso de estudio de en Soja cosechada en la República Argentina (Isolation and identification of the mycoflora in cereals and oilseeds: A case of study of in Soybean harvested in the Republic Argentina). Zelaya M., Gonzalez H.H.L., Resnik S. y Pacin A. Bienal de Ciencia y Tecnología 2005 de la Provincia de Buenos Aires, 8-10 de Noviembre, ciudad de La Plata.

- Food Intake Estimation In 236 Blood Donors In General Rodríguez, Buenos Province, Argentina. Castillo M.D., Ciancio Bovier E.V., Pacin A.M., Resnik S.L., Villa D. Conference: Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006. Villa Carlos Paz, Córdoba, Argentina.

- Ochratoxin A In Human Plasma In Buenos Aires Province, Argentina. Motta E., Ciancio Bovier E.V., Pacin A.M., Resnik S.L., Villa D. Conference: Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006. Villa Carlos Paz, Cordoba, Argentina.

- Contamination by Aflatoxins, Zearalenone and Deoxynivalenol in corn and the fractions obtained in the wet milling process. M.D. Castillo, A.M. Pacin, G.A. Molto, S.L. Resnik. Conference: Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 Marzo 2006. Villa Carlos Paz, Córdoba, Argentina.

- Insecticide effect on the mycoflora of soybean RR isolated from the Pampean region in Argentina. Frusteri L.M., Gonzalez H.H.L., Zelaya M.J., Resnik S.L., Pacin A.M., Martinez M.J. Conference: Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006, Villa Carlos Paz, Córdoba, Argentina.

- Micoflora contaminante y ocurrencia natural de micotoxinas en avena cosechada en la provincia de Entre Ríos, Argentina (Mycoflora and micotoxins natural occurrence in oats harvested in Entre Rios province, Argentina). Sacchi C.A., Broggi L.E, Resnik S.L., González H.H.L., y Pacin A.M. V Congreso Latino Americano De Micotoxicología - V Clam – XII Encuentro Nacional De Micotoxinas - Enm 2006 – IV Simposium De Almacenaje Cualitativo De Granos Del Mercosur - IV Sag-Mercosul. Florianópolis, SC, Brasil, 18-21 de Junio del 2006.

- Tricotecenos tipo A y B y micoflora contaminante asociada en el trigo cosechado en la provincia de Buenos Aires (Trichotechenes type A and B and mycoflora contaminant associated in the wheat harvested in Buenos Aires province). Frusteri L.M., Molto G.A., Pacin A., González H.H.L., Resnik S.L., y Masana M.O. Congreso Internacional de Ciencia y Tecnología de los Alimentos. Córdoba, 2006. 15-17 de Noviembre del 2006. pp. 232-233.

Dissemination conferences

- Evaluación de riesgo a la intoxicación por micotoxinas. Dra. Ana Pacin, Conferencia. Jornadas Interdisciplinarias de Toxicología Alimentaria auspiciadas por Asociación Argentina de Tecnólogos Alimentarios (AATA), Asociación Toxicológica Argentina (ATA), Universidad Argentina de la Empresa (UADE), UADE, Lima 717, Ciudad Autónoma de Buenos Aires, 9, 20-21 de Septiembre del 2005.

- Mycotoxins, a present enemy in the food. Resnik Silvia and Pacin Ana. The IVth Students National Congress of Biochemistry and Biotechnology. National university of the Litoral. On 1st October 2005. Santa Fé.

- Implicancias de las Micotoxinas en la Salud Animal y Humana. Ana Pacin, Conferencia. Mycotox Project INCO ICA4-CT-2002-10043, General Pico La Pampa, 1 de Diciembre del 2005.

- ¿Existe un diagnóstico sobre micotoxinas en soja en Argentina? Ana Pacin Disertación. Workshop “Calidad de la producción y granos con valor agregado” Mercosoja 2006, 3er Congreso de Soja del MERCOSUR Bolsa de Comercio de Rosario, Rosario, Santa Fe, Argentina, 27-30 de Junio del 2006.

Participation in scientific events

- First Meeting of Mycotox Project. “The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries”, ref ICA4-CT-2002-10043. 17-19 February 2003, Montevideo, Uruguay.

- Second Annual Meeting of Mycotox Project. “The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries”, ref ICA4-CT-2002-10043. 4-7 de Octubre de 2004, Montevideo, Uruguay.

- Reunión de Trabajo sobre Micotoxinas. 29 de Septiembre del 2004. Salón Carillo - SECyT, Buenos Aires, Argentina. Organizado por Agencia Nacional de Promoción Científica y Tecnológica.

- Taller de Micotoxinas y Pesticidas. Programas Estratégicos Nacionales. "Calidad y Seguridad alimentaria, contaminantes en alimentos". Buenos Aires, Argentina, 7 de Octubre del 2005.

- Third Annual Meeting of Mycotox Project. "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries", ref ICA4-CT-2002-10043. 13-14 de Marzo del 2006, Córdoba, Argentina. Reunión Villa Carlos Paz.

- Curso: Similitudes y diferencias entre las micotoxinas más conocidas. Profesor: Dra. Ana M. Pacin, Colaborador: Mg. Marcelo Castillo. Salón de Capacitación AATA, Ciudad Autónoma de Buenos Aires, 23-24 de Junio del 2005 (10 horas)

- Curso: Micotoxinas en Alimentos (Mycotoxins in Foods). Asociación Argentina de Tecnólogos en Alimentos. Fundación de Investigaciones Científicas Teresa Benedicta de la Cruz. Luján, Argentina, 15 de Junio del 2006. Profesoras: doctoras Ana M. Pacin y Silvia Resnik. Asistentes: Tec. Gabriela Cano, Tec. Daniela Taglieri y Bioq. Manuel Zelaya. (10 horas)

- Myco-Globe conference. Advances in research on togigenic fungi and mycotoxins in South America ensuring food and feed safety in a myco-globe context. 15-17 de Marzo del 2006. Villa Carlos Paz, Córdoba. Argentina.

- V Congreso Latino Americano De Micotoxicología - V Clam – XII Encuentro Nacional De Micotoxinas - Enm 2006 – IV Simposium De Almacenaje Cualitativo De Granos Del Mercosur - IV Sag-Mercosul. Florianópolis, SC, Brasil, 18-21 de Junio del 2006.

- Meeting with Dr Nadine Zakhia (general coordinator), Lujan, Argentina, 1st December 2006.

Future publications

- Trichothecene type A and B and related mycoflora in wheat harvested in Buenos Aires Province, Argentina. Gonzalez H.H.L., Molto G., Pacin A., Resnik S., Zelaya M., Masana M.

- Fumonisins behaviour on laboratory-scale corn wet milling process. Funes Gustavo J., Bello M.O., Resnik S.L., Pacin A.M., Cano Gabriela.

- Effect of industrial wet milling process on the distribution of aflatoxins, deoxynivalenol, fumonisin b_1 and b_2, and zearalenone in corn fractions. Funes Gustavo, Castillo Marcelo D., Molto Gustavo A., Pacin Ana M., Resnik Silvia L.

- Effect of maize cleaning by sieving before storage. Resnik S., Pacin A.

- Mycoflora and mycotoxin natural occurrence in oats from Entre Rios and Buenos Aires provinces, Argentina. Sacchi C., González H.H.L., Broggi L.E., Pacin A., Resnik S.L., Cano G. and Taglieri D.

Partner 11 – UCON DBND

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

2003 – 2006 ACTIVITIES

Year 2003

Participation in WP 1
Purchase material to perform analytical work was the first task; then the FAPAS samples were analyzed and Fumonisins analytical method started to be developed. Lack of accuracy in analytical results was the main problem presented in this point.

Participation in WP 2
Methodology for analysis of OTA in blood was implemented y validated. Three zones of the country were chosen to collect human blood samples; this task was part of a post graduate student, Katherine Muñoz S., as her Master Thesis.

Participation in WP 3
Identification of the mill to study distribution of mycotoxin during dry milling was done; according to it structure a sampling plan was designed, unfortunately two main problems made impossible get the objectives, no mycotoxin were found and changes in administration of the mill did not allow to apply the sampling plan. Wet milling was discarded because there not exist in Chile that kind of mill.

Participation in WP 4&5
Partner 11, UCON, supported to partner 7 giving assistance to do the analytical work of samples collected for the HACCP studies, Ricardo Villegas was incorporated to the HACCP team.

Year 2004

Participation in WP 1 and WP 3
In this period, methodologies for quantitative analysis of Ochratoxin A, Aflatoxins B_1, B_2, G_1, G_2, Zearalenone, Fumonisins B_1 and B_2 were implemented. The analytical methods for sample analysis involve different purification systems like Solid Phase Extraction columns and immune affinity columns. The analytical methods included:

- High Performance Liquid Chromatography
- High Performance thin Layer Chromatography
- Gas Chromatography with Electron Capture Detector (ECD)

During 2004 validation of DON analysis by HPTLC was completely finished.
In relation to WP 3, Collico Mill samples collected by Partner 7, INIA Chile, were analyzed; these samples were submitted to mycological analysis, many of these samples looked with rather low DON content, at ppb level. Part of these samples were sent to Argentina for confirmation, two of them resulted positives at a level under 0.3 ppm. Confirmation of these samples was performed in Sweden with HPLC–MS and finally all resulted negatives to any thrichotecene. This year a fusariosis problem appeared in other part of the country, INIA investigated the problem, took wheat samples, which ones were rapidly analyzed resulted with high DON content, about 5 ppm. In relation to distribution of DON in dry milling, no positive samples were found.

That was the reason that all studies according to WP 3 were taken in the Doctoral Thesis of Gisela Ríos in France, "Influence of grain structural properties and milling steps on the mycotoxin distribution in the grain and out coming fractions"

Participation in WP 2
In this period sampling analysis and confirmation of Ochratoxin A in 88 human plasma samples collected in the agricultural South-Center zone of Chile, was finished.

Participation in WP 4&5
Hazard analysis of mycotoxins (identification of cereal commodity/mycotoxin combinations that present an unacceptable risk to human health or present a constraint to trade. Determination at which step(s) in the commodity flow diagram the mycotoxin hazard enters or increases to an unacceptable level, requiring control).

Sampling and analysis of wheat in South Zone of Chile.
Period: 2002-2005
Nº samples: 199
Kind of sample: Wheat
Place of Collection: Collico Mill, Valdivia, Región de Los Lagos.
Mycotoxin: Deoxynivalenol

Crop	***Nº samples***	***DON***	***Range***
2002-2003	41	Neg.	---
2003-2004	30	Neg.	---
2003-2004*	40	Neg.	---
2004-2005	60	Neg.	---
2004-2005**	28 (+22)	0.052 ppm	0-0.090
Total	**199**		

* Samples collected from the transporting trucks at the entrance of the Mill
** Sample obtained from the Central zone. Lot stored in bad conditions with high content of humidity.

Years 2005-2006

Participation in WP 1
Implementation and validation of methodology for Fumonisins B_1 and B_2 analysis was implemented in a Pregraduate Thesis of a Chemistry and Pharmacy student (Carolina Sepúlveda) and this method was used for the analysis of samples collected in WP 4.
During 2006 the main activities of WP 1 were related with diffusion of results obtained with implementation and validation of different methods for mycotoxin analysis. The presentation of posters was made at scientific events at Florianopolis and Merida.
Between August 21-28, an interchanging of laboratory experience between UCON (Chile) and LATU (Uruguay) was held in the University of Concepcion. In this opportunity came to UCON, an interesting interchange of procedures for the mycotoxin analysis using HPTLC for DON and Aflatoxins and HPLC-FLD for OTA was produced. From Uruguay attended Jacqueline Cea and Chiemi Moriyama.

Participation in WP 2
Validation of different methodologies for the analysis of Ochratoxin A in cereals, wine and other matrices.

During 2006 alternative analytical methodologies were implemented and validated for the analysis of Ochratoxin A for different matrixes, wheat, oat, pork meat and blood.

Liquid-Liquid extraction of Ochratoxin A in cereals and based products.

10 g of sample are mixed with 5 phosphoric acid 0.1M and 60 ml of dichloromethane and filtered in a glass filter. 10 ml of the filtered are evaporated until dryness and reconstituted with 600 µl of Acetonitrile: water: acetic acid (41+58+1 v/v/v). Additionally the sample is cleaned with n-hexane. *(Reference: Solfrizzo M., Avantaggiato G., Visconti A., 1998. Use of various clean-up procedures for the analysis of ochratoxin A in cereals. Journal of chromatography A 815, 67-73).*

Immune affinity columns for the clean up of ochratoxin A in cereals and based products.

10 g of sample are extracted with a water and acetonitrile; 40+60 v/v solution. After filtering, 8 ml of the filtered are mixed with 88 ml of PBS and the mixture is passed through the column at a flow of 1 ml/min. The column is washed with 20 ml of milli-Q water. Ochratoxin A is eluted with 3 ml of a mixture of methanol + acetic acid 92+2 v/v. The sample is evaporated until dryness and reconstituted in 250 µl of acetonitrile: water: acetic acid (41+58+1 v/v/v). *(Reference: Beretta B., De Domenico R., Gaiaschi Ballabio C., Galli C.L., Gigliotti C., Restan P., 2002. Ochratoxin A in cereal-based baby foods: occurrence and safety evaluation. Food Additives and Contaminants 19 (1), 70-75).*

SPE for the analysis of ochratoxin A in wines.

An aliquot of 10 ml of wine is diluted with 5 ml of distilled milli-Q water. The mixture is passed through the C18 column, previously conditioned with 5 ml methanol followed of 5 ml of milli-Q water. The column is washed with 2 ml of water and 2 ml of a mixture of methanol +water 60+40 v/v and Ochratoxin A is eluted with 3 ml of methanol. The sample is evaporated until dryness and reconstituted in 250 µl of acetonitrile: water: acetic acid (41+58+1 v/v/v). *(Reference: Hernández M.J., García-Moreno M., Durán E., Guillén D., Barroso C., 2006. Validation of two analytical methods for the determination of Ochratoxin A by reversed-phased high-performance liquid chromatography coupled to fluorescence detector in musts and sweet wines from Andalucia. Analytica Chimica Acta 566, 117-121).*

Liquid-liquid extraction of Ochratoxin A from human plasma.

1 ml of the human plasma is mixed with 5 ml of $MgCl_2$ in HCl 0.05 M. Ochratoxin A is extracted with 3 ml of chloroform and 2 ml of the organic phase are evaporated until dryness. The extract is reconstituted in 250 µl of acetonitrile: water: acetic acid (41+58+1 v/v/v). *(Reference: Leibniz-Institute for Working Environment and Human Factors at the University of Dortmund IfADo).*

Ochratoxin A in pork muscle, kidney and liver. Liquid-liquid extraction.

A method using liquid-liquid extraction for the analysis of OTA in muscle, liver and kidney of pork, was implemented and validated. Once the method was validated, sampling of commercial pork products was done.

2.5 g of sample are extracted with 10 ml of ethyl acetate + phosphoric acid 99+1 v/v. The extract is filtered by a glass filter and 5 ml of the solution are reduced under a N_2 until a volume of 3 ml. 3 ml of $NaHCO_3$ 0.5 M are added to the organic phase and the organic phase is discarded. The aqueous phase is pH adjusted until 2.5. Ochratoxin A is re-extracted with 5 ml of ethyl acetate + phosphoric acid. The organic phase is evaporated until dryness and reconstituted with 250 µl of acetonitrile: water: acetic acid (41+58+1 v/v/v). (Reference: Monaci L., Tantillo G., Palmesano F., 2004. Determination of ochratoxin A in pig tisúes by liquid-liquid extraction and clean-up and high-performance liquid chromatography. Anal Bioanal Chem 378, pp. 1777-1782).

Results of this research:
Results of these analyses showed some degree of contamination with OTA that could explain the resulted obtained in human blood samples for WP 2.

Pork Product	***Place of sampling***	***OTA ppb***
Chops	Butcher shop	< LOD
Chops	Butcher shop	< LOD
Chops	Retail market	< LOD
Chops	Retail market	< LOD
Loin	Butcher shop	0.24
Loin	Butcher shop	0.17
Kidney	Butcher shop	0.10
Kidney	Butcher shop	0.11

Participation in WP 4&5

Sampling and Analysis of Flour.
Period: 2005-2006
Nº samples: 34
Kind of sample: Flour for human consumption
Place of collection: South Zone (Valdivia)
Central Zone (Pullehue-Curanipe)
South- Central Zone (Concepción)
Mycotoxin: Ochratoxin A (OTA)

Collection	***Nº of samples***	***Average OTA***	***Range***
Valdivia 2005	17 (15+)	0.200	
Curanipe 2006	12 (8+)	0.111	
Concepcion 2006	5	0.189	
Valdivia 2002 Wheat	60	Negative	Negative
Total flours	**34**		

Activities related to dissemination (participation in WP 6)

September-November 2005
Organisation of a training course for Food Inspectors of Concepcion and Talcahuano.
Title: “Actualization in technology, Control and Regulation on foods”.
This course counted with the participation of 40 inspectors and chiefs of departments of Health Service Concepcion and Health Service Talcahuano.
Thematic:

- Basic Guidelines on quality, sanitation and hygiene: BPA, BPM, HACCP, ISO 9000, ISO 14000, ISO 18000, POSS, etc.
- Importance of Mycotoxins for Human and Animal health.
- International and Local Regulations.
- Diffusion of MYCOTOX project.
- Diffusion of research performed on mycotoxins.
- Delivery of necessary lineaments for control and analysis of mycotoxins in foods both in local market and imported foods.

December 2005
Lecture organized by the University of Concepcion in the frame of The World Day for Alimentation. Lecturer: Dr. Ricardo Madariaga

April 2006, Chillán
International Seminary: “Calidad y Seguridad Alimentaria, una estrategia para competir en mercados exigentes”.
Seminary focused to researchers, enterprisers, farmers, authorities of trading, health, agriculture, cattle and students.
Lecturer: Mr. Ricardo Villegas F.
Title: “Sistemas de aseguramiento de calidad en cereales: ejemplo para la cadena de trigo en Latinoamerica”

18-21 June 2006, V Congreso Latinoamericano de Micotoxicología, Florianopolis, Brasil
Presentation of Posters with the results of research on mycotoxins obtained in the frame of MYCOTOX Project.
Mycotoxins and MICOTOX Project
Ricardo Villegas, Katherine Muñoz, Mario Vega, Ricardo Madariaga.
Faculty of Pharmacy.

- Cromatografía Planar (HPTLC) como herramienta para el estudio de producción de Ocratoxina A por algunas especies fúngicas.
- Estudio de hongos y Fumosina B_1 presentes en maíz para ensilaje y su relación con la altura de corte en el campo.
- HPTLC como herramienta para el estudio de producción de Ocratoxina A por algunas especies fúngicas en trigo, café y otros medios de cultivo.
- Deoxinivalenol en Chile. Estudio de 3 años.

Participation in project meetings and scientific events

- Project kick off meeting, Montevideo (Uruguay), 17-19 February 2003, Montevideo,

Uruguay.
- Project meeting of WP 4&5 in Buenos Aires (Argentina). 20-22 August 2003.
- IV Congreso Latinoamericano de Micotoxicología, La Habana (Cuba) 24-26 September 2003.
- Second annual meeting of MYCOTOX Project, 4-7 October 2004, Montevideo, Uruguay.
- COLACRO X, Campos de Jordao, Sao Paulo (Brazil) 20-23 October 2004.
- 27 Mycotoxin Workshop, 13-15 June 2005, Dortmund, Germany.
- V Congreso Latinoamericano de Micotoxicología, Florianópolis, Brazil
- COLACRO XI. Congreso Latino Americano de Cromatografía y Ciencias afines, Mérida, México.

Publications

2003
- Vega M, Saelzer R., Ríos G., Herlitz E., Bastías C., "Chile, micotoxinas, globalización. Las micotoxinas ¿Son un problema emergente?
- Corréa T., Vargas E., Cea J., Vega M., Resnik S., Souza M. L., Freitas-Silva O., Zakhia N., "Sistema de gerenciamento de la calidad para el control de micotoxinas en las cadenas de producción y procesamiento de cereales de los países del Cono Sur."

2004
- Vega H.M., Muñoz S.K., Ríos G., "Determinación de Ocratoxina A en sangre humana, estudio preliminar para estimar riesgo de exposición"
- Ríos G., Muñoz S.K., Vega H.M. "Ocratoxina A en Cereales, estudio de dos procedimientos de análisis."

2005
- Ochratoxin A in cereals, study of two procedures of analysis, (poster), Germany.
- Determination of Ochratoxin A in Human Blood. Preliminary Study to estimate risk exposure in Chile, (poster), Germany.
- Pacín Ana, Resnik Silvia, Vega Mario, Saelzer Roberto, Ciancio Bovier Emilia, Rios Gisela, Martinez Natalia. Occurrence of Ochratoxin A in wines in the Argentinian and Chilean markets ARKIVOC 2005 (xii) 214-223.

2006
- Katherine Muñoz, Mario Vega, Gísela Ríos, Sara Muñoz, Ricardo Madariaga. Preliminary study of Ochratoxin A in human plasma in agricultural zones of Chile and its relation to food consumption. Journal of Food and Chemical toxicology 44 (2006) 1884-1889.
- Deoxinivalenol en Chile, Estudio de tres años, (poster), Brasil.
- Estudio de hongos y Fumonisina B_1 y B_2 presentes en maíz para ensilaje y su relación con la altura de corte en el campo, (poster), Brasil.
- Producción de Ocratoxina A por algunas especies fúngicas en trigo, café y otros medios de cultivo, (poster), Brasil.
- Aplicación de la Cromatografía Planar para el estudio de producción de Ocratoxina A, por algunas especies fúngicas en trigo, café y otros medios de cultivo, (poster), Mexico.

Research lines originated from MYCOTOX project

- *Study of fungus population and Fumonisins present in maize for silage and it relationship with the height cut.*

Authors: Universidad de Concepción in collaboration with INIA Chile (partner 7)
Participants: Mario Vega, Ricardo Madariaga, Katherine Muñoz, Ernesto Jahn, Ricardo Villegas.
Publication in preparation:

- *Determination of Fumonisins and mould screening in maize for silage and its relation with the height cut.*

- *Validation of an Analytical Methodology for determination of Ochratoxin A in cereals and derivatives. (Pregraduate thesis in Chemical Pharmacy Carolina Sepúlveda).*

Paper in preparation to be published.

- *Validation of a methodology for determination of Ochratoxin A and it metabolites in pork's muscle, liver and kidney (Pregraduate thesis in Chemical Pharmacy Alejandra Opazo).*

Participants: Universidad de Concepción (Mario Vega H., Ricardo Villegas F., Katherine Muñoz S.).
Paper in preparation to be published.

Training

- Gisela Rios, University of Concepción, Chile (partner 11). Participation in WP 3.
PhD student in France under the supervision of partners 1 and 11. Topic: Study of the influence of grain structural properties and milling steps on the mycotoxin distribution in the grain and outcoming fractions

- Katherine Muñoz, University of Concepción, Chile (partner 11), PhD in Pharmaceutical Sciences degree. Participation in WP 2. Topic: OTA determination in human blood samples and relationships with the Chilean food diet.

- Carolina Sepúlveda, University of Concepción, Chile (partner 11), Pre-graduated student. Participation in WP 2. Topic: Implementation of an analytical technique for OTA determination in wheat and derived products.

- Alejandra Opazo, University of Concepción, Chile (partner 11), Pre-graduated student. Participation in WP 2. Topic: Validation of an analytical technique by liquid-liquid extraction for OTA determination in pork muscle, liver and kidney.

Partner 12 - LATU

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

TITLE OF PROJECT:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries.

Mycotoxin Department of Technological Laboratory of Uruguay participated in MYCOTOX Project in three workpackages: WP 1 and WP 4&5 as colaborator.
The work plan laid out at the first "Kick Off" Meeting in Montevideo (17-21 February 2003) was fully followed and all assigned tasks were completed for the whole project duration.

Participation in WP 1:

NIR Methodology
LATU has available for using an Infratec 1241 Grain Analyzer Foss Tecator (NIT). It would be extremely usefull to use it for determining mycotoxin.
When Dr. D. Bastianelli (CIRAD, partner 1) visited Mycotoxin Department at the project start, explained NIR/NIT theory and a joint work on evaluation of NIT methodology for Deoxynivalenol was planned and carried out.
Mycotoxin Department of LATU determinated DON by visual Thin Layer Chromatography (TLC) and High Performance Liquid Chromatography (HPLC) detection in 24 samples: 13 wheat flour, 6 wheat(whole grain powder), 3 bran and 2 by products.
The range of the values was 413 µg/kg-11322 µg/kg
Those samples were sent to Dr. Bastianelli with the purpose to start a NIR mycotoxin study.
The report done by Bastianelli concluded that more samples should be processed in order to demonstrate that the prediction model detects really a trace contamination.
Following its implementation and positive evaluation, LATU sent 48 wheat samples at different levels of DON content from those collected on field within WP 4&5 (see annex VII).

Protocols with the detail of infrastructure and analytical protocols for each mycotoxin were sent to coordinator during the period 2003- 2004. (Annex I).
After the changing of the WP 1 coordinator, WP 1 partners were asked to fulfill new questionnaires with analytical methods to be used during the WP 1 studies. The information requested was related to matrix, mycotoxins, and the quality assurance status of the analytical methods. (Annex II).

Quality documents
Procedure related to the preparation and determination of concentration of standards, for aflatoxins, zearalenone, ochratoxin A, ergot alkaloids as well as procedure related to performance parameters recovery percentage, detection limit, quantitation limit, precision were written by LATU and sent to WP 1 Coordinator and WP 1 members.

FAPAS reference materials were purchased for conducting the interlaboratory studies for Aflatoxin B_1, B_2, G_1, G_2 in maize T0446, Deoxynivalenol in wheat flour T2210, Zearalenone in maize T2209 and Fumonisin B_1 and B_2 in maize T 2208. Results of the studies were reported to coordinator. (Annex III). A specific questionnaire was asked to be completed. (Annex IV).

A WP 1 meeting was carried out at the IV Congreso Latinoamericano de Micotoxicología in La Habana Cuba (24-27 September 2003). All assigned tasks were

completed.

Laboratory for Quality Control and Food Safety, Ministry of Agriculture, Brasil showed not to have received the corresponding data sheet, so LATU was asked to send them the assigned value of each of the FAPAS reference materials received. (Annex V Table 1).

As LATU is a routine participant of FAPAS Programme, it was asked to send to the coordinator the statistical studies of each of the rounds in which we participated in the period 2002-2003 related to Aflatoxin B_1, B_2, G_1, G_2, Deoxynivalenol, Zearalenone and Fumonisin B_1 and B_2. This was sent by mail.

During the period 2003-2006 participation in all planned FAPAS tests, analyzing 32 sample test materials for: aflatoxin B_1, B_2, G_1, G_2, zearalenone, ochratoxin A, deoxynivalenol, aflatoxin M_1, and fumonisin B_1, B_2 was undertaken in order to maintaining the quality assurance of the results and the accreditation of the analytical methods. For all we obtained satisfactory results within the accepted z score values. (Annexes V&VI).

As part of the WP 1 tasks, it took place the participation in the interlaboratory comparison among WP 1 members .

TOXIN	SAMPLES
Aflatoxins	D, E, H, J
Zearalenone	I, K, L
Ochratoxin A	M,O,Q
Deoxynivalenol	N,P,R

All interlaboratory samples were analysed and results sent to coordinator, (annex VI). Results were reported completing the Analytical Work Questionnaire and the Proficiency Testing Results form. For sample R it was obtained z score of -2.15, in spite of obtaining a value of 0.01 for sample N that both are the same reference material. As corrective action, internal reference material was analysed. Values obtained were acceptable according the internal control chart parameters.
Similar situation is applicable to sample M and Q for which the z score obtained was -0.38.

Materials and reagents were purchased for conducting the interlaboratory studies of WP 1 and the analysis of WP 4&5 samples (immunoaffinity columns, TLC plates, HPLC consumables, glassware, solvents and reagents, fungus analysis reagents).

Validation of a deoxynivalenol HPLC method and comparison with visual TLC method.
Mycotoxin Department of LATU as national reference laboratory, developed a no expensive HPLC method for determining Deoxynivalenol (DON) in wheat, barley and by products. Results obtained by HPLC detection were compared to those obtained from

accredited to ISO17025 TLC method by United Kingdom Accreditation Service (UKAS). The extraction and clean-up was based on method 986.17AOAC 2000: chapter 49, and the HPLC detection based on J.AOAC 70(3), 1987:479-483, both with modifications. The performance parameters evaluated were those suggested in the AOAC International, 1998. Peer-Verified Methods: manual on Policies and Procedures.

Results obtained for performance parameters were acceptable and the method accepted for accreditation by ISO17025 by UKAS. Both methods allow the analyst to do the same extraction and clean-up procedure, with the possibility of undistinguish detection by TLC or HPLC, obtaining comparable results.

This method was included as reference method for the analysis of the wheat samples of WP 4&5.

Participation in WP 4&5

Analysis of wheat samples collected by the WP 4&5 team
DON analysis was performed in 87 samples of wheat provided by WP 4&5 team.
Study was performed by different analytical methods, TLC detection, HPLC detection, immunoaffinity clean up.
Results were reported by accredited methods. (Annex VII).

Study of clean up procedures using Charcoal-Alumina-Celite (7+5+3) Column; Immunoaffinity Column R-Biopharm Rhone and Strata X 33 mm polymeric sorbent Phenomenex Column were performed to determine Deoxynivalenol by HPLC was performed. Considering as reference analytical method the internal protocol PEC.TOX.063 accredited by United Kingdom Accreditation Service (UKAS) following the ISO 17025 requirements, and based on AOAC method 986.17 (chapter 49, 2002) for extraction and clean-up and on J.AOAC 70(3), 1987:479-483 for the high performance liquid chromatography (HPLC) detection, two more clean up methods were evaluated. In all of them PEC.TOX.063 detection procedure was carried out. A poster was presented on this work (Villa Carlos Paz, March 2006).

Fungus were also analysed in some of those samples. Two posters were presented on this work (Villa Carlos Paz, March 2006).

Analysis of wheat flour samples collected by WP 4&5 team
A survey of mycotoxin content in different wheat flour samples collected from the market was performed as part of the WP 4&5 surveillance. DON analysis was performed in 84 samples of wheat flour. Aflatoxin B_1, B_2, G_1 and G_2, zearalenone, ochratoxin A were determinated in 32 samples and ergotamine tartrate, ergosine, ergonovine maleate, ergocristine, ergokryptine and ergocornine were determinated in 30 samples. Results are showed in annex VIII.

These analysis were performed joint with internal quality wheat flour samples. Accuracy studies showed acceptable results analysing FAPAS material and internal samples. Precision studies were acceptable following the Horwitz equation.

Training activities and scientific exchanges with other partners

- A training on fungus was carried out at the University of Buenos Aires (partner 9) 8-19 November 2004. We thank Dr. Silvia Resnik for her kindness.

- A training activity at the Universidad de Concepción in Chile (partner 11) was carried out (21–25 August 2006). Exchange of knowledge related to different analitycal methods for aflatoxins B_1, B_2, G_1 and G_2, zearalenone, ochratoxin A and fumonisins was possible during the visit. It was extremely useful that the analyst involved in the project participated in this training method activity to discuss problems, to be trained "hand on" in the different methods of analysis with feedback on results and local laboratory conditions. We thank Dr Mario Vega for his kindness.

Participation in scientific events, meetings and produced publications

- A summary of the work performed related to the wheat study was presented in 2006 at the University of Science of Uruguay, Cammarota L.

One conference and three posters were presented as diffusion of MYCOTOX work at the Myco-Globe Conference: Advances in research on toxigenic fungi and mycotoxins in South American ensuring food and feed safety in a Myco-Globe context, 15-17 March 2006, Villa Carlos Paz, Argentina.

- Up-date on Worldwide Regulations for Mycotoxins. The MERCOSUR Harmonization of Limits on Mycotoxins with the International Regulations. Cea J. Myco-Globe Conference. Carlos Paz, Argentina. 2006.

- Study Of Clean Up Procedures Using Charcoal-Alumina-Celite Column, Immunoaffinity Column And Strata x Column To Determine Deoxynivalenol By High Performance Liquid Chromatography In Wheat. Cea J., Cammarota L. Myco-Globe Conference. Carlos Paz, Argentina, 2006.

- Relationship Between The Level Of Deoxynivalenol Contamination In Wheat And The Fungal Infection. Cea J., Martinez M. Myco-Globe Conference. Carlos Paz, Argentina, 2006.

- INCO-DEV MYCOTOX Project "The development of a food quality management system for the control of mycotoxins in cereal production and processing chains in Latin America South Cone countries" (ICA4-CT-2002-10043): Part II - Interlaboratory control. Vargas E.A., Castro L., Dos Santos E.A., França R.C., Cea J., Vega M., Freitas-Silva O. Myco-Globe Conference. Carlos Paz, Argentina, 2006.

One poster was presented as diffusion of MYCOTOX work at the V Congreso Latinoamericano de Micotoxicología y IV Simposio en Almacenaje de Granos, 18-21 June 2006, Florianópolis, Brasil.

- Interlaboratory Control among INCO-DEV MYCOTOX Project Laboratories. Vargas E.A., Castro L., Dos Santos E.A., França R., Cea J., Moriyama Ch., Vega M., Freitas-Silva O., Souza M.L. V Congreso Latinoamericano de Micotoxicología y IV Simposio en

Almacenaje de Granos. Florianópolis, Brasil.

- A poster was proposed and approved by the Scientific Committee of the IUPAC Symposium that will be held in May 2007 in Turkey. A HACCP Plan Along The Wheat Chain To Prevent Don In Wheat Flour In Uruguay. S. Stewart, G. Gutiérrez, J. Cea.

- An informal meeting (May 2003) was undertaken with the attendance of S. Stewart, G. Gutierrez, G. Henry and J. Cea. Report of ongoing WP4 research, details of the HACCP studies were arranged and future activities coordinated.

- Mycotoxin Department (LATU) surveillance data year 2002 related to Aflatoxin B_1, B_2, G_1, G_2, Deoxynivalenol, Zearalenone and Fumonisin B_1 and B_2 was sent to WP 4&5 coordinator.

- Assistance to the workshop La Aplicación de los Principios de HACCP en la Prevención y Control de Micotoxinas was undertaken as representant of Uruguay at the IV Congreso Latinoamericano de Micotoxicología. Presentation of a HAACP study for wheat flour was presented to be discussed and studied. Registration to the workshop was paid with FAO funds.

- A second informal meeting (October 2003) was undertaken with the attendance of S. Stewart, G. Gutierrez, and J. Cea to ensure exchange of information and completion of schedule and planned goals. Results of the HAACP study for wheat flour presented at the workshop La Aplicación de los Principios de HACCP en la Prevención y Control de Micotoxinas were shared.

- Other informal meetings were undertaken with the attendance of S. Stewart and J. Cea to evaluate the design survey, sample collection, number of samples and costs.
We agreed a special low cost for the analysis to be done as partner of the same project. 87 wheat samples were analysed for DON, and other 84 wheat flour samples to evaluate the impact of storage on different mycotoxin content in the final wheat flour.

- A joint paper will be prepared for publication evaluating the effect of crop rotation and the effect of harvesting at different moments over the content of DON.

Participation in project meetings (17-19 February 2003, 4-7 October 2004 and 13-14 March 2006). Meeting with the general coordinator (28 November 2006).

ANNEX I

PROTOCOLS OF THE INFRASTRUCTURE AND ANALYTICAL PROCEDURES

Laboratory: LATU
Sample: wheat and by products, maize and by products.
Analysis (mycotoxin): **Aflatoxin B_1, B_2, G_1, G_2**
Reference: PEC.TOX.053 based on J. AOAC 77(6), 1994:1512-1521
Sample weight: 1 kg
Subsample weight: 25 g
Extration procedure (aqueous slurry or not): not, shake with solvent
Clean-up: LC-Si SPE tubes 1000 mg.
Determination: HPLC
Equipments (Mills, HPLC, Fluorimeter, etc.):

Agitador orbital “shaker” and Vortex
Romer mill with sub sampler
Rotary evaporator
Protector flex
HPLC HP 1050
Column C18
Fluorescence detector
Oven HP 1100
Visiprep for columns

PROTOCOL OF THE INFRASTRUCTURE AND ANALYTICAL PROCEDURES

Laboratory: LATU
Sample: wheat and by products, maize and by products.
Analysis (mycotoxin): **Aflatoxin B_1, B_2, G_1, G_2**
Reference: PEC.TOX.002 based on method 970.45 AOAC 2000 chapter 49
Sample weight: 1 kg
Subsample weight: 25 g
Extration procedure (aqueous slurry or not):not, shake with solvent
Clean-up: liquid-liquid extraction
Determination: TLC
Equipments (Mills, HPLC, Fluorimeter, etc.):

Agitador orbital “shaker” and Vortex
Romer mill with sub sampler
Rotary evaporator
UV Cabinet
Lamps 254 nm and 366nm
Densitometer CamagII
Protector flex

PROTOCOL OF THE INFRASTRUCTURE AND ANALYTICAL PROCEDURES

Laboratory: LATU
Sample: wheat and by products, maize and by products.
Analysis (mycotoxin): **deoxynivalenol**
Reference: PEC.TOX.063 based on J.Assoc.off.Anal. Chem 70 (3), 1987:479-483.
Sample weight: 1 kg
Subsample weight: 25 g
Extration procedure (aqueous slurry or not): not, shake with solvent
Clean-up: SPE column, charcoal: celite, alumina
Determination: HPLC

Equipments (Mills, HPLC, Fluorimeter, etc.):

Agitador orbital “shaker” and Vortex
Romer mill with sub sampler
Rotary evaporator
Protector flex
Visiprep for columns
HPLC HP 1050
Column C18
UV detector
Oven HP 1100

PROTOCOL OF THE INFRASTRUCTURE AND ANALYTICAL PROCEDURES

Laboratory: LATU
Sample: wheat and by products, maize and by products.
Analysis (mycotoxin): **deoxynivalenol**
Reference: PEC.TOX.005 based on method 986.17 AOAC 2000 chapter 49
Sample weight: 1 kg
Subsample weight: 25 g
Extration procedure (aqueous slurry or not): not, shake with solvent
Clean-up: SPE column, charcoal: celite, alumina
Determination: TLC
Equipments (Mills, HPLC, Fluorimeter, etc.):

Agitador orbital “shaker” and Vortex
Romer mill with sub sampler
Rotary evaporator
UV Cabinet
Lamp 366 nm
Protector flex
Visiprep for columns

PROTOCOL OF THE INFRASTRUCTURE AND ANALYTICAL PROCEDURES

Laboratory: LATU
Sample: maize and by products.
Analysis (mycotoxin): **Fumonisin B_1 and B_2.**
Reference: PEC.TOX.050 based on J.AOAC 75(2), 1992:313-318, method 995.15 AOAC 2000: chapter 49, Food Addit. Contam. 13, 1996:823-832.
Sample weight: 1 kg
Subsample weight: 10 g
Extration procedure (aqueous slurry or not): not, shake with solvent
Clean-up: SAX SPE tubes 100 mg.
Determination: HPLC
Equipments (Mills, HPLC, Fluorimeter, etc.):

Agitador orbital “shaker” and Vortex
Romer mill with sub sampler
Rotary evaporator
Protector flex
HPLC HP 1050
Column C18
Fluorescence detector
Oven HP 1100
Visiprep for columns

PROTOCOL OF THE INFRASTRUCTURE AND ANALYTICAL PROCEDURES

Laboratory: LATU
Sample: wheat and by products, maize and by products.
Analysis (mycotoxin): **zearalenone**
Reference: PEC.TOX.002 based on method 970.45 AOAC 2000 chapter 49
Sample weight: 1 kg
Subsample weight: 25 grams
Extration procedure (aqueous slurry or not):not, shake with solvent
Clean-up: liquid-liquid extraction
Determination: TLC
Equipments (Mills, HPLC, Fluorimeter, etc.):

Agitador orbital “shaker” and Vortex
Romer mill with sub sampler
Rotary evaporator
UV Cabinet
Lamps 254 nm and 366nm
Densitometer CamagII
Protector flex

ANNEX II

TABLE WITH THE INFORMATION OF THE ANALYTICAL METHODS performed by LATU, completed and sent to the WP 1 coordinator.

MATRIZ / MICOTOXINAS / MÉTODOS UTILIZADOS PARA CADA PARTICIPANTE DO PROJETO MYCOTOX País: ____URUGUAY (LATU)

Matriz (milho ou trigo)	**Micotoxina**	**Método e referência* (extração/purificação/ detecção/quantificação)**	**Nível de implementação ****
Maìz y trigo	Aflatoxinas	Metanol/água 55:45 al 2.5% NaCl/ particiòn lìq-lìq./ TLC/ Fluorescencia	Validado Acreditado Participaciòn em FAPAS rounds Zscore<l2l
Maìz y trigo	Aflatoxinas	Metanol/água 85:15 l/columna sílica 1g./ TLC/Fluorescencia	Validado Acreditado Participaciòn em FAPAS rounds Zscore<l2l
Maìz y trigo	Aflatoxinas	Metanol/água 85:15 l/columna sílica 1g./ HPLC/ Fluorescencia	Validado Acreditado Participaciòn em FAPAS rounds Zscore<l2l
Maìz y trigo	Zearalenona	Metanol/água 55:45 al 2.5% NaCl/ particiòn lìq-lìq./ TLC/ Fluorescencia	Validado Acreditado Participaciòn em FAPAS rounds Zscore<l2l
Maìz y trigo	Fumonisinas	Metanol/água 3:1 l/columna SAX 500mg./ HPLC/ Fluorescencia	Validado Acreditado Participaciòn em FAPAS rounds Zscore<l2l
Maìz y trigo	Deoxinivalenol	Acetonitrilo/água 84/16/ columnaalùmina, carbòn activado, celite/ TLC/ fluorescencia	Validado Acreditado Participaciòn em FAPAS rounds Zscore<l2l
Maìz y trigo	Deoxinivalenol	Acetonitrilo/água 84/16/ columnaalùmina, carbòn activado, celite/ HPLC/ UV	Validado Acreditado Participaciòn em FAPAS rounds Zscore<l2l

*indicar o solvente de extração/método de purificação/método de detecção/método de quantificação, por exemplo: metanol:água, 7:1 v/v/coluna de imunoafinidade/Cromatografia líquida de alta eficiência/fluorescência)

**indicar se a metodologia está implementada ou em fase de implementação no laboratório, e se está validada inter ou intralaboratorialmente, resultados satisfatórios com as amostras de contaminação conhecida

Observações: ----------

ANNEX III

FAPAS REFERENCE MATERIALS RESULTS SENT TO COORDINATOR

Toxin/substrate/ Identification	**Assigned value µg/kg**	**Satisfactory range µg/kg**	**Our assigned value to FAPAS Round µg/kg**	**Z score***	**Our value reported to coordinator µg/kg**
Aflatoxin B_1, B_2, G_1, G_2 in maize T0446	B_1 6.78 B_2 1.66 Totals 8.66	B_1 3.8 -9.76 B_2 0.93 -2.39 Totals 4.85-12.46	7.3 2.1 9.4	0.3 1.2 0.4	5.5 1.6 7.1
Zearalenone in maize T2209	ZON 228	ZON 137-319	308.8	1.8	246.5
Fumonisin B_1 and B_2 in maize T 2208	FB_1 879.1 FB2 305.9	FB_1 432.1-1326.1 FB_2 150.4-461.3	958.6 ----	0.4 ----	420.3 447.1
Deoxynivalenol in wheat flour T2210**	DON 463	DON 297- 630	340	-1.4	340

*satisfactory $Z \leq 2$

ANNEX IV

REPORT OF FAPAS SAMPLES sent to WP 1 Coordinator following the instructions.

RESULTS of FAPAS SAMPLES (SURPLUS TEST MATERIAL)

Laboratory: _____LATU_____
Date: 25/10/2004 Sample code: T0446_____
Mycotoxin: **aflatoxin B_1, B_2**
Matrix: Maize_____ Date of analysis: _____/_____/_____
Analyst: _____ Signature: _____
Results:

Duplicate number/μg/kg	Mycotoxin	Result (μg/kg)	Recovery (%)	Result correct by recovery (μg/kg)	Assigned value (μg/kg)	Result evaluation
1/6.3	Afla B_1	5.5	94	5.9	6.78	Z<I2I
1/1.3	Afla B_2	1.6	89	1.8	1.66	Z<I2I

In case of non conformance, do critical analysis and describe the correction actions.
Observations: ----------

RESULTS of FAPAS SAMPLES (SURPLUS TEST MATERIAL)

Laboratory: _____LATU_____
Date: 25/10/2004 Sample code: T2209_____
Mycotoxin: **Zearalenone**
Matrix: Maize_____ Date of analysis: _____/_____/_____
Analyst: _____ Signature: _____
Results:

Duplicate number μg/kg	Mycotoxin	Result (μg/kg)	Recovery (%)	Result correct by recovery (μg/kg)	Assigned value (μg/kg)	Result evaluation
1/230	Zea	246.5	85	290	228	Z<I2I

In case of non conformance, do critical analysis and describe the correction actions.
Observations: ----------

RESULTS of FAPAS SAMPLES (SURPLUS TEST MATERIAL)

Laboratory: _____LATU_____
Date: 25/10/2004 Sample code: T2208_(MYCOTOX) _____
Mycotoxin: **Fumonisina B_1, B_2**
Matrix: Maize_____ Date of analysis: _____/_____/_____
Analyst: _____ Signature: _____
Results:

Duplicate number/μg/kg	Mycotoxin	Result (μg/kg)	Recovery (%)	Result correct by recovery (μg/kg)	Assigned value (μg/kg)	Result evaluation
1/473	FB1	420.3	94	447.1	879.1	Z<I2I
1/242	FB2	239.3	89	268.9	305.9	Z<I2I

In case of non conformance, do critical analysis and describe the correction actions.
Observations:

ANNEX V

TABLE 1: **FAPAS PROFICIENCY TEST PROGRAMME RESULTS YEAR 2003**

Toxin/substrate/ Identification	**Assigned value µg/kg**	**Satisfactory range µg/kg**	**Our assigned value to FAPAS Round µg/kg**	**Z score***
Aflatoxin B_1, B_2, G_1, G_2 in maize T0446	B_1 6.78 B_2 1.66 Totals 8.66	B_1 3.8 - 9.76 B_2 0.93 - 2.39 Totals 4.85 - 12.46	7.3 2.1 9.4	0.3 1.2 0.4
Zearalenone in maize T2209	228	137 - 319	308.8	1.8
Ochratoxin A in cereal T1721	6.61	3.70 - 9.52	6.7	0.1
Ochratoxin A in cereal T1724	23.39	13.10 - 33.69	16.5	-1.3
Fumonisin B_1 and B_2 in maize T 2208	FB_1 879.1 FB_2 305.9	FB_1 432.1 - 1326.1 FB_2 150.4 - 461.3	958.6 ----	0.4 ----
fumonisin B_1, B_2 in maize T2211	FB_1 650 FB_2 230	FB_1 319- 980 FB_2 113- 347	FB_1 512 FB_2 278	-0.8 0.8
Deoxynivalenol in wheat flour T2210	463	297 - 630	340	-1.4
Aflatoxin M_1 in milk T0461	0.09	0.05-0.13	<0.5	----

*satisfactory $Z \leq |2|$

TABLE 2: **FAPAS PROFICIENCY TEST PROGRAMME RESULTS YEAR 2004**

Toxin/substrate/Identification	**Assigned value µg/kg**	**Our assigned value to FAPAS Round µg/kg**	**Z score***
Aflatoxin B_1, B_2, G_1, G_2 in maize T0459	B_1 21.1 B_2 2.09 G_1 1.3 G_2 Not set Totals 25.2	B_1 14.1 B_2 2.5 G_1 <0.7 G_2 <0.7 Totals 16.6	-1.5 0.9 ----- ----- -1.5
Aflatoxin B_1, B_2, G_1, G_2 in maize T0463	B_1 3.47 B_2 0.74 G_1 1.36 G_2 1.91 Totals 7.36	B_1 3.6 B_2 <0.7 G_1 1.1 G_2 2.8 Totals 7.5	0.2 ---- -0.9 0.2 0.1
Zearalenone in maize T 2213	417.6	271	-1.9
Zearalenone in maize T 2216	277.0	200	-1.4
Ochratoxin A in barley T 1732	5.6	4.5	-0.9
Fumonisin B_1 and B_2 in maize T2215	FB_1 520.0 FB_2 230.1	FB_1 461 FB_2 253	-0.4 0.4
Deoxynivalenol in wheat flour T 2212	2531.7	2553	0.0
Deoxynivalenol in wheat flour T 2214	898.5	1133	1.6
Aflatoxin M_1 milk T0477	0.44	0.23	-2.2

*satisfactory $Z \leq |2|$

TABLE 3: **FAPAS REFERENCE MATERIALS RESULTS 2005**

Toxin/substrate/Identification	**Assigned value µg/kg**	**Our assigned value to FAPAS Round µg/kg**	**Z score***
Aflatoxin B_1, B_2, G_1, G_2 in animal feed T0470	B_1 11.8 B_2 2.93 G_1 3.85 G_2 1.08 Totals 19.66	B_1 8.4 B_2 3.5 G_1 3.8 G_2 <0.7 Totals 15.7	-1.3 0.9 -0.1 ---- -0.8
Aflatoxin B_1, B_2, G_1, G_2 in animal feed T0478	B_1 7.28 B_2 3.01 G_1 3.48 G_2 1.89 Totals 15.7	B_1 5.20 B_2 3.10 G_1 6.0 G_2 3.30 Totals 17.6	-1.3 0.1 3.3** 3.4** 0.5
Aflatoxin B_1, B_2, G_1, G_2 in maize T0473	B_1 4.66 B_2 1.46 G_1 2.13 G_2 0.43 Totals 9.0	B_1 3.7 B_2 1.4 G_1 1.9 G_2 <0.7 Totals 7.0	-1.0 -0.1 -0.4 ---- -1.0
Zearalenone in maize T 2219	157.8	135.0	-0.7
Ochratoxin A in cereal T 1737	24.5	24.4	0.0
Ochratoxin A in cereal T 1739	8.44	9.2	0.4
Fumonisin B_1 and B_2 in maize T2220	FB_1 1966 FB_2 696	FB_1 2598.9 FB_2 591.7253	1.3 -0.6
Deoxynivalenol in maize T 2218	984	996.7	0.1
Deoxynivalenol in maize T 2222	3434	3343	-0.2
Aflatoxin M1 milk T0472	0.069	<0.2	----

*satisfactory Z≤ I2I

**corrective action was implemented

TABLE 4: **FAPAS REFERENCE MATERIALS RESULTS 2006**

Toxin/substrate/Identification	**Assigned value µg/kg**	**Our assigned value to FAPAS Round µg/kg**	**Z score***
Aflatoxin B_1, B_2, G_1, G_2 in maize T0486	B_1 22.97 B_2 1.73 G_1 NA G_2 NA	B_1 17.50 B_2 1.61 G_1 ---- G_2 ----	-1.1 -0.3 -0.4 ----
Zearalenone in maize T 2225	274	178	-1.8
Ochratoxin A in cereal T 1748	8.03	3.0	-2.8
Deoxynivalenol in maize T 2226	640	426	-2.0
Aflatoxin M_1 milk T 0487	0.218	0.2	-0.4

NA no assigned value

*satisfactory Z≤I2I

ANNEX VI

RESULTS OBTAINED FROM THE PARTICIPATION IN THE INTERLABORATORY COMPARISON AMONG WP 1 MEMBERS

Toxin/substrate/ Identification	Assigned value by coordinator, µg/kg	Satisfactory range, µg/kg	Our value reported to coordinator, µg/kg	Z score*
Aflatoxin B_1, B_2, G_1, G_2 in maize. Sample D	B_1 <0,3 B_2 ---- G_1 ---- G_2 ----	B_1 ---- B_2 ---- G_1 ---- G_2 ----	B_1 <0.7 B_2 <0.7 G_1 <0.7 G_2 <0.7	B_1 ---- B_2 ---- G_1 ---- G_2 ----
Aflatoxin B_1, B_2, G_1, G_2 in maize . Sample E	B_1 18,8 B_2 0,8 G_1 ---- G_2 ----	B_1 10,5 - 27,1 B_2 0.45 - 1,15 G_1 ---- G_2 ----	B_1 20.1 B_2 1.04 G_1 <0.7 G_2 <0.7	B_1 0.30 B_2 1.39 G_1 ---- G_2 ----
Aflatoxin B_1, B_2, G_1, G_2 in maize. Sample H	B_1 3,4 B_2 ---- G_1 ---- G_2 ----	B_1 1,9 - 4,9 B_2 ---- G_1 ---- G_2 ----	B_1 2.39 B_2 <0.7 G_1 <0.7 G_2 <0.7	B_1 -1.35 B_2 ---- G_1 ---- G_2 ----
Aflatoxin B_1, B_2, G_1, G_2 in maize Sample J	B_1 18,6 B_2 ---- G_1 ---- G_2 ----	B_1not reported B_2 ---- G_1 ---- G_2 ----	B_1 14.51 B_2 1.1 G_1 <0.7 G_2 <0.7	B_1 -1.0 B_2 ---- G_1 ---- G_2 ----
Zearalenone in maize Sample I	52	29.3 – 74.9	<60	----
Zearalenone in maize Sample K FAPAS T2213	417.6	233.9 – 601.3	254.65	-1.77
Zearalenone in maize Sample L FAPAS T2213	417.6	233.9 – 601.3	332.6	-0.93
Ochratoxin A cereal Sample M	23.4	13.1 – 33.7	12.53	-2.11
Ochratoxin A maize Sample O	9.1	5.1 – 13.1	6.0	-1.54
Ochratoxin A maize Sample Q	23.4	13.1 – 33.7	21.4	-0.38
Deoxynivalenol in wheat flour Sample N	463	259.3 – 666.7	463.7	0.01
Deoxynivalenol in wheat flour Sample P	898.5	503.2 – 1293.8	967.0	0.35
Deoxynivalenol in wheat flour Sample R	463	259.3 – 666.7	207.6	-2.51

Reported results were corrected by recoveries.

*satisfactory $Z \leq |2|$

ANNEX VII

RESULTS OF WHEAT SAMPLES COLLECTED IN FIELD WITHIN WP 4&5

	PRODUCER 1			PRODUCER 2			PRODUCER 3			PRODUCER 4			PRODUCER 5		
MOMENT	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2	T0	T1	T2
REPLICATE 1	**A1A1**	**A1B1**	**A1C1**	**A2A1**	**A2B1**	**A2C1**	**A3A1**	**A3B1**	**A3C1**	**A4A1**	**A4B1**	**A4C1**	**A5A1**	**A5B1**	**A5C1**
µg/kg	7349	3481	2321	1379*	2022*	2321	24076	18954	17197	14215	12765	12038	5222	7223	11112
Aw	0.673	0.712	0.696	0.674	0.597	0.648	0.667	0.697	0.641	0.663	0.643	0.680	0.632	0.690	0.716
REPLICATE 2	**A1A2**	**A1B2**	**A1C2**	**A2A2**	**A2B2**	**A2C2**	**A3A2**	**A3B2**	**A3C2**	**A4A2**	**A4B2**	**A4C2**	**A5A2**	**A5B2**	**A5C2**
µg/kg	6382	4642	3481	1836*	2611	2611	28430	16578	15473	5581*	14350*	15527	8767	7223	11193
Aw	0.672	0.672	0.692	0.678	0.596	0.646	0.673	0.673	0.648	0.676	0.652	0.663	0.651	0.674	0.723
REPLICATE 3	**A1A3**	**A1B3**	**A1C3**	**A2A3**	**A2B3**	**A2C3**	**A3A3**	**A3B3**	**A3C3**	**A4A3**	**A4B3**	**A4C3**	**A5A3**	**A5B3**	**A5C3**
µg/kg	6819	4642	3481	3481	2214*	2321	23629	13170	17408	14900*	17152*	12259	7221	9025	4255
aw	0.647	0.672	0.702	0.627	0.657	0.644	0.666	0.644	0.691	0.670	0.653	0.666	0.640	0.689	0.689
REPLICATE 1	**B1A1**	**B1B1**	**B1C1**	**B2A1**	**B2B1**	**B2C1**	**B3A1**	**B3B1**	**B3C1**	**B4A1**	**B4B1**	**B4C1**	**B5A1**	**B5B1**	**B5C1**
µg/kg	6832	5802	3481	1741	6356*	3417	----	6440*	3610	8510	5944*	8991*	11690*	2385	6382
Aw	0.683	0.701	0.676	0.714	0.700	0.663	----	0.660	0.633	0.638	0.621	0.633	0.628	0.634	0.618
REPLICATE 2	**B1A2**	**B1B2**	**B1C2**	**B2A2**	**B2B2**	**B2C2**	**B3A2**	**B3B2**	**B3C2**	**B4A2**	**B4B2**	**B4C2**	**B5A2**	**B5B2**	**B5C2**
µg/kg	6834	10031	2331	3997	6382	5168*	8510	6447	4384	7349	----	6834	6382	2321	5157
Aw	0.686	0.712	0.723	0.687	0.652	0.660	0.642	0.625	0.643	0.644	----	0.636	0.635	0.632	0.611
REPLICATE 3	**B1A3**	**B1B3**	**B1C3**	**B2A3**	**B2B3**	**B2C3**	**B3A3**	**B3B3**	**B3C3**	**B4A3**	**B4B3**	**B4C3**	**B5A3**	**B5B3**	**B5C3**
µg/kg	8141	11604	2321	2321	3610	3675	10504	5028	3249	11604	9281	9477	6330*	10059*	----
aw	0.691	0.687	0.725	0.688	0.639	0.657	0.656	0.638	0.637	0.633	0.640	0.644	0.631	0.622	----

Deoxynivalenol: PEC.TOX. 005, based on method 986.17 AOAC 2002: chapter 49.
*Deoxinivalenol: PEC.TOX. 063 based on AOAC 2002:986.17 and
Assoc. off. Anal. Chem. 70(3), 1987:479-483.
Level of quantitation 29 µg/kg
*Level of quantitation 62 µg/kg PEC.TOX.063

ANNEX VIII

RESULTS OF MYCOTOXIN ANALYSIS PERFORMED IN WHEAT FLOUR SAMPLES

Mill	Brand	Sample	Aflatoxin B_1, B_2, G_1 and G_2* µg/kg each one	Zearalenone** µg/kg	Ochratoxin A*** µg/kg	1**** µg/kg	2**** µg/kg	3**** µg/kg	4**** µg/kg	5**** µg/kg	6***** µg/kg
Cañuelas	Fortin	Aguada - 1.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Cañuelas	Fortin	Cerro - 1.1.2	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Cañuelas	Fortin	Nueva Helvecia - 1.1.3	<0.7	<60	<2	interf	<0.9	<1.7	<1.0	<0.8	<0.9
Cañuelas	Demás	Libertador - 1.2.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Cañuelas	Demás	Sayago - 1.2.2	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Cañuelas	Demas 0000	Libertador - 1.3.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Cañuelas	Davoto	Sayago - 1.4.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Cañuelas	Leader Price/TI	Sayago - 1.5.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Cañuelas	Leader Price/TI	Carrasco - 1.5.2	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<9.0	<0.9
San José	San Telmo	Cerro - 2.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
San José	San Telmo	Mercado Agrícola - 2.1.2	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
San José	Flor	Mercado Agrícola - 2.2.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
San José	Flor	Sayago - 2.2.2	<0.7	<60	<2	---	---	---	---	---	---
San José	Condesa	Mercado Agrícola - 2.3.1	<0.7	<60	<2	<3.8	<0.9	<1.0	<1.0	<0.8	<10.6
San José	Cololo	Nueva Helvecia	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	interf	<0.9
San José	Adria	Libertador - 2.6.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
San José	Adria	Sayago - 2.6.2	<0.7	<60	<2	---	---	---	---	---	---
San José	La Especialista	Sierra - 2.7.1	<0.7	<60	<2	<0.3 ppb	<0.9	<1.0	<1.0	<0.8	<0.9
Young	Doña Blanca	Cerro 3.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.6	<0.8	<10.6
Florida	Aceguá	Cerro 4.1.1	<0.7	<60	<2	interf	<8.2	<1.0	7.7	<8.6	11.8
Puritas	Puritas	Nueva Helvecia	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Santa Rosa	Santa Unión	Uruguay 6.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<10.6
Santa Rosa	Cefa	Colonia 1 - 6.3.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Corberi	Bio	Uruguay - 7.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Molino X	Suelta	Pando - 8.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Molino Y	Suelta	Nueva Helvecia - 9.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	interf
IIHSA	Las Acacias	Sayago 10.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
IIHSA	Blanca (San Salvador)	Carrasco 10.1.2	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
IIHSA	Integral El equilibrio	Colonia 6 - 10.4.1	<0.7	<60	<2	<4.0	<0.9	<1.7	19	<9.0	<0.9
Frutigran	Integral Frutigran	Colonia 5 - 12.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Molino Z	Suelta	Tarariras 2 - 16.1.1	<0.7	<60	<2	interf	<0.9	<1.0	<1.0	<0.8	<0.9
Miroluce S.A	Molino del Litoral	Tarariras 3 - 17.1.1	<0.7	<60	<2	<4.0	<7.0	<1.7	<7.0	<1.4	<10.6

Mill	Brand	Sample	DON (µg/kg)*****
Cañuelas	Fortín	Aguada - 1.1.1	< 29
Cañuelas	Fortín	Cerro - 1.1.2	< 29
Cañuelas	Fortín	Nueva Helvecia - 1.1.3	< 29
Cañuelas	Demás	Libertador - 1.2.1	< 29
Cañuelas	Demás	Sayago - 1.2.2	< 29
Cañuelas	Demás	Colonia 1 - 1.2.3	<120
Cañuelas	Demas 0000	Libertador - 1.3.1	< 29
Cañuelas	Demas 0000	Colonia 2 - 1.3.3	<120
Cañuelas	Devoto	Sayago - 1.4.1	< 29
Cañuelas	Leader Price/TI	Sayago - 1.5.1	< 29
Cañuelas	Leader Price/TI	Carrasco - 1.5.2	< 29
San José	San Telmo	Cerro - 2.1.1	< 29
San José	San Telmo	Mercado Agrícola - 2.1.2	< 29
San José	San Telmo	Muestra Colonia 7	< 120
San José	San Telmo	Colonia 8 - 2.1.3	< 120
San José	Flor	Mercado Agrícola - 2.2.1	29
San José	Flor	Sayago - 2.2.2	< 29
San José	Flor	Colonia 3 - 2.2.3	< 120
San José	Condesa	Mercado Agrícola - 2.3.1	< 29
San José	Condesa	Colonia 7 - 2.3.2	< 120
San José	Condesa	Semillero 2 - 2.3.3	< 120
San José	Cololó	Nueva Helvecia	< 29
San José	Cololó	Muestra - 2.4.1	< 120
San José	Cololó	Sayago - 2.4.2	< 120
San José	Cololó	Colonia 3 - 2.4.3	< 120
San José	Cololó 000	Semillero 1 - 2.5.3	< 120
San José	Adria	Libertador - 2.6.1	< 29
San José	Adria	Sayago - 2.6.2	< 29
San José	Adria	Colonia 1 - 2.6.3	< 120
San José	La Especialista	Sierra - 2.7.1	29
San José	Primor	Colonia 8 - 2.8.1	< 120
San José	La Sibarita	Colonia 1 - 2.9.1	< 120
San José	La Sibarita	Colonia 2 - 2.9.2	< 120
San José	La Sibarita	Colonia 6 - 2.9.3	< 120
San José	La Ponderosa	Semillero 1 - 2.10.1	< 120
Young	Doña Blanca	Cerro 3.1.1	58
Florida	Acegua	Cerro 4.1.1	< 29
Puritas	Puritas	Nueva Helvecia	< 29
Puritas	Puritas	Rapenor - 5.1.2	< 120
Puritas	Puritas	Rapenor - 5.1.3	< 120
Puritas	Leu. Blancanieves	Colonia 1 - 5.2.1	< 120
Puritas	Leu. Blancanieves	Colonia 2 - 5.2.2	< 120
Puritas	Leu. Blancanieves	Colonia 6 - 5.2.3	< 120
Santa Rosa	Santa Unión	Uruguay 6.1.1	< 29
Santa Rosa	Blanca	Colonia 2 - 6.2.2	< 120
Santa Rosa	Blanca	Colonia 6 - 6.2.3	< 120
Santa Rosa	Cefa	Colonia 1 - 6.3.1	< 120

Santa Rosa	Cefa	Colonia 2 - 6.3.2	< 120
Santa Rosa	Cefa	Colonia 6 - 6.3.3	< 120
Corben	Bio	Uruguay - 7.1.1	< 29
Molino X	Suelta	Pando - 8.1.1	< 29
Molino X	Suelta	Pando	< 29
Molino Y	Suelta	Nueva Helvecia - 9.1.1	< 29
IHSA	Las Acacias	Sayago 10.1.1	< 29
IHSA	Blanca (San Salvador)	Carrasco 10.1.2	< 29
IHSA	Josefina 00	Colonia 1 - 10.2.1	< 120
IHSA	Josefina 00	Colonia 2 - 10.2.2	< 120
IHSA	Josefina 00	Colonia 3 - 10.2.3	< 120
IHSA	GC 00	Colonia 1 -10.3.1	< 120
IHSA	GC 00	Colonia 2 - 10.3.2	< 120
IHSA	GC 00	Colonia 3 - 10.3.3	< 120
IHSA	Integral El equilibrio	Colonia 6 - 10.4.1	< 120
Americano	Cañuelas	Colonia 3 - 11.1.1	< 120
Americano	Cañuelas	Colonia 4 - 11.1.2	< 120
Americano	Cañuelas	Colonia 5 - 11.1.3	< 120
Frutigran	Integral Frutigran	Colonia 5 - 12.1.1	< 120
Frutigran	Integral Frutigran	Tarariras 2 - 12.1.2	< 120
Frutigran	Integral Frutigran	Tarariras 4 - 12.1.3	< 120
Tidestar S.A	La Uruguaya	Semillero 1 - 13.1.1	< 120
Rio Uruguay	Mara 00	Tarariras 1 - 14.1.1	< 120
Rio Uruguay	Mara 00	Tarariras 2 -14.1.2	< 120
Rio Uruguay	Mara 00	Tarariras 4 -14.1.3	< 120
Rio Uruguay	Molino Rio Uruguay 000	Tarariras 2 - 14.2.1	< 120
Rio Uruguay	Molino Rio Uruguay 000	Tarariras 4 - 14.2.2	< 120
Rio Uruguay	Molino Rio Uruguay 000	Tarariras 7 - 14.2.3	< 120
Rio Uruguay	Maxi 000	Tarariras 2 - 14.3.1	< 120
Rio Uruguay	Maxi 000	Tarariras 4 - 14.3.2	< 120
Rio Uruguay	Maxi 000	Tarariras 6 - 14.3.3	< 120
Calprose	Calprose	Tarariras 1 - 15.1.1	< 120
Calprose	Calprose	Tarariras 2 - 15.1.2	< 120
Calprose	Calprose	Tarariras 5 - 15.1.3	< 120
Molino Z	Suelta	Tarariras 2 - 16.1.1	< 120
Molino Z	Suelta	Tarariras 2 - 16.1.2	< 120
Miroluce S.A	Molino del Litoral	Tarariras 3 - 17.1.1	< 120

*, **, ***Aflatoxins B_1, B_2, G_1 y G_2: PEC.TOX. 002 based on method 970.45 AOAC 2005: chapter 49.

***Detection limit-------0.7 μg/kg each one**

****Detection limit-------60 μg/kg**

*****Detection limit------2 μg/kg**

****Ergot Alkaolids: PEC.TOX.061 based on Retention of Ergot Alkaloids in Wheat During Processing, J.E.Fajardo, J.E.Dexter, M.M.Roscoe, T.W.Nowicki: Cereal Chem.72 (3), 1995: 291-298.

1 Ergonovine maleate **Detection limit 0.3 μg/kg; quantification level 3.8 μg/kg**
2 Ergosine **Detection limit 0.9 μg/kg; quantification level 7.0 μg/kg**
3 Ergotamine tartrate **Detection limit 1.0 μg/kg; quantification limit 1.7 μg/kg**
4 Ergocornine mesilate **Detection limit 1.0 μg/kg; quantification limit 1.6 μg/kg; quantification level 7.0 μg/kg**

5 Ergocriptine **Detection limit 0.8 μg/kg; quantification limit 1.4 μg/kg**
6 Ergocristine **Detection limit 0.9 μg/kg; quantification level 10.6 μg/kg**

*****Deoxynivalenol): PEC.TOX. 005 based on method 986.17 AOAC 2005: chapter 49
Quantification level: 29 μg/kg
***Detection limit: 20 μg/kg**

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INCO: International Scientific Cooperation Projects (1998-2002)

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

Title:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries

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Final Other relevant information

- Links were made with the European Cluster on Mycotoxins at the start of the project, with the help of Dr Monica Olsen, scientific advisor of the MYCOTOX project and member of the European Cluster. The general coordinator participated in the Third European Mycotoxin Cluster Workshop, 2-4 June 2003, Uppsala, Sweden and presented a dissemination poster on the MYCOTOX project.

- Links were made with the French Cluster on Mycotoxins through the RARE Fusariotoxin project funded by the French Ministry for Research. Some members of this consortium were also members of the European Cluster on Mycotoxins. This opened the way to scientific collaborations with French institutions or laboratories and logistic support in some specific areas (e.g. the use of a pilot milling plant, the share of analytical techniques, the infrastructure for welcoming the Chilean PhD student (Ms Gisela Ríos, partner 11) in 2004 and information exchanges on the application of HACCP method throughout various commodity agrichains in the Northern countries).

- A scientific collaboration started in 2004 (and continued beyond the project's end) with the French National Institute for Agricultural Research INRA (Institut National de Recherche Agronomique) through the joint supervision (INRA and CIRAD partner 1) of the Chilean PhD, Ms Gisela Ríos, coming from the University of Concepción, Chile (partner 11) (within the framework of WP 3). The collaboration was done with INRA Montpellier (for technological aspects), INRA Bordeaux (for molecular biology tools) and INRA Rennes (for wheat resistance to *Fusarium* infection).

- Links were made with the CEREFER project ("*Meeting Consumer Requirements for Cereal-Based Fermented Foods with Improved Nutritional and Sanitary Quality and Shelf Life in Africa*") funded by the European Commission through the FP5 INCO-DEV Programme. The CEREFER project organised a conference on small-scale producing units of traditional fermented foods, held in Jaén (Spain), 6-8 September 2004 (http://www.ujaen.es/huesped/foodsafe/). The general coordinator of MYCOTOX project (ICA4-CT-2002-10043) was member of the Conference Scientific Committee and gave an oral presentation on mycotoxins in fermented foods.

- Links were made with the MYCO-GLOBE project *("Integration of Mycotoxin and Toxigenic Fungi Research for Food Safety in Global System")* funded by the European Commission (FP6, Specific Support Action). The MYCOTOX general coordinator acted as a member of the steering committee of MYCO-GLOBE. She also participated in the Launch Conference (22 October 2004, Brussels, Belgium) and in the first international conference of this project (*"Reducing Impact of Mycotoxins in Tropical Agriculture with Emphasis on Health and Trade in Africa"),* held in Accra, Ghana, 13-16 September 2005.

In order to foster the relationships between the MYCOTOX and MYCO-GLOBE projects, all MYCOTOX consortium actively participated in the second international conference *("Advances in Research on Toxigenic Fungi and Mycotoxins in South America Ensuring*

Food and Feed Safety in a Myco-Globe Context") organised by the MYCO-GLOBE project and held in Villa Carlos Paz (Cordóba), Argentina, 15-17 March 2006. Many oral presentations and posters were provided by MYCOTOX partners. The EC officer for MYCOTOX, Dr Maria João Fernandes, participated in this event. For optimising financial resources, the MYCOTOX consortium took advantage of this scientific event and organised its annual meeting two days before the conference (13-14 March 2006).

The MYCOTOX general coordinator also participated in the international conference of MYCO-GLOBE *("Advances on Genomics, Biodiversity and Rapid Systems for Detection of Toxigenic Fungi and Mycotoxin")* held in Monopoli (Bari), Italy, 26-29 September 2006.

- The MYCOTOX general coordinator acted as an interface for initiating contacts between some partners of the MYCOTOX consortium and a French enterprise (ECCLOR S.A.) interested in collaborations in South America upon cereal storage and OTA management within the silos.

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Meetings

During the whole project duration, several meetings were held in order to ensure good and fluid communication, information exchange and discussion of the advances. Details on these meetings are given in the section “Management” of this final report. Meetings minutes were annexed to the previously delivered annual meetings.

All meetings performed during the whole project are listed below:

1. Kick-off (First Annual) meeting held in Montevideo (Uruguay), 17-19 February 2003.

2. Mid-year progress meeting specific for WP 4&5 held in Buenos Aires (Argentina), 20 22 August 2003.

3. Meetings for WP 1, 3, 4&5 held in la Habana (Cuba), 22-26 September 2003.

4. Internal regional workshop on the “Formulation of socio economic approach, methods and instruments”, held in Campinas (Brazil), 1-2 December 2003.

5. Second Annual Meeting held in Montevideo (Uruguay), 4-7October 2004.

6. Specific meeting of the socio-economists involved in the project, Mar de Plata, (Argentina), 4-5 November 2004.

7. *Other informal meetings were also held among the socio-economists* involved in the project during participation in regional scientific events and conferences (12-15 October 2004 in Montevideo (Uruguay), 27-29 July 2005 in Ribeirão Preto (Brazil), 15-17 March 2006 in Villa Carlos Paz (Argentina) and 10-13 June 2006 in Buenos Aires (Argentina).

8. *Regular visits* were done by the general coordinator to the project’s pilot sites and meetings were then held with the consortium members, and the associate institutions and stakeholders involved in the project: Argentina (August 2003, November 2005), Uruguay (October 2004), Brazil (February 2005), and Chile (December 2005).

9. *Regional meetings* were regularly held between the two CIRAD (partner 1) scientists (Catherine Brabet and Guy Henry) respectively outposted in Brazil and Argentina with the partners in the 4 South Cone countries (2003, 2004, 2005 and 2006).

10. *Specific meetings* were regularly held among the partners (from “analytical” WP 1&2&3 and “field” WP 4&5&6) in each Southern Cone country, according to the needs of each Work Package advances and activities.

11. *A final trip was done by the general coordinator to the Southern Cone* by the end of the project (23 November to 3 December 2006) in order to discuss the last outputs, final reporting and further valorisation through scientific papers and conferences. Meetings were held in Belo Horizonte (Brazil), Buenos Aires and Luján (Argentina), Santiago (Chile) and Colonia (Uruguay).

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Papers and publications

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Papers and publications

Publications in peer-reviewed scientifics journals

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Occurrence of ochratoxin A in wines in the Argentinian and Chilean markets

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This manuscript is dedicated to our valued colleague Prof. Rosa Lederkremer

Abstract

Wine contaminated with ochratoxin A (OTA) has been reported all over the world. Sixty-eight wine samples were analysed to assess OTA wine contamination in various regions of Argentina and Chile. In addition, some imported wines were analysed. Wine samples were collected at manufacturers' stock and retail markets in Argentina in 2003 and Chile in 2002. A high-performance liquid chromatographic method with fluorescence detection and two different immunoaffinity clean-up columns were employed, with recoveries higher than 90% (Argentina: LOD:0.008 µg/l, LOQ:0.015 µg/l; Chile: LOD:0.012 µg/l, LOQ: 0.04 µg/l). None of the analysed wines produced in Argentina or Chile were contaminated. The presence of OTA in wines would appear to be a lesser problem in Argentina and Chile than in other countries, but it still could contribute to OTA exposure of human populations and more studies of the occurrence of OTA in wine should be done.

Keywords: Mycotoxins, ochratoxin A, wines, immunoaffinity clean-up, high-performance liquid chromatographic method

Introduction

Ochratoxin A (OTA) is a mycotoxin produced by some species of the genera *Aspergillus* and *Penicillium*, and can contaminate a wide variety of foods.[1,2] According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA),[3] *OTA is produced by a single Penicillium species, P. verrucosum, by Aspergillus ochraceus and several related Aspergillus species, and by*

A. carbonarius, with small percentage of isolates of the closely related A. niger. OTA has been found in cereals and derived products (e.g. beer), legumes and pulses. It can also appear in other commodities such as coffee, cacao, nuts, spices, dried fruit, wine, etc., and in animal-derived products.[4]

Wine contamination with OTA has been reported all over the world.[3,5-15] However, no similar study has been performed on Argentinian and Chilean wines. The Codex Alimentarius Commission reported that wine is the second most important source of human exposure to OTA following cereals, giving a total dietary intake of 15%.[16]

Because OTA is known to have toxicological effects in humans and animals, such as nephrotoxic, immunotoxic, genotoxic and carcinogenic effects,[4,17,18] several countries have specific regulations for OTA content in a variety of commodities at levels ranging from 1 to 50 μg/kg for foods.

The JEFCA met in Geneva on February 6-15, 2001,[3] where it retained the previously established provisional tolerable weekly intake of 100 ng/kg body weight, pending the results of ongoing studies on nephrotoxicity and carcinogenicity mechanisms, and recommended a further review of OTA during 2004. The Commission of the European Community considered that *"it would be prudent to reduce exposure to ochratoxin A as much as possible, ensuring that exposures are towards the lower end of the range of tolerable daily intakes of 1.2–1.4 ng/kg b.w"*.[19] Recently, the European Commission proposed a maximal limit for OTA in wine of 2 μg/l.

The increased awareness of the potential risk for consumer health due to OTA exposure through wine consumption requires each country to carry out systematic measurements of OTA levels of the wines offered in the domestic market.

Because red wines tended to have higher OTA concentration than white wines,[15,16,20,21] the aim of this work was to obtain a preliminary overview of OTA contamination in red wines consumed in Chile and Argentina.

Results and Discussion

None of the red wines produced in Argentina or Chile analysed by us presented contamination. Other authors found two out of seven Argentinian red wines analysed with OTA at 0.028 and 0.042 μg/l; and two out of five Chileans wines contaminated with 0.028 and 0.07 μg/l.[15] Soleas et al.[11] reported that five out of 17 Argentinian and eight out of 42 Chilean red wines were contaminated at levels below 0.05 μg/litter.

On the other hand, Da Rocha et al.[22] showed that only 8 out of 48 isolates of *Aspergillus niger* produced OTA in the range of 32 to 77 μg/g, and none of the other *Aspergillus* species isolates from Argentinian grapes were OTA producers. Magnoli et al.[23] studied OTA production by 63 species of *Aspergillus* section *Nigri* isolated from wine grapes in Argentina. *A. niger* var. *niger* (19 strains out of 44), *A. niger* var. *awamori* (5 strains out of 15), and *A. faetidus* (1

strains out of 4), were OTA producers in the range of 0.002 µg/l to 0.045 µg/l. Both studies used YES medium at 30ºC during 10 days to test toxigenic capacity.[22,23]

With the goal of confirming the presence of OTA in wine, we also analysed imported wines from European regions in which contamination had been found.[6,8,13,14] Figure 1 shows the chromatograms obtained from an imported wine sample naturally contaminated with OTA (a) and the OTA methyl ester derivative (b).

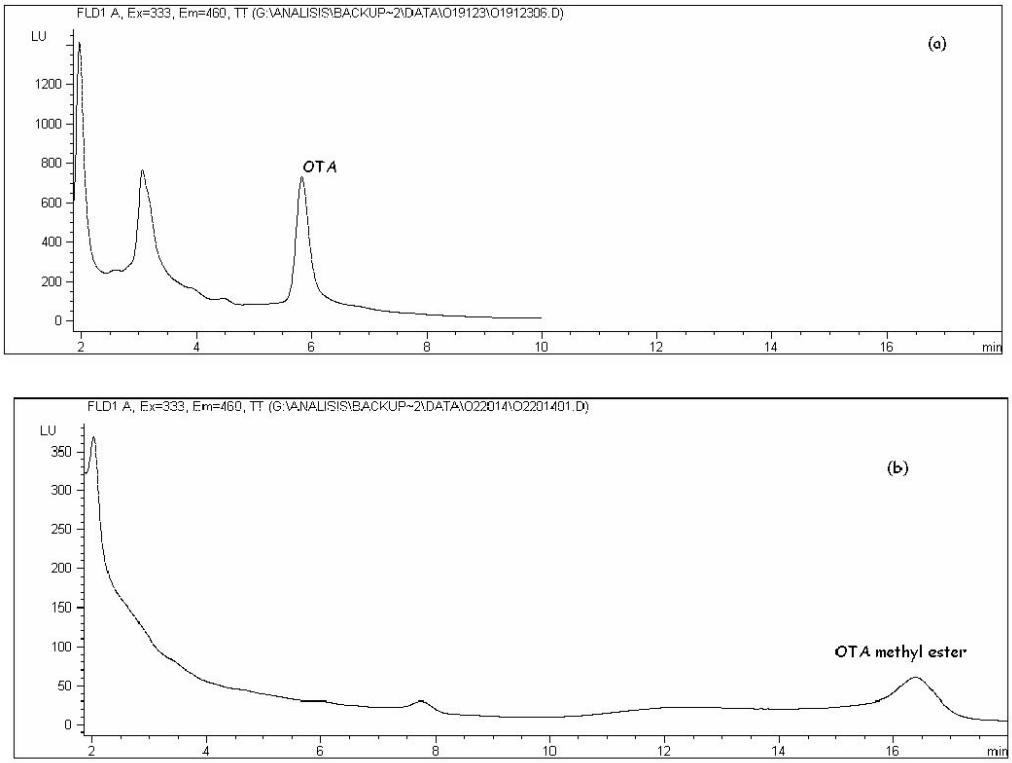

Figure 1. Chromatogram of: a) dessert wine sample with 1.32 µg/l OTA; b) OTA methyl ester derivative of the dessert wine.

Figure 2 shows the wine consumption trend from 1985 to 2001 for Argentina, Chile and the average of 15 European countries.

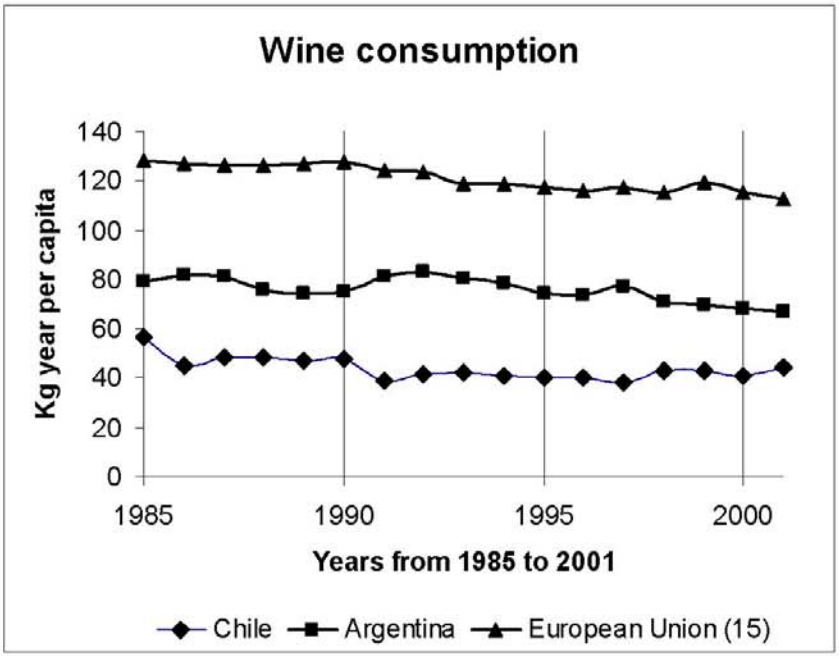

Figure 2. Wine consumption in Argentina, Chile and the European Union (15 countries).

The mean intake for those years was 121.34 kg year per capita for European countries, 76.06 kg year per capita for Argentina and 44.03 kg year per capita for Chile.[24] Taking into account the wine consumption in both South American countries, and based on the present results, it seems possible that wine intake is not an important OTA for the Argentinian and Chilean populations, in comparison with European people.

Although the number of imported wine samples analysed was limited, our results showed that OTA contaminations in European red wines was in the range of the SCOOP study.[20] From those countries which provided discriminated information to the SCOOP study, the mean concentration in red wine was 0.17 µg/l, and the mean level found in the sixteen European red wines analysed by us was 0.0315 µg/l. The Argentinian population may be more exposed than the Chilean population due to the consumption of imported wine, and because the average wine intake in Argentina is higher than in Chile (Figure 2).

Conclusions

This paper presents a preliminary report on OTA contamination of wines from Chile and Argentina. The presence of OTA in wines would appear to be a lesser problem than other countries, but it still contributes to OTA exposure. The results of this study confirm the

importance of continuing research in this direction, not only because of the serious human health concerns related to OTA exposure, but also due to the fact that both these countries export wines.

Experimental Section

General Procedures. Samples. Eighty-four samples of red wines were bought in manufacturers' stock and retail markets in the Argentinian cities of Luján and Buenos Aires (54 domestic and 16 imported wines) in 2003, and in the Chilean city of Concepción (14) in 2002. Table 1 shows details for all the South American wine samples. Information regarding the origin of the commercial samples was obtained from the bottle labels.

Table 1. Red wine samples for Argentina and Chile

Winery N°	Year Crop	Grape variety	Origin	
			Country	Region
1	1996	Merlot	Argentina	Mendoza
1	1999	Merlot	Argentina	Mendoza
1	2000	Cabernet Sauvignon, Merlot, Malbec	Argentina	Mendoza
1	2000	Malbec	Argentina	Mendoza
1	2000	Barbera	Argentina	Mendoza
1	2000	Cabernet Sauvignon	Argentina	Mendoza
1	2001	Unknown	Argentina	Mendoza
2	2000	Syrah	Argentina	Mendoza
2	2000	Cabernet Sauvignon	Argentina	Mendoza
2	2000	Merlot	Argentina	Mendoza
2	2000	Malbec	Argentina	Mendoza
2	2000	Cabernet Sauvignon, Merlot, Malbec	Argentina	Mendoza
2	2002	Unknown	Argentina	Mendoza
2	2002	Malbec	Argentina	Mendoza
2	2002	Burgundy	Argentina	Mendoza
3	2000	Syrah	Argentina	Mendoza
4	2000	Merlot	Argentina	Mendoza
4	2000	Syrah	Argentina	Mendoza
4	2001	Malbec	Argentina	Mendoza
4	2002	Pinot Noir	Argentina	Mendoza

Table 1. (Continued)

4	2002	Unknown	Argentina	Mendoza
4	2002	Unknown	Argentina	Mendoza
4	2002	Cabernet Sauvignon	Argentina	Mendoza
5	2000	Cabernet Sauvignon	Argentina	Mendoza
6	1999	Cabernet Sauvignon	Argentina	Mendoza
6	1999	Cabernet Sauvignon	Argentina	Mendoza
6	1999	Malbec	Argentina	Mendoza
6	2000	Malbec	Argentina	Mendoza
6	2002	Unknown	Argentina	Mendoza
6	2002	Unknown	Argentina	Mendoza
6	2002	Burgundy	Argentina	Mendoza
6	2002	Unknown	Argentina	Mendoza
7	2002	Cabernet Sauvignon	Argentina	San Juan
7	2002	Merlot	Argentina	San Juan
7	2002	Unknown	Argentina	San Juan
8	1998	Unknown	Argentina	Mendoza
8	1998	Sangiovese Merlot Malbec	Argentina	Mendoza
8	2001	Malbec	Argentina	Mendoza
9	2001	Burgundy Bonarda-Malbec	Argentina	Mendoza
9	2002	Unknown	Argentina	Mendoza
9	2002	Cabernet Sauvignon	Argentina	Mendoza
10	2000	Cabernet Sauvignon	Argentina	Mendoza
10	2001	Malbec	Argentina	Mendoza
10	2001	Malbec-Cabernet Sauvignon	Argentina	Mendoza
10	2001	Unknown	Argentina	Mendoza
11	2000	Merlot	Argentina	Patagonia
11	2000	Malbec	Argentina	Patagonia
11	2002	Unknown	Argentina	Patagonia
12	2002	Cabernet Sauvignon	Argentina	San Juan
12	2002	Unknown	Argentina	San Juan
13	2002	Merlot-Cabernet Sauvignon-Malbec	Argentina	Mendoza
13	2002	Unknown	Argentina	Mendoza
14	2002	Borgoña	Argentina	Mendoza

Table 1. (Continued)

14	2002	Cabernet Sauvignon-Malbec	Argentina	Mendoza
15	1999	Cabernet-Carmenère	Chile	Maipo Valley
16	1998	Cabernet Sauvignon	Chile	Itata Valley
17	1998	Cabernet Sauvignon	Chile	Rapel Valley
18	2000	Cabernet Sauvignon	Chile	Limarí Valley
19	2000	Merlot	Chile	Limarí Valley
20	2000	Cabernet Sauvignon	Chile	Maule Valley
21	2000	Merlot	Chile	Curicó Valley
21	2000	Cabernet Sauvignon	Chile	Curicó Valley
22	2000	Pinot	Chile	Pirque
23	1999	Cabernet Sauvignon	Chile	Lontué Valley
23	1999	Malbec	Chile	Lontué Valley
24	1999	Cabernet Sauvignon	Chile	Maipo Valley
25	1999	Merlot	Chile	Rapel Valley
26	2000	Cabernet Sauvignon	Chile	Colchagua Valley

Analysis for ochratoxin A. OTA was purchased from Sigma-Aldrich (USA). The standard solutions were made in benzene:acetic acid (99:1) according to the established concentration using a UV spectrophotometer at 333 nm (molar absorptivity: 5500). The required quantity was evaporated to dryness and dissolved in the mobile phase as indicated under chromatographic conditions.

Clean-up. Two different immunoaffinity clean-up columns were used in Argentina and Chile. Both procedures are briefly summarized.

Argentina. The extraction and quantification were based on Castellari et al.[25] with minor modifications. The column (Ochraprep, Rhône Diagnostics Technologies) was placed on an SPE vacuum manifold (Baker), and was first washed with 5 ml of PBS before use (PBS: dissolve 7.02 g NaCl, 0.201 g KCl, 1.14 g Na_2HPO_4, 0.26 g NaH_2PO_4, and 0.5 g NaN_3 in 1 l HPLC-grade water; adjust pH to 7.4).[26] Then 10 ml of wine, adjusted to pH 7.8 using 1 M NaOH, was diluted with 10 ml of PBS and introduced into the column at a flow-rate of about 1-2 drops per second. The eluted extract was introduced once more into the column. The column was successively washed with 10 ml of PBS and 10 ml HPLC-grade water at a flow-rate of about 3-4 drops per second and dried with air. OTA was then slowly eluted, using back-flushing three times in each fraction (4.5 ml and three 1.5 ml fractions), from the column with HPLC-grade MeOH at a flow-rate of about 1 drop per second. The eluted extract was evaporated into a silanised glass vial under vacuum at 40°C and the residue was re-dissolved in 250 µl of mobile phase.

Chile. The extraction and quantification were based on Visconti et al.[7] with minor modifications.

A 10-ml wine sample was diluted with 10 ml of water containing PEG 6000 (1%) and $NaHCO_3$ (5%), mixed and filtered through a Whatman GF/A glass micro-fibre filter. A 5 ml aliquot of diluted extract was cleaned up through an Ochratest immunoaffinity column (Vicam) at a flow-rate of about 1 drop per second. The column was washed with 5 ml solution containing NaCl (2.5%) and $NaHCO_3$ (0.5%), followed by 5 ml distilled water at a flow-rate of 1-2 drops per second.

The OTA was eluted with MeOH (2 ml) into a glass vial. The eluted extract was evaporated under a nitrogen stream and re-dissolved in 250 µl HPLC mobile phase.

Apparatus and Chromatographic conditions. OTA (Figure 3) detection was achieved at 333 nm excitation, 460 nm emission wavelength for both countries. Injection volume of the samples was 100 µl. A calibration curve was established by injecting six standard solutions with OTA concentrations ranging from: 0.026 to 2.65 µg/l (R^2: 0.9997) and 0.1 to 10 µg/l (R^2: 0.9977) in Argentina and Chile[27], respectively. Recovery experiments were performed in both laboratories on OA-free wines samples spiked with different OA levels. A mean recovery of OA was greater than 90 %[27]. Results were not corrected for recovery.

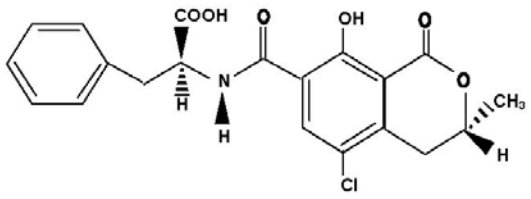

Figure 3. Chemical structure of Ochratoxin A.

Detection limits of the methods employed were 0.008 µg/l and 0.012 µg/l, and quantification limits were 0.015 µg/l in and 0.04 µg/l in Argentina and Chile[27], respectively.

Two different sets of equipment were used in both countries.

Argentina. Hewlett-Packard 1100 model equipped with fluorescence detector and Hypersil (125 x 4 mm) column packed with 5 µm BDS C-18 and Lichrocart guard column, packed with 5 µm RP-18. The computer program used for chromatographic analysis was Chemstation A.08.03.

The system was operated at 40°C, with a mobile phase consisting of MeCN: water:AcOH (85:114:1 v/v/v) at a rate of 1 ml/min. The retention time of OTA was approximately 5.8 min.

Chile. Merck-Hitachi MODEL with fluorescence detector and Waters 746 Integrator and a Waters Symmetry RP-18 (150 x 3.9 mm, 5 µm) column and Symmetry guard column C-18 (3,9 x 20 mm, 0.5 µm).

The mobile phase was MeCN: water:AcOH (99:99:2 v/v/v) at a rate of 1 ml/min. The retention time of OTA was approximately 6.2 min.

Confirmation of OTA by derivatisation as the methyl ester. OTA standard solutions and samples were derivatised by forming the methyl ester of the mycotoxin. Slight modifications

were made to the Grosso et al. procedure.[28] Briefly, an aliquot of the MeOH elution phase from the immunoaffinity column was evaporated to dryness and re-suspended in 120 µl of a 12% MeOH solution of BF_3 (Baker C701-07). After heating for 15 min at 60 ºC, the derivative was analysed by HPLC using the same chromatographic conditions as for ochratoxin A. Retention time of the OTA methyl ester (Figure 4) was 16.3 min. Detection and quantification limits expressed as OTA were 0.017 µg/l and 0.028 µg/l, respectively.

Acknowledgements

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References and Footnotes

1. Pitt, J. *Appl. Environ. Microbiol.* **1987,** *53*, 266.
2. Bucheli, P.; Taniwaki, M.H. *Food Addit. Contam.* **2002,** *19*, 655.
3. JECFA. Safety evaluation of certain mycotoxins in food, prepared by the Fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives 2001, Series 47, 410.
4. Krogh, P. In: *Mycotoxins in Food*; Krogh, P. Ed.; Academic Press, 1987; p. 97.
5. Zimmerli, B.; Dick, R. *Food Addit. Contam.* **1996**, *13*, 655.
6. Burdaspal, P.A.; Legarda, T.M. *Alimentaria* **1999**, *299*, 107.
7. Visconti, A.; Pascale, M.; Centonze, G. *J. Chromatogr. A*, **1999**, *864*, 89.
8. Otteneder, H.; Majerus, P. *Food Addit. Contam.* **2000,** *17*, 793.
9. Filali, A.; Ouammi, L.; Betbeder, A.M.; Baudrimont, I.; Soulaymani Benayada, R.A.; Creppy E.E. *Food Addit. Contam.* **2001,** *18*, 565.
10. Pietri, A.; Bertuzzi, T.; Pallaroni, L.; Piva, G. *Food Addit. Contam.* **2001,** *18*, 647.
11. Soleas, G.J.; Yan, J.; Goldberg, D.M. *J. Agric. Food Chem.* **2001,** *49*, 2733.
12. Cabañes, F.J.; Accensi, F.; Bragulat, M.R.; Abarca, M.L.; Castella, G.; Minguez, S.; Pons, A. *Int. J. Food Microbiol.* **2002**, *79*, 213.
13. Lopez de Cerainy, A.; González-Peña, E.; Jimenez, A.M.; Bello, J. *Food Addit. Contam.* **2002,** *19*, 408.
14. Shephard, G.S.; Fabiani, A.; Stockenström, S.; Mshicieleli, N.; Sewram, V. *J. Agric. Food Chem.* **2003,** *51*, 1102.

15. Rosa, C.A.R.; Magnoli, C.E.; Fraga, M.E.; Dalcero, A.M.; Santana, D.M.N. *Food Addit. Contam.* **2004,** *21*, 358.
16. Codex Alimentarius Commission, *Position paper on Ochratoxin A.* CX/FAX 99/14, 1998.
17. IARC Monographs on the Evaluation of Carcinogenic Risks to Humans **1993,** *56* (Lyon: IARC), 489.
18. Dirheimer, G. *Rev. Med. Vet.* **1998,** *149*, 605.
19. Commission of the European Community. Commission of the European Community, Scientific Committee on Food Opinion on Ochratoxin A, CS/CNTM/ MYC/14 Final, Annex II to Document XXIV/2210/98 (Brussels), 1998.
20. SCOOP. Assessment of dietary intake of Ochratoxin A by the population of EU Member States Task 3.2.7, 2002. *europa.eu.int/comm/food/fs/scoop/3.2.7_en.pdf*
21. Siantar, D.P.; Halverson, C.A.; Kirmiz, C.; Peterson, G.F.; Hill, N.R.; Dugar, S.M. *Am. J. Enol. Vitic.* **2003,** *54*, 170.
22. Da Rocha Rosa, C.; Palacios, V.; Combina, M.; Fraga, M.E.; De Olivera Rekson, A.; Magnoli, C.E.; Dalcero, A. *Food Addit. Contam.* **2002,** *19*, 408.
23. Magnoli, C.; Violante, M.; Combina, M.; Palacio, G.; Dalcero, A. *Lett. Appl. Microbiol.* **2003,** *37*, 1.
24. FAO, Food Balance Sheet http://faostat.fao.org/faostat/form?collection=FBS&Domain=FBS&servlet=1&hasbulk=&version=ext&language=EN
25. Castellari, M.; Fabbri, S.; Fabiani, A.; Amati, A.; Galassi S. *J. Chromatogr. A* **2000,** *888*, 129.
26. Scott, P.M.; Kanhere, S.R.; Lau, B.P.Y.; Lewis, D.A.; Hayward, S.; Ryan, J.J.; Kuiper-Goodman, T. *Food Addit. Contam.* **1998,** *15*, 555.
27. Saelzer, R.; Vega, M.; Retamal, A.; Ríos, G.; Herlitz, E. *Noticias Técnicas del laboratorio, NTL* 2002**,** *2*, 6 & 9.
28. Grosso, F.; Saïd, S.; Mabrouk, I.; Fremy, J.M.; Castegnaro, M.; Jemmali, M.; Dragacci, S. *Food Chem. Toxicol.* **2003,** *41*, 1133.

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FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

Title:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries

ANNEXES

Papers and publications

Oral presentations in conferences and congress

ORGANIZACIÓN DE LAS NACIONES UNIDAS PARA LA AGRICULTURA Y LA ALIMENTACIÓN

ORGANIZACIÓN MUNDIAL DE LA SALUD

Documento de Sala 36
Español solamente

Conferencia Regional FAO/OMS sobre Inocuidad de los Alimentos para las Américas y el Caribe

San José, Costa Rica, 6-9 de diciembre de 2005

LA PREVENCIÓN DE RIESGOS EN LAS CADENAS AGROALIMENTARIAS

(Preparado por el Instituto Nacional de Tecnología Agropecuaria (INTA), dependiente de la Secretaria de Agricultura, Ganadería, Pesca y Alimentos de Argentina)

Marco Institucional

La posibilidad de demostrar la inocuidad de las producciones agropecuarias a través de una evaluación riesgos es fundamental para que no se vea afectada la salud y la nutrición de la población, así como para asegurar el acceso a los mercados internacionales**.** En este sentido el Instituto Nacional de Tecnología Agropecuaria (INTA); como organismo descentralizado de investigación tecnológica dependiente de la Secretaría de Agricultura Ganadería Pesca y Alimentos de la Nación Argentina; a través de su Plan Estratégico Institucional **(2)** implementado en Programas Nacionales que integran las cadenas productivas y Áreas Estratégicas de Investigación por disciplinas ha establecido la necesidad de dirigir acciones coordinadas respecto de la evaluación del riesgos de contaminación biológica o química, ya que es un tema común que atraviesa la mayor parte de las cadenas agroalimentarias (CAA) a las que el INTA apoya. En la situación actual se ha determinado que no existe un diagnóstico suficientemente adecuado sobre los puntos críticos donde se incorporan las contaminaciones, derivadas de acciones del hombre o naturales, en la producción agroalimentaria. También se ha establecido que existe una necesidad de implementar acciones preventivas efectivas del riesgo biológico y químico para las distintas cadenas y regiones productivas. Estas acciones preventivas debieran enmarcarse progresivamente dentro de un sistema de gestión de la seguridad alimentaria tal como el Análisis de Peligros y Puntos Críticos de Control (APPCC).

Peligros y situaciones de riesgo en las cadenas agroalimentarias

La lista de peligros de contaminantes potenciales en las CAA es muy extensa y creciente de acuerdo a la incorporación de nueva información**,** sin embargo hay problemas prioritarios a los que nos referiremos brevemente a continuación. En lo respectivo a la contaminación biológica de las cadenas agroalimentarias la misma incluye tanto la contaminación fúngica como la bacteriana y la parasitaria. La primera a través de la presencia de hongos en la producción primaria puede ocasionar diversos tipos de daños, tales como, disminución de la germinación, decoloración, calentamiento, marchitamiento, pudrición de granos además de la eventual aparición de micotoxinas. Estas toxinas tienen efectos perjudiciales para la salud, por su toxicidad y económicos; debido a que disminuyen la disponibilidad de alimentos proteicos, por la morbi-mortalidad de animales de producción y por otra parte, ocasionan pérdidas derivadas de la comercialización de materias prima **(4).** En cuanto a los peligros de enfermedades bacterianas transmitidas por los alimentos resalta la presencia potencial del grupo de *Escherichia coli* productor de toxina Shiga -STEC, cuyo prototipo es *E. coli* O157:H7. Este grupo de microorganismos es capaz de producir distintos cuadros clínicos cuya forma más severa es el Síndrome Urémico Hemolítico (SUH). En Argentina se cuenta con laboratorios de referencia a nivel regional en la detección y caracterización de este grupo de patógenos en clínica humana lo cual ha permitido avanzar en la epidemiología y caracterización de cepas STEC **(7).**

Por otra parte la preponderancia detectada en países centrales del serotipo O157:H7 en brotes y casos asociados con el consumo de alimentos ha llevado a que diversas agencias de control de la seguridad alimentaria enfocaran sus esfuerzos de control en alimentos en la detección de este serotipo principalmente **(11).**

En este sentido Argentina ha aportado investigaciones respecto de la incidencia y caracterización de cepas STEC en el ganado bovino en pie. En uno de estos trabajos **(6)** se recuperaron cepas de STEC de un 39% de los 200 animales estudiados, en ese mismo estudio la prevalencia del serotipo O157:H7 fue del 0.5%. En esta línea, el INTA ha iniciado estudios más exhaustivos para la evaluación del riesgo de *Escherichia coli* productor de toxina Shiga en la cadena de la carne bovina. El desarrollo de estudios respecto de la prevalencia de este grupo de bacterias, en los distintos pasos de la producción, procesamiento y comercialización de la carne, es una necesidad imperiosa para la evaluación de riesgos de este peligro, lo que también facilitará la implementación de medidas efectivas de control y vigilancia diseñadas en el marco de un plan APPCC.

En lo referido a los contaminantes químicos existe el consenso que la contaminación con plaguicidas y micotoxinas se convierte en preocupante dado que ciertos países de destino pueden utilizar su presencia en embarques como barrera para-arancelaria, o como legítimo requisito de inocuidad **(9)**. Las acciones que está desarrollando INTA conjuntamente con otros organismos de Ciencia y Técnica **(10)** pretenden seleccionar algunas de las grandes cadenas agroalimentarias con el objeto de analizar el valor de las producciones, el consumo nacional, la exportación y especular sobre el "costo de la no seguridad alimentaria" visto desde los riesgos en salud pública y su efecto en la comercialización. Para el análisis de riesgo se utilizará la información toxicológica disponible, las normativas en el ámbito nacional e internacional, los hábitos de consumo, la caracterización epidemiológica de los productos, la utilización de agroquímicos, ambientes y tecnologías de poscosecha y procesamiento en el caso de productos alimenticios elaborados. La implementación del Programa se realiza mediante Proyectos Integrados de Impacto Regional ó Nacional que priorizarán en una primera etapa aquellos productos o subproductos de mayor relevancia en el consumo interno y en la exportación y de mayor riesgo de contaminación

Desafíos y Herramientas para la gestión sanitaria de los agroalimentos

Dada la naturaleza de la contaminación química y microbiológica a lo largo de las CAA los desafíos a superar en la evaluación de riesgos, y en la posterior prevención y control, son variados y complejos, por lo que se requiere un enfoque integrado y multidisciplinario. Un buen ejemplo de ello es el control de las micotoxinas en las cadenas productoras de cereales, donde es necesaria la colaboración de, entre otros, fitopatólogos, especialistas en post cosecha, diseño de maquinaria agrícola, microbiólogos, y tecnólogos alimentarios para alcanzar una adecuada evaluación de la influencia de las prácticas agroindustriales en el riesgo sanitario **(4).** Otro de los desafíos encontrados es la carencia de metodología analítica rápida y precisa para relevar la contaminación con residuos químicos y microbiológicos en las distintas etapas de la CAA. Es también un desafío la profundización, integración y sistematización de toda la información que se genera respecto de la influencia de las prácticas productivas e industriales en el riesgo sanitario a lo largo de la CAA. Dichas acciones de integración debieran darse en el marco de sistemas de gestión como el APPCC y de la implementación previa o simultánea de Buenas Prácticas de Agrícolas y de Manufactura (BPA, BPM) ya que los mismos son capaces de integrar las CAA desde la materia prima hasta el producto final con un enfoque sistematizado de la seguridad alimentaria.

Para la mejora de estos sistemas de gestión de la seguridad alimentaria es ineludible el desarrollo de instrumentos de anticipación conocidos como modelos predictivos aplicables en distintos puntos de la CAA. Así los modelos predictivos aplicados a la respuesta de los microorganismos frente a factores ecológicos de los alimentos, denominada Microbiología Predictiva, son una herramienta reconocida para el desarrollo de los estudios de evaluación de riesgo y APPCC **(1, 12).** Sintéticamente la microbiología predictiva se basa en el desarrollo de modelos matemáticos que predicen la respuesta microbiana:

crecimiento, inactivación / supervivencia, o producción de toxina, bajo un conjunto limitado de factores tales como temperatura, pH y actividad de agua **(5).** La capacidad de predecir el crecimiento o la muerte de un microorganismo bajo condiciones que no fueron experimentalmente ensayadas, constituye una ventaja con respecto a los estudios de desafío los que implican la realización de múltiples ensayos con base estadística, lo que además de costoso en tiempo y dinero no resulta de aplicación a otras condiciones que las ensayadas. Otro punto de aplicación de los modelos predictivos en la CAA es la predicción de la degradación de agroquímicos en base a condiciones ambientales lo que permite establecer períodos de carencia seguros durante la cosecha para no sobrepasar los límites residuales permitidos en la comercialización. Un tercer aspecto de la aplicación de los modelos predictivos es el de la predicción de la aparición de enfermedades fúngicas a campo por condiciones de riesgo ambiental los que constituyen un instrumento extremadamente valioso para un racional empleo de tratamientos antifúngicos (http://www.sinavimo.gov.ar). Un cuarto tipo de modelos lo integran los modelos para la biotransformación de fármaco-veterinarios que permiten establecer períodos de carencia adecuados en este caso para medicamentos de uso veterinario. La información que se genera con el desarrollo de estos modelos es relevante para la agroindustria, el consumidor, y los organismos de control de alimentos al permitirles contar con herramientas para estimar riesgo sanitario cuantitativamente.

Ejemplos de Cooperación Regional y Nacional

Dentro del marco de la cooperación regional latinoamericana en el que el INTA se inserta debemos señalar la importancia de compartir proyectos de investigación con otros organismos de Ciencia y Técnica de la región, incluyendo los INIAs, unidos por una problemática común a la seguridad alimentaria de los países de la zona. Un ejemplo de ese accionar lo constituye el proyecto MYCOTOX **(8)** para el estudio de diversos aspectos de la prevención de la contaminación con micotoxinas en las cadenas cerealeras de Uruguay, Chile, Brasil y Argentina basado en la implementación de un APPCC. Este proyecto financiado por la Unión Europea tiene características destacables que pueden replicarse en otros emprendimientos cooperativos. Una de estas características es la organización de una red de laboratorios regionales con metodologías analíticas unificadas y validadas lo que potencia la capacidad analítica de cada uno de los participantes. Otro aspecto notable es el fuerte componente socioeconómico del proyecto destinado a analizar las restricciones a la implementación de los sistemas de gestión agroalimentaria en las cadenas de abasto regionales. Estos estudios basados en, entre otros, encuestas a productores agropecuarios de la región y observaciones a campo han demostrado la importancia de contar con incentivos de mercado apropiados y una adecuada legislación nacional para una exitosa implementación de los sistemas preventivos de gestión como el APPCC **(3).** En el ámbito nacional INTA realiza acciones sinérgicas y cooperativas con los otros componentes de Sistema Nacional de Ciencia y Técnica (SNCyT) en comisiones para la formulación de proyectos conjuntos en el marco de la prevención de los riegos de contaminación química y biológica con la finalidad de establecer un sistema de investigación diagnóstica, monitoreo permanente, trazabilidad y certificación de inocuidad de los principales productos de origen agropecuario para el consumo humano y animal **(10).**

Conclusiones: Integración y Prevención

Los organismos internacionales que pautan la seguridad alimentaria a nivel mundial han enfatizado la necesidad de basar sus normativas de seguridad alimentarias, y comerciales, en evaluaciones objetivas de riesgo microbiológico. Estas evaluaciones deben ser realizadas con información epidemiológica, de estudios de incidencia, y del efecto de las prácticas agroindustriales y procedimientos tecnológicos e higiénicos realizados en cada país. Es para ello necesario el estudio de los niveles de prevalencia de los contaminantes químicos y microbiológicos en los distintos pasos de la producción y comercialización de los alimentos así como la identificación de los factores que los aumentan o disminuyen a lo largo de la CAA. Esta información es necesaria además para el desarrollo de estudios y planes APPCC adaptados a las condiciones de producción y procesamiento locales. En este camino de las experiencia de cooperación realizadas por INTA surge la necesidad la integración multidisciplinaria y regional así como el hecho de que de la realización de proyectos de investigación en

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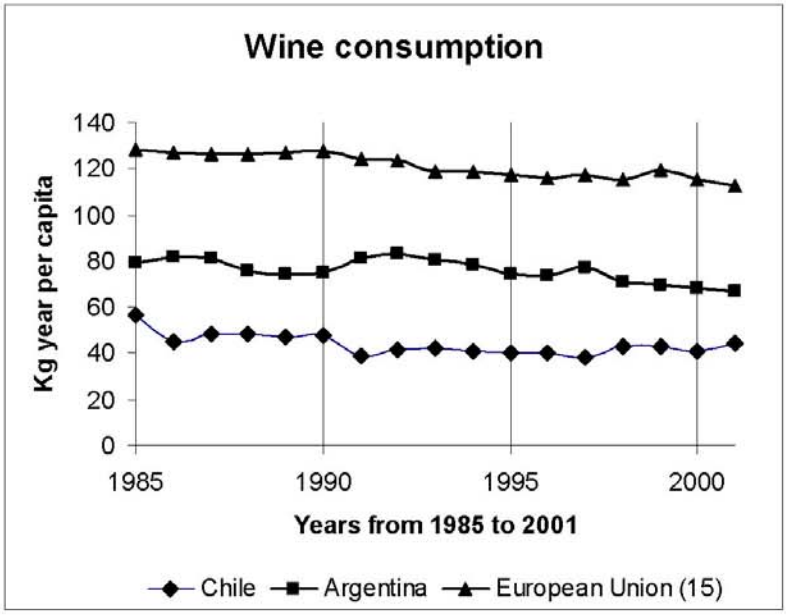

Figure 2. Wine consumption in Argentina, Chile and the European Union (15 countries).

The mean intake for those years was 121.34 kg year per capita for European countries, 76.06 kg year per capita for Argentina and 44.03 kg year per capita for Chile.[24] Taking into account the wine consumption in both South American countries, and based on the present results, it seems possible that wine intake is not an important OTA for the Argentinian and Chilean populations, in comparison with European people.

Although the number of imported wine samples analysed was limited, our results showed that OTA contaminations in European red wines was in the range of the SCOOP study.[20] From those countries which provided discriminated information to the SCOOP study, the mean concentration in red wine was 0.17 µg/l, and the mean level found in the sixteen European red wines analysed by us was 0.0315 µg/l. The Argentinian population may be more exposed than the Chilean population due to the consumption of imported wine, and because the average wine intake in Argentina is higher than in Chile (Figure 2).

Conclusions

This paper presents a preliminary report on OTA contamination of wines from Chile and Argentina. The presence of OTA in wines would appear to be a lesser problem than other countries, but it still contributes to OTA exposure. The results of this study confirm the

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ANNEXES

Papers and publications

Posters presented in congresses

Resume presented as poster in 'ENCONTRO NACIONAL DE MICOTOXINAS", Piracicaba city, 30th June to 02nd July, 2004.

DESENVOLVIMENTO E PADRONIZAÇÃO DE FERRAMENTAS ANALÍTICAS EFETIVAS PARA DETERMINAÇÃO DE MICOTOXINAS EM CEREAIS E SUBPRODUTOS

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INTRODUÇÃO

Este trabalho faz parte do Projeto MYCOTOX (ICA4-CT-2002-10043) "O desenvolvimento de um sistema de gestão da qualidade de alimentos para o controle de micotoxinas nas cadeias de produção e processamento de cereais nos países do cone sul da América Latina", iniciado em 2003. O projeto, coordenado pelo CIRAD da França, envolve a participação de instituições nos seguintes países: Reino Unido, Suécia, Argentina, Brasil, Chile e Uruguai. O objetivo geral do projeto é melhorar a competitividade dos cereais produzidos pelos países do cone sul da América Latina no comércio interno e internacional pelo controle da ocorrência de micotoxinas em milho, trigo e derivados utilizados para consumo humano e animal. Como resultados esperados pelo Projeto podem ser citados a redução do risco para o consumidor final e benefícios crescentes para os países produtores de grãos.

O projeto MYCOTOX compreende 6 planos de ação: WP1 - Desenvolvimento e padronização de ferramentas analíticas eficazes para determinação de micotoxinas em cereais e subprodutos, WP2 – Análise do risco e da exposição humana à ocratoxina A, WP3 – Análise dos procedimentos de moagem como potenciais pontos críticos de controle, WP4 – Análise de perigos, WP5 – Identificação e validação de medidas de controle de micotoxinas e WP6 – Desenvolvimento de um Sistema de Manejo de Qualidade dos Alimentos. Por meio do WP1 vêm sendo estabelecidos os controles intra e interlaboratorial como medidas para a garantia da qualidade analítica dos dados gerados por métodos cromatográficos em uso pelos laboratórios participantes do Projeto, como também os critérios para a reportagem dos resultados para que os dados possam ser corretamente utilizados e interpretados. Devido à distribuição não homogênea das micotoxinas nas amostras, uma atenção especial é dada também ao procedimento de amostragem. É importante que este procedimento esteja claramente definido, seja único e seguido por todos aqueles que realizarão a coleta das amostras referentes ao Projeto MYCOTOX. A obtenção de dados de monitoramento significativos requer a coleta de amostras representativas de lotes de amostra criteriosamente selecionados, os quais são representativos de regiões bem definidas (país ou região dentro de um país).

OBJETIVOS

- Contribuir para a constituição de uma rede latino-americana de laboratórios para análise de micotoxinas;
- Contribuir para melhorar o desempenho e a performance analítica dos laboratórios participantes do Projeto MYCOTOX na determinação de micotoxinas (aflatoxinas, zearalenona, fumonisinas, deoxynivalenol e ocratoxina A) em amostras de milho e trigo por métodos cromatográficos:
  - Assegurar que os resultados analíticos serão obtidos dentro de um critério de aceitabilidade, garantindo a qualidade e confiabilidade dos dados analíticos gerados e contribuindo para a harmonização dos procedimentos analíticos;
  - Assegurar que os resultados analíticos serão reportados de forma harmonizada e apropriada.
- Uniformizar o procedimento de amostragem a ser utilizado para a coleta das amostras que serão analisadas no Projeto MYCOTOX para que os resultados obtidos no monitoramento possam ser corretamente obtidos e utilizados.

MATERIAL E MÉTODOS

O Projeto está sendo implementado conforme as fases descritas a seguir:

Fase 1 – Inventário dos métodos cromatográficos utilizados pelos laboratórios do Projeto MYCOTOX, em formulário padronizado, contendo informações sobre as técnicas em uso e características dos métodos.

Fase 2 – Avaliação dos métodos pela análise de amostras de referência de contaminação conhecida, FAPAS "surplus test material", comparando os resultados de recuperação e desvio padrão com aqueles obtidos na validação dos métodos e considerando os critérios de aceitabilidade para avaliação interna da aplicabilidade e adequação dos métodos.

Fase 3 – Produção de amostras de referência (amostras testes homogêneas naturalmente contaminadas) preparadas e homogeneizadas, com o valor de contaminação mais provável estimado segundo Horwitz (1995), Thompson e Wood (1993), ISO/IEC 43 (1997), Thompson (2000), FAPAS (2002) e Vargas *et al*. (2001).

Fase 4 – Implementação dos controles inter e intralaboratoriais entre os laboratórios parceiros do Projeto MYCOTOX para comparação, padronização e validação dos métodos analíticos cromatográficos em uso (avaliação da reprodutibilidade e repetibilidade):

- Controles intralaboratoriais: analise de amostras de referência (amostras branco de milho e trigo artificialmente contaminadas com solução padrão da micotoxina de interesse) juntamente com as amostras advindas das várias ações do Projeto;
- Controles interlaboratoriais: participação em ensaios de proficiência utilizando as amostras produzidas na Fase 3 para o controle de aflatoxinas e zearalenona em milho e amostras de referência FAPAS para o controle de deoxynivalenol em trigo e fumonisinas em milho.

Quando os resultados se apresentarem "questionáveis" ou "insatisfatórios" deverão ser tratados como não-conformidades (análise das causas, propostas ações corretivas e verificada a implementação das ações e eliminação da não-conformidade).

Fase 5 – Harmonização dos procedimentos de reportagem de resultados.

Fase 6 – Harmonização dos procedimentos de amostragem de acordo com plano já publicado e aceito internacionalmente (FAO, *Codex Alimentarius*, USDA, União Européia ou JECFA) considerando a capacidade dos laboratórios em termos de infraestrutura e equipamentos necessários ao processamento e armazenamento das amostras.

RESULTADOS E DISCUSSÃO

O WP1 alcançou até o momento os seguintes resultados:

Fase 1 - Inventário dos métodos cromatográficos utilizados pelos laboratórios parceiros na determinação de aflatoxinas, zearalenona, fumonisina, deoxynivalenol e ocratoxina A em milho e trigo;

Fase 2 – Aquisição das amostras do FAPAS "surplus test material": zearalenona em milho (T2209), fumonisinas B_1 e B_2 em milho (T2208 e T2211), aflatoxinas B_1 e B_2 em milho (T0446 e T0453) e deoxynivalenol em trigo (T2210). As amostras foram enviadas e analisadas pelos laboratórios participantes do WP 1. Os resultados foram enviados para a coordenação do WP 1 para compilação e tratamento estatístico (cálculo da recuperação e desvio padrão). O Laboratório que apresentou resultados fora da faixa esperada está implementando modificações no método para a identificação e correção da não conformidade.

Fase 3 – Iniciada a produção de amostras de referência (amostras testes homogêneas de aflatoxinas e zearalenona em milho).

Fase 4 – Elaboração dos protocolos que nortearão a implementação dos controles inter e intralaboratoriais e estes comunicados aos laboratórios parceiros do WP 1. Foi elaborado o cronograma para as próximas fases dos controles.

Fases 5 e 6 – Elaboração dos protocolos para discussão entre os parceiros do Projeto MYCOTOX referentes aos procedimentos de reportagem de resultado e de amostragem.

CONCLUSÕES

Fase 1 – O inventário dos métodos cromatográficos deverá ser reformatado, inserindo informações mais detalhadas sobre os métodos;

Fase 2 – Os métodos utilizados pelos laboratórios do WP1 apresentaram resultados satisfatórios em sua maioria. Deverão ser compilados ainda os resultados dos laboratórios do WP4 e WP5 e os resultados referentes a deoxynivalenol;

Fase 3 – A produção de amostras de referência deverá ser continuada;

Fase 4 – Os protocolos dos controles inter e intralaboratoriais contribuíram para a harmonização dos procedimentos e discussão entre os laboratórios participantes do Projeto MYCOTOX. O cronograma da fase 4 e um plano de estudo estão sendo elaborados pela coordenação do WP1 para comunicação aos laboratórios e implementação;

Fases 5 e 6 – Os protocolos referentes aos procedimentos de reportagem de resultado e de amostragem deverão ser discutidos e harmonizados entre os parceiros do projeto.

BIBLIOGRAFIA

CEN – European Committee for Standardization, 1999, CEN Report: Food Analysis. Biotoxins: Criteria of analytical methods of mycotoxins, Brussels. CR 13505:1999 E. 8p.

Codex Alimentarius Comission. CL 2001/5-MAS March 2001, Report of 23rd Session of Codex Committee of Analysis and Sampling. Budapest, Hungary, 26 a 02 de March 2001.
FAO- Plano de Amostragem para Análise de Aflatoxinas em Milho e Amendoins. FAO Alimento e Nutrição, Boletim 55, 1993
FAPAS - FOOD ANALYSIS PERFORMANCE ASSESSEMENT SCHEME. Protocol for the FAPAS. Organisation and Analysis of Data, CSL. 5th ed., April 1997
Food and Agriculture Organization (1997) FAO Food and Nutrition Paper 64, FAO, Viale Della Terme di Caracalla 00100, Rome, Italy. 43p.
Food and Agriculture Organization (1997) FAO Food and Nutrition Paper 55, FAO, Viale Della Terme di Caracalla 00100, Rome, Italy. 75p.
Food and Agriculture Organization (FAO), World Health Organization (WHO) (2001) FAO Food and Nutrition Paper 74 and Who Food Additives Series 47, 701p. prepared by the fifty-sixth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).
GILBERT, J. Quality Control Measures for Mycotoxin Laboratories, FDA Workshop on Mycotoxins, 22-26, July, 2002, Maryland, USA.
HORWITZ W. Protocol for the design, conduct and interpretation of collaborative studies, Pure & Applied Chemistry, vol. 60, no. 6, pp. 855-864, 1988.
HORWITZ W., ALBERT R., NESHEIM S.; 1993; Reliability of Mycotoxin Assays – An Update; Journal of AOAC International; 76; 3.
ISO/IEC Guide 43-1 (1997) Proficiency testing by interlaboratory comparisons – Part 1: Development and operation of proficiency testing schemes, second edition.
IUPAC - INTERNATIONAL UNION PURE AND APPLIED CHEMISTRY. Harmonized guidilines for internal anality control in analytical chemistry laboratories prepared by Michael Thompson and Roger Wood. Pure and Applied Chemistry. v. 67, N. 4, p. 649-666, 1995.
JECFA – JECFA sampling working paper 2, elaborated by Coker & Whitaker, Revised Draft 1, February, 14th, 2001.
Report to the Standing Committee on the Food Chain and Animal Health on the Relation Between Analytical Results, Measurement Uncertainty, Recovery Factors and the Provisions in EU Food and Feed Legislation.
THOMPSON M., ELLISON, S. L. R., FAJGELJ, A., WILLETTS, P., WOOD, R. Harmonized guidelines for the use of recovery information in analytical measurement, Technical Report of IUPAC – International Union of Pure and Applied Chemistry, Pure & Applied Chemistry, vol.71, no.2, pp. 337-348, 1999.
THOMPSON, M, WOOD, R. (1993) International Protocol for Proficiency Testing of (Chemical) Analytical Laboratories. J. Assoc. Off. Anal. Chem. 76, 4, 926-940.
THOMPSON, M., Recent trends in inter-laboratory precision at ppb and sub-ppb concentrations in relation to fitness for purpose criteria in proficiency testing, Analyst, 125, 385-386, 2000.
União Européia - Diretiva 27/2002/CE da Comissão de 13 de março de 2002, Jornal Oficial das Comunidades Européias, L75/44.
União Européia - Diretiva 98/53/CE da Comissão de 16 de julho de 1998, Jornal Oficial das Comunidades Européias, L201/93.
VARGAS, E. A., SANTOS, E.A., PITTET, A., 2001, Collaborative Study Submitted for consideration by AOAC International: D-2 Protocol - Determination of ochratoxin A in green coffee by immunoaffinity column clean-up and HPLC. Ministério da Agricultura e do Abastecimento, Laboratório de Micotoxinas/LAV-MG, Brasil. 28p.
WEIGERT, P., GILBERT, J., PATEY, A., L., KEY, P. E., WOOD, R., BARYLKOPIKIELNA, N. Analytical quality assurance for WHO GEMS/Food EURO programme – results of 1993/1994 laboratory proficiency testing. Food Additives and Contaminants, v. 14, n. 4, p. 399-410, 1997.

Interlaboratory Control Involving Participant Laboratories within the INCO-DEV MYCOTOX PROJECT 2003-2005 "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries"

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Key words: validation reference material, interlaboratory control, maize, wheat, aflatoxins B_1, B_2, G_1, G_2, ochratoxin A, zearalenone, fumonisins B_1, B_2, tricothecenes.

An interlaboratory control (IC) program was implemented among project partner laboratories from Brazil, Uruguay, Chile and Argentina aiming to give confidence to the data generated by the project; to contribute to the harmonization of analytical procedures and to improve of laboratory's proficiency in mycotoxin analysis. The maize aflatoxin B_1, B_2, G_1, G_2 (AFLs) and zearalenone (ZON) reference materials were prepared. The homogeneity of the reference materials was investigated by analysis of variance (ANOVA), according to the International Harmonized Protocol for the Proficiency Testing of (Chemical) Analytical Laboratories as established by ISO 43-1. Four homogeneous maize materials were prepared: ZON and AFL blanks and two naturally contaminated for AFLs. These samples were used to method validation methods and as reference samples for the interlaboratory control. Additional FAPAS test materials for AFLs, ochratoxin A (OTA), ZON, fumonisins B_1, B_2 (FB) and deoxynivalenol (DON) were also used in order to implement the interlaboratory control, and to validate the methods and validate the reference materials. In each round, participant laboratories received refrigerated parcels containing: reference and blank samples for spiking purpose, test material receipt form, method instructions, result reporting sheets and analytical work questionnaire in 3 rounds. The results were evaluated by using z-score function. As a result: the laboratories have improved their methods and, in some cases, methods were replaced; an inventory of the analytical methods used by the laboratories in the region was prepared and is available; and a a network of mycotoxin has being consolidated in the Mercosur region.

Resume approved to be presented as porter at XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, May, 21-25th, Istambul, Turkey

A HACCP Plan Along The Wheat Chain To Prevent Don In Wheat Flour In Uruguay

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Fusarium head blight (FHB) has become the most significant disease of wheat in Uruguay in the last decade, not only due to yield losses (up to 44%) but due to hazards imposed to human health by its mycotoxins. There were two mayor outbreaks of FHB during two consecutive years, 2001 and 2002, mainly due to above normal precipitations by late September and October. Main *Fusarium* specie found in wheat in the country is *F.graminearum* which is a mayor DON producer. Hazard Analysis of the Critical Control Point (HACCP) is the food management system that identifies, assesses hazards and tries to control them. A HACCP plan was put in place to control DON mycotoxin in wheat chain, from the field to the mill. Good Agricultural Practices (GAP) are essential, especially due to the fact that *Fusarium* is a field fungus that grows in the field, from wheat anthesis throughout grain filling. The effect of crop rotation as a GAP on DON content was validated. Moreover, four critical control points (CCP) were identified in the wheat chain; CCP1 at the traders´ reception, CCP2 at the mills reception, CCP3 at mixing wheat silos and CCP4 at mixing flour silos.

Key words: wheat, *Fusarium,* FHB, Mycotoxin, DON, HACCP, GAP, CCP

Presented at the XII International IUPAC Symposium on Mycotoxins and Phycotoxins, 21-25 May 2007, Turkey (www.atal.tubitak.gov.tr/iupac2007-mycotoxin)

DESENVOLVIMENTO DOS PONTOS CRÍTICOS DE CONTROLE PARA PREVENIR A CONTAMINAÇÃO POR MICOTOXINAS AO LONGO DA CADEIA PRODUTIVA DO MILHO

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INTRODUÇÃO

A implementação do APPCC (Análise de Perigos e Pontos Críticos de Controle) no segmento das indústrias de alimentos e rações vem sendo difundida tendo como objetivo a garantia da qualidade e da segurança dos produtos acabados, além, da redução das perdas.

O milho assume papel de vital importância, pois constitui a principal fonte de nutrientes e também de micotoxinas para as aves, uma vez que servem de substrato para o crescimento dos fungos e a conseqüente produção destas toxinas. O Brasil é o terceiro produtor de milho com 42.157.000 toneladas, sendo a região Sul, a de maior participação representando 37% da produção brasileira. Desta região, o Paraná lidera com 66% da produção. A região Sul também é considerada a maior produtora de aves de corte do país. Trinta e seis porcento da produção de milho é direcionada para a fabricação de rações para aves.

Este trabalho teve como objetivo o desenvolvimento dos Pontos Críticos de Controle Potenciais a fim de verificar a contaminação por micotoxinas ao longo do processo de fabricação de ração destinada a alimentação de frangos de corte, em uma fábrica localizada no Estado do Paraná, a qual tem como principal matéria-prima, o milho.

METODOLOGIA

Este projeto foi realizado no âmbito do projeto Mycotox, projeto europeu de cooperação coordenado pelo Cirad, envolvendo parceiros institucionais europeus (França, Reino-Unido, Suécia) e paises do Cone Sul (Brasil, Chile, Argentina, Uruguai) e visando o desenvolvimento de um sistema de gestão integrada da qualidade para o controle das micotoxinas nas cadeias de trigo e milho dos países do Cone Sul da América Latina (Brasil, Chile, Argentina e Uruguai).

O presente estudo foi realizado em uma Fábrica de Ração integrada a Granjas, localizada no Noroeste do Paraná.

A metodologia utilizada para definir os Pontos Críticos de Controle Potenciais foi primeiramente conhecer de forma detalhada as características da matéria-prima e do produto final, além de todo o sistema funcional do processo de fabricação de ração realizado na indústria, através de entrevistas e aplicação de questionários aos responsáveis de cada setor.

RESULTADOS

Diversos aspectos foram relevantes para se determinar os Pontos Críticos de Controle Potenciais, dentre eles destacam-se que os fungos que colonizam os grãos são agrupados em duas categorias: a) fungos do campo e b) fungos de armazenamento, portanto a contaminação pode ocorrer tanto durante a pré-colheita como na pós-colheita. Outro ponto importante considerado, foram as condições favoráveis ao desenvolvimento dos fungos e sua conseqüente produção de micotoxinas, como umidade, temperatura, período de armazenamento em silo, condições físicas dos grãos, dentre outros. Estudando todos estes aspectos, os Pontos Críticos encontrados foram: recepção do milho, visando verificar a qualidade dos grãos provindos do campo, diferenciando os diversos tipos de fornecedores; o processo de limpeza e secagem do milho, com o objetivo de estudar o impacto destes sistemas sobre o conteúdo de micotoxinas; armazenagem do milho em silo e estocagem da ração em silos das granjas, permitindo avaliar o impacto do sistema de armazenagem (Tabela 1 e Figura 1).

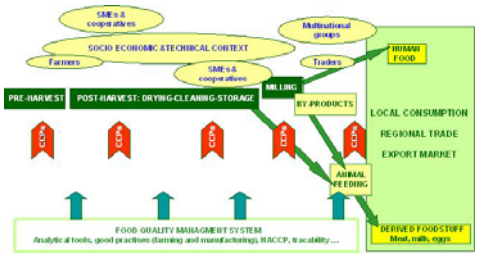

Figura 1 – Pont

Tabela 1 – P

PONTOS CRÍTICOS	POPULAÇÃO
1. RECEPÇÃO	A: Produtores com entrega direta do milho pós-colheita B: Produtores que secam e estocam o milho no campo C: Cooperativas e Cerealistas D: Rejeitados e/ou grãos ardidos > 6%
2. SISTEMA DE LIMPEZA E DE SECAGEM	A: Antes da pré-limpeza B: Depois da pré-limpeza / antes da secagem C: Antes da pós-limpeza / depois da secagem D: Depois da pós-limpeza E: Descarte da pré-limpeza
3. SISTEMA DE ESTOCAGEM DO MILHO EM GRÃO NO SILO	A: Sistema Silo nº2
4. SISTEMA DE ESTOCAGEM DA RAÇÃO NA GRANJA	A: Granja com sistema manual de alimentação B: Granja com sistema automático de alimentação

CONCLUSÃO

Sendo o APPCC uma ferramenta que busca a garantia da qualidade da matéria-prima e do produto final, torna-se extremamente importante a sua correta aplicação. Pois quando este sistema operacional é bem aplicado, se torna bastante útil para o monitoramento do processo, em que é possível identificar exatamente em qual ponto da operação são necessárias as medidas preventivas para seu controle.

REFERÊNCIAS

ABIMILHO – Associação Brasileira das Indústrias do Milho. Disponível em: <http: //www.abimilho.com.br>. Acesso em: 19 de setembro de 2005.

BENNETT, J.W. Mycotoxins, mycotoxicoses, mycotoxicology and mycopathology. *Mycopathlogia*, v.100, p.3–5, 1987.

CHRISTENSEN, C. M. *Storage of Cereal Grains and Their Products*. 2nd ed. American Association of Cereal Chemists, St.Paul, Minnesota, 1974.

CONAB – Companhia Nacional do Abastecimento. Disponível em: <http://www.conab.gov.br>. Acesso em: 19 de setembro de 2005.

ILSI - International Life Sciences Institute - *A Simple Guide to Understanding and Applying the Hazard Analysis Critical Control Point Concept*, 3nd edition, Europe, Monograph Series, Brussels: ILSI Europe, 2004.

USDA – United States Department of Agriculture. Disponível em: <http://www.usda.gov>. Acesso em: 20 setembro de 2004.

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MICOFLORA CONTAMINANTE Y PRESENCIA DE TRICOTECENOS TIPO A Y B EN TRIGO COSECHADO EN LA PROVINCIA DE BUENOS AIRES

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INTRODUCCION

Los mohos son la contaminación microbiana más común en granos y semillas, y su presencia puede derivar en la producción de micotoxinas. El conocimiento acerca de los mohos asociados a granos y semillas es importante para orientar la búsqueda de contaminación en matrices alimenticias. Se conocen especies fúngicas, pertenecientes principalmente a los géneros *Fusarium, Aspergillus* y *Penicillium*, que pueden ser capaces de producir micotoxinas que originan manifestaciones patológicas, al ser ingeridas en los alimentos por la población humana y animal.
En Argentina el trigo pan (*Triticum aestivum* L.) es destinado principalmente a la alimentación humana [1], esta es la razón por la que son importantes los estudios de la ocurrencia natural de tricotecenos tipo A, como las Toxinas T-2, HT-2, T-2 tetraol y T-2 triol, diacetoxiscirpenol (DAS) y neosolaniol (Neo) y tipo B como el deoxinivalenol (DON), 3 y 15 acetil deoxinivalenol (ADON) y nivalenol (NIV) y la micoflora contaminante asociada.

OBJETIVOS

El objetivo de este trabajo es conocer y comparar la micoflora interna presente y la ocurrencia de algunos tricotecenos tipo A y B en trigo pan cosechado en distintas localidades del noroeste de la provincia de Buenos Aires.

MATERIALES Y METODOS

Muestreo. Un total de 68 muestras de trigo pan recién cosechado (≈3 kg) fueron colectadas al azar durante la cosecha 2004 en Arrecifes (12 muestras), Bragado (14), Junín (14), Pergamino (14) y San Antonio de Areco (14), provenientes de campos experimentales de INTA en las zonas citadas. Submuestras cuarteadas al azar de 500 g cada una, se destinaron al análisis de la micoflora y de micotoxinas.

Aislamiento de los mohos. Se realizó según la metodología propuesta por González y col. [2] en agar YGC (Yeast Extract-Glucose-Chloramphenicol Agar, Merck N° 16000).

Identificación de los mohos. Los aislamientos de los mohos fueron identificados de acuerdo a las siguientes metodologías propuestas: especies de *Fusarium* de acuerdo a Nelson y col. [3]; especies de *Aspergillus*, *Penicillium* y otros mohos según Pitt y Hocking [4]. Se calculó la frecuencia de aislamiento (*Fr*) y la densidad relativa (*DR*) de géneros y de especies como está descripto en González y col. [2].

Análisis de tricotecenos. El análisis de los tricotecenos se realizó empleando columna Romer para la extracción y limpieza de los extractos, cromatografía gaseosa y confirmación por capa delgada [5]. En resumen se pesaron 25g de muestra y se agregaron 100ml de acetonitrilo:agua (84:16). Se agitaron durante 3 min. a alta velocidad. La mezcla se filtró a través de un papel de filtro Whatman N° 4. Se tomaron 5ml del filtrado y se trasvasaron a un tubo de 10ml y se realizó el cleanup con columna ROMER #227. Se evaporó en un baño maría a 40°C, bajo vacío. Se resuspendió el residuo en 200µl de acetato de etilo : metanol (95:5). Se tomaron 100µl y 100µl de una solución toluénica de 2amino5clorobenzofenona. Se evaporó a sequedad bajo nitrógeno, se le agregó catalizador y heptafluorbutiril anhidrido ácido como derivatizante. Se agitó 30 segundos y se calentó a 60°C durante 30 min. Se neutraliza y se separa la fase toluénica. Se identificaron las toxinas de acuerdo a la relación entre el tiempo de retención de cada una de ellas y el del estándar interno. Se calcula la concentración de la micotoxina de acuerdo a una curva de calibración y se confirma por cromatografía planar.

Análisis estadístico. Para analizar posibles diferencias en las frecuencias de aislamiento (*Fr*) de las especies fúngicas de interés micotoxicológico se utilizó el test exacto de Fisher [6]. Para ver diferencias de la contaminación con tricotecenos se usó el test de la mediana, empleando el programa Statistix 7.0 [6].

RESULTADOS Y DISCUSION

Las *Fr* y *Dr* de las especies fúngicas aisladas de trigo en la Provincia de Buenos Aires en 2.004 se muestran en la tabla 1. Se puede observar que *Alternaria alternata* (4.348 aislados), *Fusarium graminearum* (1.244), *Fusarium poae* (132) y *Fusarium semitectum* (75) son las especies predominantes. Es de destacar que *Aspergillus flavus* y *Penicillium citrinum* se aislaron con muy bajos niveles de *Fr* y *DR*.
En la tabla 2 se detallan las *Fr* y *DR* de todas los mohos aislados en cada región. Cualitativamente la micoflora interna identificada es similar a la observada en trabajos previos en trigo pan y candeal realizados en otras localidades de las provincias de Buenos Aires, Córdoba, Santa Fe y La Pampa [2] [8].
No se observaron diferencias estadísticas en la comparación de las *Fr* de las especies de interés micotoxicológico entre localidades.
Se analizaron los tricotecenos presentes y se confirmó la presencia de DON (mínimo de muestras positivas: 7 µg/kg, máximo: 2.438 µg/kg, media muestras positivas: 347,8 µg/kg), HT-2 (3,5 µg/kg, 41 µg/kg, 12,0 µg/kg), T-2 triol (21 µg/kg, 123 µg/kg, 47,1 µg/kg), acetil DON (14 µg/kg, 43 µg/kg, 19,5 µg/kg). Nivalenol detectado en tres muestras no se pudo confirmar por cromatografía planar. No se detectó la presencia de toxinas T-2, T-2 tetraol, diacetoxiscirpenol, ni neosolaniol.
El DON fue la micotoxina predominante. En la figura 1 se muestra el Box Plot de la contaminación de DON en las regiones estudiadas. Se puede observar que el nivel de contaminación de DON en San Antonio de Areco fue el más alto (mediana: 974,0 g/kg), seguido de Bragado (mediana: 213,0 g/kg). Estadísticamente la comparación de la mediana de la contaminación de DON en San Antonio de Areco y en Bragado mostró diferencias altamente significativas ($p < 0,01$) entre sí y cuando se compararon con el resto de las localidades.

Tabla 1. Frecuencia de aislamiento (*Fr*) y densidad relativa (*DR*) de mohos aislados de trigo pan en el noroeste de la Provincia de Buenos Aires en 2.004.

Especies	*Fr* (%)	*DR* (%)
Alternaria alternata	100,0	63,9
Aspergillus flavus	4,4	0,06
Arthrinium phaeospermum	8,8	0,3
Bipolaris sorokiniana	41,2	0,9
Cladosporium cladosporioides	63,2	1,6
Curvularia lunata	16,2	0,3
Epicoccum nigrum	91,2	8,4
Eurotium chevalieri	29,4	0,6
Fusarium		
F. acuminatum	1,5	0,02
F. avenaceum	2,9	0,2
F. culmorum	7,4	0,1
F. equiseti	4,4	0,1
F. graminearum	100,0	18,3
F.poae	66,2	1,9
F. sambucinum	7,4	0,3
F. semitectum	36,8	1,1
Mucor racemosus	1,5	0,1
Nigrospora oryzae	35,3	1,3
Penicillium citrinum	13,2	0,3
Phoma spp.	2,9	0,1
Trichoderma harzianum	2,9	0,03
Ulocladium spp.	1,5	0,01

Tabla 2. Frecuencia de aislamiento (*Fr*) y densidad relativa (*DR*) en porcentaje, de las especies fúngicas aisladas de trigo pan en cinco localidades del noroeste de la Provincia de Buenos Aires en 2.004.

	ARRECIFES		BRAGADO		JUNN		PERGAMINO		SAN ANTONIO DE ARECO	
N °DE AISLAMIENTOS	1.200		1.400		1.400		1.400		1.400	
ESPECIES	*Fr*	*DR*	*Fr*	*DR*	*Fr*	*DR*	*Fr*	*DR*	*Fr*	*DR*
Alternaria alternata	100,0	65,7	100,0	67,1	100,0	76,7	100,0	66,5	100,0	44,0
Aspergillus flavus	8,3	0,3	7,1	0,07	7,1	0,07	nd	nd	nd	nd
Arthrinium phaeospermum	25,0	0,8	7,1	0,07	nd	nd	nd	nd	14,3	0,5
Bipolaris sorokiniana	41,7	0,8	28,6	0,5	28,6	0,6	64,3	1,6	42,9	0,8
Cladosporium cladosporioides	66,7	1,7	71,4	1,9	28,6	0,9	6403	1,6	85,7	1,9
Curvularia lunata	8,3	0,3	42,9	0,6	21,4	0,3	nd	nd	7,1	0,07
Epicoccum nigrum	100,0	11,3	92,9	7,5	78,6	4,9	92,9	10,6	92,9	8,4
Eurotium chevalieri	33,3	0,6	21,4	0,4	21,4	0,8	42,9	0,6	28,6	0,9
Fusarium acuminatum	nd	nd	nd	nd	nd	nd	nd	nd	7,1	0,07
Fusarium avenaceum	nd	nd	10.53	0.25	nd	nd	nd	nd	14,3	1,1
Fusarium culmorum	16,7	0,2	nd	nd	nd	nd	7,1	0,6	14,3	0,4
Fusarium equiseti	nd	nd	7,1	0,1	nd	nd	14,3	0,4	nd	nd
Fusarium graminearum	100,0	13,3	100,0	16,6	100,0	10,6	100,0	13,4	100,0	36,9
Fusarium poae	83,3	2,8	85,7	2,6	57,1	1,7	42,9	1,0	64,3	1,8
Fusarium sambucinum	25,0	0,9	7,1	0,1	nd	nd	nd	nd	7,1	0,3
Fusarium semitectum	25,0	0,5	21,4	0,5	50,0	1,6	57,1	1,9	28,6	0,9
Mucor racemosus	nd	nd	nd	nd	nd	nd	7,1	0,6	nd	nd
Nigrospora oryzae	33,3	0,7	42,9	1,5	21,4	0,9	35,7	1,3	42,9	2,1
Penicillium citrinum	25,0	0,4	14,3	0,3	21,4	0,8	7,1	0,07	nd	nd
Phoma spp	nd	nd	nd	nd	7,1	7,1	0,4	0,07	nd	nd
Trichoderma harzianum	nd	nd	7,1	0,07	16,67	7,1	0,07	nd	nd	nd
Ulocladium spp.	nd	nd	nd	nd	7,1	0,07	nd	nd	nd	nd

nd: no detectado

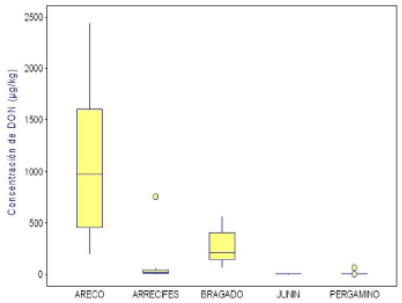

Figura 1. Box Plot de la concentración de deoxinivalenol (DON) en muestras de trigo cosechado en localidades del noroeste de la Provincia de Buenos Aires en 2.004.

CONCLUSIONES

Las especies fúngicas prevalentes y potencialmente micotoxicogénicas fueron: *Alternaria alternata*, *Fusarium graminearum*, *Fusarium poae* y *Fusarium semitectum*. La micotoxina predominante entre los tricotecenos analizados fue DON.
Las localidades San Antonio de Areco y Bragado presentaron mayor nivel de contaminación de DON.
La alta incidencia de *Alternaria alternata*, entre los mohos aislados, amerita que se estudie la ocurrencia natural de las toxinas de *Alternaria* en trigo.

BIBLIOGRAFIA

1.Pacin A, Elena Martínez EJ, Pita Martín de Portela ML, Neira MS. Consumo de alimentos e ingesta de algunos nutrientes en la población de la Universidad Nacional de Luján, Argentina. 1999. *Archivos Latinoamericanos de Nutrición*; 49:31-39.

2.González HHL, Pacin A, Resnik SL, Martínez EJ. 1996. Deoxynivalenol and contaminant mycoflora in freshly harvested Argentinean wheat in 1993. *Mycopathologia*; 135: 129-134.

3.Nelson PE, Toussoun TA & Marasas WFO. 1983. *Fusarium* species: an illustrated manual for identification. The Pennsylvania State University Press, University Park, Pennsylvania.

4.Pitt JI and Hocking AD. 1997. Fungi and Food Spoilage. Blackie Academic & Professional, London-New York.

5.Croteau SM, Prelusky DB and Trenholm HL. 1994. Analysis of trichothecenes mycotoxins by gas chromatography with Electron Capture Detection. *Journal of Agricultural and Food Chemistry*; 42: 928-934.

6.Conover WJ. Practical Nonparametric Statistic. 1980, John Wiley & Sons, New York.

7.Statistix Version 7.0. Analytical Software. 2000, Tallahassee, Florida.

8.González HHL, Martínez EJ, Pacin A, Resnik SL. 1999. Relationship between *Fusarium graminearum* and *Alternaria alternata* contamination and deoxynivalenol occurrence on Argentinean durum wheat. *Mycopathologia*; 144: 97-102.

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CONTAMINACION POR FUMONISINAS EN LAS FRACCIONES OBTENIDAS EN LA MOLIENDA HUMEDA DE MAIZ

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INTRODUCCION

El maíz producido en Argentina representa casi el 30% del total de la producción de cereales y oleaginosas (aproximadamente 10 millones de toneladas por año). El maíz exportado representa aproximadamente el 50% de dicha producción (Broggi y col., 2.002). En nuestro país este grano es utilizado como alimento para animales (80%), el resto es procesado en molienda húmeda (17%) y seca (3%).

La molienda húmeda permite obtener como productos de maíz: almidón, germen, "gluten feed" y "gluten meal". A partir de estos se producen: almidones modificados, aceite, alimento para animales y productos de azúcar. La producción en Argentina (en miles de toneladas por año) es aproximadamente de almidón 70, fructosa 300, glucosa 150, otros azúcares 70, aceite de maíz 30 y 180 de "gluten feed" y "meal" (C.A.F.A.G.D.A., 2.000).

Las fumonisinas (Figura 1) son los contaminantes naturales más importantes encontrados en los maíces argentinos (González y col., 1.999; Pacin y col., 2.001; Solovey y col., 1.999).

OBJETIVOS

El objetivo del trabajo es conocer cómo el proceso de molienda húmeda afecta la contaminación por fumonisinas en las distintas fracciones (almidón, germen, "gluten feed" y "gluten meal") obtenidas a partir del maíz original contaminado naturalmente.

MATERIALES Y METODOS

Muestras de maíz naturalmente contaminadas, provenientes de una planta de procesamiento industrial, fueron analizadas para determinar la contaminación del maíz que ingresa al proceso de molienda húmeda. Se seleccionaron silos que tuvieran contaminación por fumonisina B_1 (FB_1) y fumonisina B_2 (FB_2) a través de un análisis de toxinas previo al procesamiento. Se estudiaron 21 procesos que consistieron en la molienda de 120 toneladas por proceso. Para ello se hizo un muestreo del grano y de todas las fracciones obtenidas; dicho muestreo fue en forma aleatoria y reduciendo dicha muestra hasta hacer una muestra representativa y adecuada para el trabajo de laboratorio.

Para el análisis de fumonisinas, se extraen 50g de maíz con 100 ml metanol:agua (3:1) y, para las otras fracciones se agrega metanol de acuerdo al contenido acuoso para mantener la relación 3 de metanol por uno de agua (AOAC, 1.996). Se licúa por 3 minutos. Se filtra y se ajusta a pH= 5,8 (NaOH 1 M) y se centrifuga 10 minutos. Se toman 10 ml del filtrado y se pasan por una columna de extracción, prelavada con 5 ml de metanol y 5 ml de metanol:agua (3:1) a flujo 2 ml/min. Se lava con 5 ml metanol:agua (3:1) y 5 ml de metanol. Las toxinas se eluyen con 10 ml de ácido acético:metanol (1:99) a flujo 1 ml/min. Se evapora. Se resuspende en 2.000 µl de metanol:acético (99:1). Se toman dos alicuotas de 800 µl. Se secan y uno de los tubos se resuspende en 1.000 µl de acetonitrilo:agua 1:1. Se utiliza muestreador automático para la determinación, la derivatización es previa a la inyección. Se inyecta 54 µl del derivado (20 µl de la muestra). Tiempo retención entre 6,3 y 7,3, para FB, y 13 a 15 para FB_2 (límite detección: FB, 10 µgkg y FB_2 6 µgkg límite cuantificación: 18 µgkg y 30 µgkg).

RESULTADOS Y DISCUSION

En la Tabla 1 se encuentran el rango de contaminación así como los valores de mediana y número de fracciones analizadas. La contaminación del maíz argentino se observa habitualmente en las diferentes fracciones (González y col., 1.999; Solovey y col., 1.999; Pacin y col., 2.001).

En la Figura 2 se muestran las relaciones de concentración de las distintas fracciones respecto a la contaminación del maíz a la entrada de la maceración así como la distribución de las mismas por medio de Box Plots.

La relación de contaminación del maíz entre FB, y FB_2 encontrada en este trabajo es aproximadamente de 2,7. Con respecto a las fracciones Bennett y col (1.996) observaron en las fracciones estudiadas que el gluten presenta igual concentración de FB_2 que FB, y la fibra mayor FB_2. A pesar del bajo número de muestras analizadas (2) por estos autores, esta tendencia a presentar algunas fracciones mayor contaminación de FB_2 se detectó en el gluten meal, fracción equivalente al gluten del trabajo citado.

Es de destacar que el almidón (Figura 2) no presenta contaminación por fumonisinas y que todas las fracciones presentan menor concentración que el maíz original, contrariamente a lo publicado por Broggi y col. (2.002) en el proceso de molienda seca del maíz donde se encuentran fracciones con mayor concentración de fumonisinas que el maíz original.

También se puede observar que la fracción mas contaminada en el proceso industrial de molienda húmeda es el "gluten meal", mientras que en el procesamiento a escala planta piloto son el germen y las aguas de maceración (Funes y col., 2.000)

Cuando se realiza un balance de masa de las fumonisinas presentes en todas las fracciones, con respecto a la cantidad de fumonisinas en el maíz que ingresa en el proceso de molienda industrial, la reducción de la contaminación observada es del 94 y 87% para FB_1 y FB_2 respectivamente.

CONCLUSIONES

El proceso de molienda húmeda de maíz, presenta diferencias importantes en la concentración de fumonisinas remanentes en las distintas fracciones obtenidas cuando se realiza a escala planta piloto o a escala industrial. En el proceso a escala planta piloto las fracciones mas contaminadas son el germen y las aguas de maceración mientras que en el proceso a escala industrial la fracción más contaminada es el "gluten meal".

En el proceso de molienda seca se produce una distribución de las fumonisinas en las fracciones obtenidas, mientras que en el proceso de molienda húmeda hay una considerable reducción.

BIBLIOGRAFIA

- AOAC, 1996, Official Method 995.15 Fumonisins B1, B2, and B3 in corn Liquid Chromatographic Method AOAC-IUPAC Method. First Action 1995. Natural Toxins Chapter 49, pp 49 AOAC Official Method of Analysis (1995) Supplement March.

- Bennett G.A; Richard J.L; and Eckhoff S.R. 1996, Distribution of fumonisins in food and feed products prepared from contaminated corn. In Fumonisins in food. Edited by Jackson et al. Plenum Press New York, 317-322.

- Broggi, L.E, Resnik S.L, Pacin A.M, González H.H.L, Cano G. and Taglieri D., 2002, Distribution of fumonisins in dry-milled corn fractions in Argentina. Food Additives and Contaminants 19(5), 465-469.

- C.A.F.A.G.D.A., 2000, Cámara Argentina de Fabricantes de Almidón, Glucosa y Afines.

- Lauren D.R and Ringrose M.A., 1997, Determination of the fate of three fusarium mycotoxins through wet milling of maize using an improved HPLC analytical technique. Food Additives and Contaminants, Vol. 14, N°5, 435-443.

- Funes G.J, Bello M.O, Taglieri D, Resnik S.L y Pacin A.M., 2000, Influencia del proceso en la contaminación por fumonisinas de las distintas fracciones de la molienda húmeda de maíz III Congreso Latinoamericano de Micotoxicología. Córdoba, República Argentina, 6-11 de Noviembre. P25.

- González H.H.L, Martínez E.J, Pacin A.M, Resnik S.L and Sydenham E.W., 1999, Natural co-occurrence of fumonisins, deoxynivalenol, zearalenone and aflatoxins in field trial corn in Argentina. Food Additives and Contaminants 16(12): 565-569.

- Pacin A.M, Broggi L.E, Resnik S.L. and González H.H.L., 2001, Mycoflora and mycotoxins natural occurrence in corn from Entre Rios province, Argentina. Mycotoxin Research 17: 31-38.

- Solovey M.M.Samar de, Somoza C., Cano G., Pacin A. and Resnik S., 1999, A survey of fumonisins, deoxynivalenol, zearalenone and aflatoxins in corn-based food products in Argentina. Food Additives and Contaminants 16(8): 325-329.

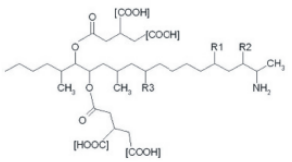

Figura 1. Fórmula estructural de las fumonisinas B_1-B_4:
Fumonisina B_1: R1= OH; R2= OH; R3= OH
Fumonisina B_2: R1= H; R2= OH; R3= OH
Fumonisina B_3: R1= OH; R2= OH; R3= H
Fumonisina B_4 R1= H; R2= OH; R3= H

Tabla 1. Resultados del estudio de la contaminación por fumonisinas en el maíz y las distintas fracciones obtenidas de la molienda húmeda.

FRACCIONES	N °	MINIMO	MAXIMO	MEDIANA
MAIZ FB1	19	1.548	14.287	6.139
GERMEN FB1	18	334	2.584	1.211
GLUTEN MEAL FB1	18	0	3.520	2.062
GLUTEN FEED FB1	11	0	2.648	1.020
FIBRA HUMEDA SIN AGUA DE MACERACION FB1	16	0	1.091	386
FIBRA HUMEDA CON AGUA DE MACERACION FB1	4	180	3.591	1.398
SUSPENSION DE ALMIDON FB1	17	0	205	0
AGUA DE MACERACION FB1	16	0	2.079	492
MAIZ FB2	19	385	6.199	2.170
GERMEN FB2	18	0	2.506	1.032
GLUTEN MEAL FB2	18	0	5.298	3.317
GLUTEN FEED FB2	11	0	868	224
FIBRA HUMEDA SIN AGUA DE MACERACION FB2	16	0	2.087	413
FIBRA HUMEDA CON AGUA DE MACERACION FB2	4	189	797	575
SUSPENSION DE ALMIDON FB2	17	0	198	0
AGUA DE MACERACION FB2	16	0	347	158

FB_1: Fumonisina B_1; FB_2: Fumonisina B_2

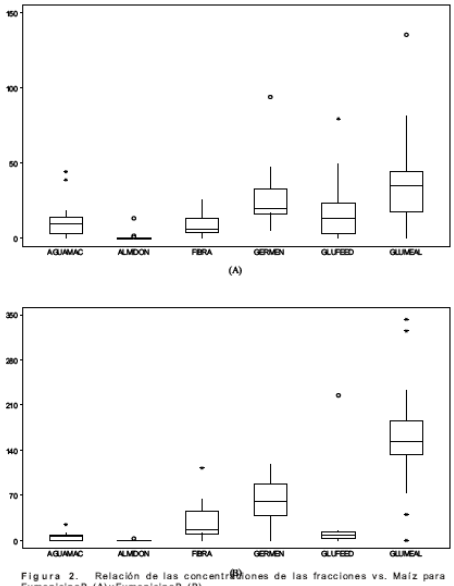

Figura 2. Relación de las concentraciones de las fracciones vs. Maíz para Fumonisina B_1 (A) y Fumonisina B_2 (B)

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ESTIMACIÓN DE LA INGESTA DE ALIMENTOS EN 210 DONANTES DE SANGRE EN LA CIUDAD DE MAR DEL PLATA

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INTRODUCCION

La ocratoxina A (OTA), micotoxina carcinogénica en ratones, posee actividad nefrotóxica, inmunotóxica y teratogénica [1]. La OTA ha sido clasificada por la Agencia Internacional de Investigación del Cáncer en el grupo 2 B, posible sustancia carcinogénica en humanos (figura 1).
OTA fue hallada como contaminante de cereales: trigo, centeno, maíz, cebada y avena [2] [3]; otros alimentos, como diversas especies de habas y legumbres, café, cacao y frutas secas han sido también halladas contaminadas por OTA. La contaminación secundaria ocurre, como consecuencia de la acumulación de metabolitos tóxicos, en carne, vísceras y subproductos (salchichas, huevos) de animales alimentados con cereales contaminados por OTA, y que son ingeridos por la población, ya que los mecanismos habituales de cocción no la degradan totalmente [4] [5].
Por esta razón, si bien muchas de las operaciones relacionadas con el procesamiento de alimentos disminuyen el grado de contaminación por OTA, no la destruyen completamente y por eso es posible hallarla en café, productos derivados del cacao, especias, vino y cerveza [6] [7] [8].
La estimación de la exposición se basa, entre otros conocimientos, en la medición de fuentes conocidas de OTA en la dieta. En la Argentina, los niveles hallados de contaminación de vino por esta toxina fueron nulos o muy bajos [9] [10], esporádicamente se detectó OTA con bajos niveles en alimentos balanceados en Córdoba. Un estudio del "Joint Food Safety and Standards Group" realizado en muestras de maíz que ingresaron al puerto de Gran Bretaña entre 1.998 y 1.999 indica que de las 37 muestras provenientes de la Argentina solamente una muestra presentaba niveles de contaminación entre 0.1 y 1.0 g/kg [11]. La baja o nula ocurrencia puede estar relacionada con una reducción durante la elaboración de ciertos alimentos o con la escasa presencia de hongos productores en esta región.

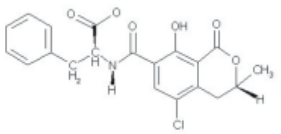

Figura 1. Estructura química de la ocratoxina A

MATERIALES Y METODOS

Se realizó una encuesta alimenticia a 210 donantes de sangre (150 hombres y 60 mujeres) que concurrieron durante el mes de febrero al Centro Regional de Hemoterapia de la ciudad de Mar del Plata, por medio de un formulario que permite recoger información sobre la frecuencia de consumo y las cantidades ingeridas. En la encuesta se consignó la edad, peso, talla y estado sanitario. Se encuestó sobre 43 tipos de alimentos, de éstos se incluyeron 18 alimentos que se consideraron importantes como fuente de contaminación por OTA. Entre ellos cerveza, vino, café, polenta, mezcla de cereales, carne de ave, otras carnes, avena, legumbres, frutas secas, pasta de maní, fiambres crudos, fiambres cocidos, panificados, galletas, fideos secos, chocolate, riñón de cerdo. El tamaño de la porción de los diferentes alimentos fue convertido a peso teniendo en cuenta las porciones estándares.
Análisis estadístico: Se determinaron los valores medios, mediana y desviaciones estándar (*DS*) de los alimentos consumidos. Para el análisis de las cantidades, se consideró la ingesta mensual para cada ítem, convirtiendo las frecuencias de consumo: diaria=30, semanal=4,25, mensual=1 esporádico=0,25 y nunca=0.

RESULTADOS Y DISCUSION

El peso medio de los encuestados fue de 77,4 Kg. (*DS*: 15,38), altura media 170,3 cm. (*DS*: 8,68) y edad media 38,1 años (*DS*: 11.99). Teniendo en cuenta aquellos alimentos que son considerados a nivel mundial como fuente de contaminación de OTA, los resultados de la encuesta muestran que el 84% de la población consume diariamente alimentos panificados, el 65% galletas, el 26% café y el 20,6% vino. Y semanalmente el 83% consume fideos, el 73% aves de corral, el 45% y el 46% fiambres crudos y fiambres cocidos respectivamente y el 17% consume café (tablas 1-3). Las cantidades mensuales ingeridas de alimentos capaces de estar contaminados por OTA, podrían agruparse en productos manufacturados con cereales: panificados 7,35 kg (*DS*: 4,24) y galletas 2,51 kg (*DS*: 2,04), y vino cuyo consumo es de 2,64 lt (*DS*: 4,5 l); seguidos carne de aves de corral (1,3 kg *DS*: 1,98), fideos (969,88 g, *DS*:1007,5), soja (397,97 g *DS*:788,2), carne de cerdo (365,19 g, *DS*: 783,4), fiambres ya sea crudos (330,99 g, *DS*: 559,8) o cocidos (362,9 g, *DS*: 641,8), harina de maíz (264,62 g, *DS*: 532,9) que no tuvo un consumo importante, probablemente debido a que la encuesta se realizó en verano y café (206,68 g, *DS*: 490,6). La cantidad de otros alimentos es escasa como para ser tenida en cuenta, como vía de exposición.

Tabla 1. Frecuencia de consumo de alimentos en 210 donantes de sangre.

ALIMENTO	DIARIO		SEMANAL		MENSUAL		ALGUNAS VECES		NUNCA	
	*N	%	N	%	N	%	N	%	N	%
TE	192	94,1	5	2,5	0	0,0	0	0,0	7	3,4
PRODUCTOS PANIFICADOS	174	84,5	25	12,1	2	1,0	4	1,9	1	0,5
AZUCAR	160	78	3	1,5	0	0,0	4	2,0	38	18,5
VEGETALES CRUDOS	135	65,9	57	27,8	3	1,5	1	0,5	9	4,4
GALLETAS	134	65,0	35	17,0	2	1,0	15	7,3	20	9,7
FRUTAS FRESCAS	129	62,9	55	26,8	7	3,4	5	2,4	9	4,4
FRUTAS ENLATADAS	107	52,2	49	23,9	10	4,9	19	9,3	20	9,8
JUGOS	105	51,2	42	20,5	7	3,4	11	5,4	40	19,5
CARNE DE VACA	96	47,6	97	47,1	7	3,4	2	1,0	2	1,0
LECHE Y SUBPRODUCTOS	82	39,8	62	30,1	23	11,2	13	6,3	26	12,6
VEGETALES COCIDOS	74	35,4	86	41,7	14	6,8	2	1,0	30	14,6
MERMELADA	71	34,6	53	25,9	6	2,9	19	9,3	56	27,3

N: número de personas

OBJETIVO

El objetivo del trabajo es identificar y cuantificar aquellos alimentos que a nivel mundial presentan contaminación por OA para asociar posteriormente el consumo de los mismos, con la presencia de OTA en plasma de dadores de sangre.

Tabla 2. Frecuencia de consumo de alimentos en los 210 donantes de sangre.

ALIMENTO	DIARIO		SEMANAL		MENSUAL		A VECES		NUNCA	
	N	%	N	%	N	%	N	%	N	%
JUGO DE FRUTAS	67	33,3	29	14,4	5	2,5	12	6,0	88	43,8
TODO TIPO DE QUESOS	58	28,2	120	58,3	8	3,9	11	5,3	9	4,4
CAFE	53	26,2	35	17,3	9	4,5	34	16,8	71	35,1
DULCES	43	21,1		24,0	10	4,9	46	22,5	56	27,5
VINOS	42	20,6	40	19,6	4	2,0	11	5,4	107	52,5
SOJA	32	15,6	38	18,5	19	9,3	14	6,8	102	49,8
DULCE DE LECHE	29	14,2	45	22,1	21	10,3	38	18,6	71	34,8
EDULCORANTES	27	13,2	4	2,0	0	0,0	0	0,0	173	84,8
CEREALES	24	11,8	29	14,2	11	5,4	21	10,3	119	58,3
CERVEZA	20	9,8	72	35,1	10	4,9	21	10,2	82	40,0
FIAMBRE COCIDO	19	9,2	95	46,1	38	18,4	32	15,5	22	10,7
FIAMBRE CRUDO	17	8,3	92	45,1	34	16,7	27	13,2	34	16,7
AVES DE CORRAL	17	8,3	150	72,8	18	8,7	8	3,9	13	6,3
SNACK SALADOS	16	7,9	76	37,6	30	14,9	40	19,8	40	19,8
TALLARINES	15	7,3	170	82,5	7	3,4	4	1,9	10	4,9

N: número de personas

Tabla 3. Frecuencia de consumo de alimentos en los 210 donantes de sangre.

ALIMENTO	DIARIO		SEMANAL		MENSUAL		A VECES		NUNCA	
	N	%	N	%	N	%	N	%	N	%
CHOCOLATE	12	5,9	53	25,9	16	7,8	28	13,7	96	46,8
HUEVOS	11	5,4	157	77,3	10	4,9	16	7,9	9	4,4
ARROZ Y DERIVADOS	10	4,9	151	73,3	16	7,8	13	6,3	16	7,8
PASTELERIA	8	3,9	51	25,1	48	23,6	63	31,0	33	16,3
FRUTAS DESECADAS	7	3,4	18	8,8	11	5,4	15	7,3	154	75,1
FRUTAS SECAS	6	3,0	10	5,0	10	5,0	43	21,4	132	65,7
BEBIDAS DIETETICAS	5	2,4	3	1,5	1	0,5	5	2,4	191	93,2
AVENA Y DERIVADOS	5	2,4	8	3,9	10	4,9	8	3,9	175	85,0
POROTOS, LENTEJAS, GARBANZOS	4	2,0	41	20,2	32	15,8	41	20,2	85	41,9
SOPAS	4	1,9	14	6,8	11	5,8	11	5,3	165	80,1
PASTA DE MANI	1	0,5	19	9,4	25	12,4	32	15,8	125	61,9
BEBIDAS ALCOHOLICAS	1	0,5	6	3,0	0	0,0	1	0,5	195	96,1
POLENTA Y PRODUCTOS DERIVADOS DEL MAIZ	1	0,5	57	27,8	36	17,6	44	21,5	67	32,7
OTRAS CARNES	1	0,5	64	31,1	66	32,0	37	18	38	18,4
CARNE DE CERDO	0	0,0	21	10,2	37	18,0	67	32,5	81	39,3
RINON DE CERDO	0	0,0	0	0,0	3	1,5	16	8,0	181	90,5

N: número de personas

CONCLUSIONES

Basados en la información sobre alimentos que son considerados a nivel mundial como fuente de contaminación de OTA, la exposición de la población encuestada podría ocurrir debido al consumo de productos panificados, vino, galletas, y en menor cantidad carne de aves, fideos; otros alimentos como fiambres, carne de cerdo, café, no se consideran relevantes en su aporte a la exposición, y el consumo de riñón de cerdo es nulo por lo que tampoco contribuiría a la exposición de OTA.
La importancia de continuar llevando a cabo encuestas de este tipo radica en la posibilidad de estimar la exposición de la población a intoxicaciones a través de consumo de alimentos contaminados, así como identificar aquellos alimentos sobre los que se debería analizar la presencia de OTA.

BIBLIOGRAFIA

1. Kuiper-Goodman, T., Scott,P. 1989. Risk assessment of mycotoxins Ochratoxin A. Biomedical and environmental. Sciences, 2. 179-248.
2. Trucksess, M.W., Giler, J., Young. K., White, K.D. & Page, S.W. 1999 Determination and survey of Ochratoxin A in wheat, barley, and coffee. 1997 J. AOAC Int., 82, 85-89.
3. Jorgensen, K., Rasmussen, G. & Thorup, I. 1996 Ochratoxin A in Danish cereals 1986-1992 and daily intake by the Danish population. Food Addit. Contam., 13. 95-104.
4. Hult, K., Hokby, E., Sellyey, G., Rutqvist, L. & Gatenbeck, S. 1992 Ochratoxin A occurrence in slaughter-pigs in Sweden and its use us a tool for feed screening programs. J. Environ. Pathol. Toxicol. Oncol., 11,39-40.
5. Patel, S., Hazel, C.M., Winterton, A.G.M. & Gleande, A.E. 1997 Survey of Ochratoxin A in UK retail coffee. Food Addit. Contam., 14,217-222.
6. Krogh, P., 1987, Ochratoxin in food. Mycotoxins in Food, edited by Krogh, P. London: Academic Press, pp. 97-121.
7. JECFA, 2001, Safety evaluation of certain mycotoxins in food, prepared by the Fifty-sixth meeting of the Join FAO/WHO Expert Committee on Food Additives. Series 47 pp. 410-413.
8. Boudra, H., Le Bars, P. & Le Bars, J. 1995 Thermostability of Ochratoxin A in wheat under two moisture conditions. App. Environ, Microbiol. 61, 1156-1158.
9. Pacin A., Resnik S., Vega M., Saelzer R., Ciancio Bovier E., Rios G., and Martinez N. 2004. Occurrence of Ochratoxin A in wines in the Argentinean and Chilean markets. Enviado a publicar.
10. Rosa, C.A.R., Magnoli, C.E., Fraga, M. E., Dalcero, A. M., and Santana, D. M. N., 2004, Occurrence of Ochratoxin A in wine and grape juice marketed in Rio de Janeiro, Brazil. Food Additives and Contaminants, 21, 358-364.
11. Food surveillance, information sheets 1998-1999. http://archive.food.gov.uk/maff/archive/food/survlist/1998surv.htm#nat

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MICOFLORA CONTAMINANTE Y OCURRENCIA NATURAL DE MICOTOXINAS EN EL MAÍZ ALMACENADO Y LOS SUBPRODUCTOS DEL PROCESO DE INDUSTRIALIZACIÓN POR MOLIENDA SECA

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INTRODUCCIÓN

En la República Argentina el maíz (*Zea mays* (L.)) es el cereal de mayor producción. Durante el período 1997/98 alcanzó un total de 19.362.755 Tn representando el 29,2 % de la producción de cereales y oleaginosas. Durante dicho período se exportaron un total de 10.966.791 Tn (56,6 % de la producción) (S.A.G.P. y A., 1999). En la provincia de Entre Ríos, este cereal fue el de mayor producción durante dicho período, correspondiéndole el 29,7 % del total de cereales y oleaginosas producidos en esta provincia, con un valor de 1.020.000 Tn (Cámara Arbitral de Cereales de Entre Ríos, 1998). El mismo representa una importante fuente de ingreso [illegible] economía regional.

INCO-DEV Project Contract number ICA4-CT-2002-10043
Period: January 1, 2003 – December 31, 2006

OBJETIVOS

Los objetivos del presente trabajo fueron: analizar la micoflora contaminante y la probabilidad de aparición de micotoxinas en el maíz y las distintas fracciones [illegible] molienda seca de este cereal, luego de un período variable de almacenamiento de la cosecha 1998/99.

MATERIAL Y MÉTODOS

Las muestras fueron obtenidas de un establecimiento elaborador de la provincia de Entre Ríos. Las distintas fracciones tomadas de este cereal fueron: maíz entero, maíz quebrado, harina "C", rebacillo y harina de maíz. Total 75 muestras.

ANÁLISIS MICOLÓGICO

Maíz entero y maíz quebrado

100 granos + Sn NaOCl 5 % 1' ⇒ lavar 3 veces ⇒ plaquear en agar estracto de levadura-glucosa-doranfenicol (YGCA) ⇒ incubar a 25 °C en la oscuridad por 5 días ⇒ transferir las colonias a agar papa-dextrosa (PDA) en stands y cuadraditos de agar Czapek Dox e incubar a 25 °C por 5 días. Los aislados resultantes fueron identificados de acuerdo con: especies de *Fusarium* (Nelson et al., 1983); especies de *Penicillium* y *Aspergillus* (Pitt y Hocking, 1997); otros géneros (Pitt y Hocking, 1997).

Harina "C", rebacillo y harina de maíz

Se prepararon diluciones en agua destilada estéril (1/10, 1/100 y 1/1000) y se plaquearon en YGCA. Luego se siguió la misma metodología descripta anteriormente.

ANÁLISIS DE DESOXINIVALENOL (DON), AFLATOXINAS (AF) Y ZEARALENONA (ZEN)

Método Nº MYB402a (ROMER)
25 g de muestra + 100 ml de acetonitrilo:agua (84:16)
⇓
Agitar 3'
⇓
Filtrar a través de papel Whatman Nº 4
⇓
Transferir 5 ml del filtrado a un tubo de ensayo de 15 x 85 mm
⇓
Pasar 4 ml del filtrado a través de una columna Myosep 224 ROMER
⇓
Evaporar a sequedad en un baño de 60° C bajo vacío
⇓
Redisolver el residuo en 100 µl de tolueno:acetonitrilo (95:5)
⇓
Sembrar en placas de silicagel (MERCK 1.055553)
⇓
Desarrollar en tolueno:acetona (1:1)
⇓
Visualizar las manchas de AF y ZEN por fluorescencia (AF: 366 nm, ZEN: 254 nm)
Secar las placas al aire, rociar con Sn ACl_4 al 20 % en etanol:agua (1:1) y secar a 150° C por 10'. Estimar los niveles de DON (Rf ± 0.5) por comparación con estándares bajo luz UV de longitud de onda larga

ANÁLISIS DE MICOTOXINAS
ANÁLISIS DE ZEARALENONA (ZEN)

ROMER LABS. Método Nº MYE501
25 g de muestra + 12,5 ml de H_3PO_4 0.1 M + 125 ml diclorometano
⇓
Licuar 1'
⇓
Filtrar por papel Whatman Nº 4
⇓
Transferir 5 ml del filtrado a un tubo de ensayo
⇓
Agregar 4 ml de N-hexano
⇓
Transferir el total del extracto a una columna MycoSep 212 ROMER
⇓
Descartar la solución filtrada y lavar la columna con tres alícuotas de 5 ml de diclorometano
⇓
Descartar la solución
⇓
Eluir con 20 ml de diclorometano:ácido fórmico 99:1
⇓
Llevar a sequedad bajo vacío a 60° C
⇓
Resuspender el residuo en 100 µl de tolueno:ácido acético 99:1
⇓
Sembrar 50 µl en una placa de TLC silicagel 60 (MERCK 1.05553)
⇓
Desarrollar en tolueno:metanol:ácido acético (18:1:1)
⇓
Observar la zearalenona bajo luz UV (360 nm) (fluorescencia celeste-verdosa)

ANÁLISIS DE FUMONISINAS POR HPLC CON DETECTOR DE FLUORESCENCIA

MÉTODO AOAC 995.15 FIRST ACTION 1995
50 g de muestra + 100 ml metanol:agua (3:1)
⇓
Blender 5'
⇓
Centrifugar 10' (500G)
⇓
Filtrar por papel Whatman Nº 4
⇓
Ajustar a pH =7 (NaOH 1M)
⇓
Activar una columna SAX con 5 ml de metanol y 5 ml de metanol:agua (3:1)
⇓
Pasar 10 ml de filtrado
⇓
Lavar con 5 ml de metanol:agua (3:1) y 3 ml de metanol
⇓
Eluir con 10 ml de ácido acético:metanol (1:99) a una velocidad de 1ml/min.
⇓
Evaporar a sequedad a 60° C con nitrógeno
⇓
Redisolver el residuo en 200 µl de metanol
⇓
Derivatizar 25 µl con 225 µl de OPA (o-Ftaldialdehyde)
⇓
Inyectar 10 µl en LC antes de 1 min.

Excitación: 335 nm — Emisión: 440 nm
Fase móvil: metanol:NaH_2PO_4 0,1M (77:23) pH = 3,3 (ác. Ortofosfórico)
Columna: fase reverse C-18, 5 µm, 15 cm x 4,6 mm, 23° C
Velocidad de flujo: 1ml/min — Volumen de inyección: 10µl

RESULTADOS

En la Figura 1 se observan los principales hongos toxicogénicos aislados de las distintas fracciones de la industrialización del maíz por molienda seca. Dentro de los hongos toxicogénicos *F. verticillioides* es el de mayor prevalencia tanto en el maíz original como en sus distintas fracciones concentrándose en el rebacillo. No se detectó aflatoxinas, ocratoxina ni deoxinivalenol en las muestras del maíz entero o sus fracciones. En la Figura 2 se observa la distribución de fumonisinas en las distintas fracciones alcanzando el nivel más alto en el rebacillo. Los rangos alcanzados fueron para FB1: entre 53.8 y 5964.0 µg/kg en el maíz entero, entre 11.9 y 457.0 µg/kg en el maíz quebrado, entre 109.1 y 1500.6 µg/kg en la harina "C", entre 357.4 y 18353.0 µg/kg en el rebacillo y entre 20.70 y 530.20 µg/kg en la harina de maíz. En la tabla 1 se observan las relaciones estadísticas halladas para las diferentes concentraciones de fumonisinas B1 (FB1), B2 (FB2) y B3 (FB3) en las diferentes fracciones, utilizando un método no paramétrico (Statistix 1.0,1996).

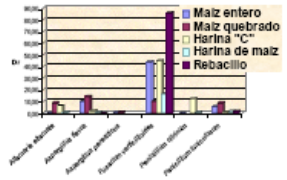


Figura 1. Principales hongos toxicogénicos aislados en las diferentes fracciones de la industrialización del maíz por molienda seca. DI: Densidad relativa

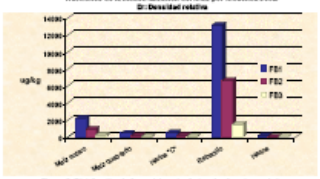


Figura 2. Distribución de fumonisinas (ug/kg) en las fracciones de la molienda seca del maíz.

CONCLUSIONES

Existe relación entre porcentaje de contaminación con *F. verticillioides* y niveles de concentración de fumonisinas en el grano entero y el rebacillo en las muestras analizadas.

El rebacillo que es utilizado para consumo animal es la fracción más contaminada y la harina de maíz que se destina a consumo humano es la fracción con menor grado de contaminación. Ross *et al.* (1991) informaron que niveles de $FB^1 \geq 10$ mg/g pueden ser letales para los caballos. De acuerdo a esta información, el rebacillo contiene niveles elevados de FB^1 (promedio: 4406.60 mg/kg, rango: 367.40 - 18353 mg/kg). Estos niveles podrían significar un serio riesgo al utilizarse esta fracción en alimentos balanceados.

Los resultados determinados en la harina de maíz son más bajos que los determinados por Doko y Visconti en 1993 y 1994 en Italia (rango: 400-3700 mg/kg) y en UK por Patel *et al.* en 1997 (16-2124 mg/kg), similares a los hallados por Pitet *et al.* en Suiza en 1992 (ND-800 mg/kg en grits de maíz) y más bajos que los determinados por Solovey *et al.* en 1997 en productos a base de maíz en comercios de Buenos Aires (Harina de maíz: 60-2860 mg/kg de fumonisina B^1). En base al consumo diario estimado de este producto por niños de 1 a 5 años consumidores de este producto (400 g) (Fochi *et al.*, 1998) la nuestra diaria promedio de fumonisina B^1 sería de 2.12 mg/kg peso. Este valor es 10 veces más alto que el calculado en Canadá (Kuiper-Goodman *et al.*, 1996). Estos valores deben ser tenidos presentes por el amplio consumo de este producto por niños y ancianos.

Tabla 1. Diferencias significativas y altamente significativas entre FB1, FB2 y FB3 (ug/kg) en las diferentes fracciones de la industrialización del maíz por molienda seca.

		Maíz entero			Maíz quebrado			Harina "C"			Harina de maíz			Rebacillo		
		FB1	FB2	FB3	FB1	FB2	FB3	FB1	FB2	FB3	FB1	FB2	FB3	FB1	FB2	FB3
Maíz entero	FB1															
	FB2															
	FB3															
Maíz quebrado	FB1	*								*			*			
	FB2		**							**						
	FB3			**												
Harina "C"	FB1															
	FB2															
	FB3															
Harina de maíz	FB1	**								**						
	FB2		*							**						
	FB3			*												
Rebacillo	FB1	**			*					**			*			
	FB2		**			**				**			**			
	FB3						**									

* p < 0,05
** p < 0,01

- Cámara Arbitral de Cereales de Entre Ríos. Año 1998.
- Doko MB, Visconti A. 1993. Fumonisin contamination of corn and corn based foods in Italy. *UK Workshop on Occurrence and Significance of Mycotoxins*. April 21-23, London.
- Doko MB, Visconti A. 1994. Occurrence of fumonisins B1 and B2 in corn and corn-based human foodstuff in Italy. *Food Add. and Contam.*, 11, 433-439.
- INDEC Secretaría de Agricultura, Ganadería, Pesca y Alimentación. 1999.
- Kuiper-Goodman T, Scott PM, McEwen NF, Lombaert GA and Ng W. 1996. Approaches to the risk assessment of fumonisins in corn-based foods in Canada. *Fumonisins in Food*, Edited by Lauren Jackson, Jonathan De Vries and L. B. Bullerman, *Advances in Experimental Medicine and Biology*, vol. 392, pp. 369-393.
- Nelson PE, Toussoun TA & Marasas WFO. 1983. *Fusarium species. An Illustrated Manual for Identification*. The Pennsylvania State University Press, University Park & London.
- Pocar A, Martínez ME, Pichia ML and Neira MS. 1999. Consumo de alimentos en la población de la Universidad Nacional de Luján. Aporte energético y proteico. *La Alimentación Latinoamericana*, 221, 28-35.
- Patel S, Hazel CM, Winterton AG and Gaudie AE. 1997. Surveillance of fumonisins in UK maize foods and other cereals. *Food Add. and Contam*, 14, 187-191.
- Pitt JI & Hocking AD. 1997. *Fungi and food spoilage*. Blackie Academic & Professional, London.
- Pitet A, Parisod V, Schellenberg M. 1992. Occurrence of fumonisins B1 and B2 in corn-based products from the Swiss market. *J. Agric. Food Chem.*, 40, 1352-1354.
- Ross PF, Rice LG, Plattner RD, Osweiler GD, Wilson TM, Owens DL, Nelson PA, Richard JL. 1991. Concentrations of fumonisin B1 in feeds associated with animal health problems. *Mycopathologia*, 114, 129-135.
- Statistix Versión 1.0. Copyright 1996. Analytical Software. Window versión 95.
- Solovey MME, Sconcia C, Caro G, Pacín A and Resnik S. 1999. A survey of fumonisins, deoxynivalenol, zearalenone and aflatoxins contamination in corn-based food products in Argentina. *Food Add. and Contam.*, 16 (8), 325-329.

INCO: International Scientific Cooperation Projects (1998-2002)

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

Title:

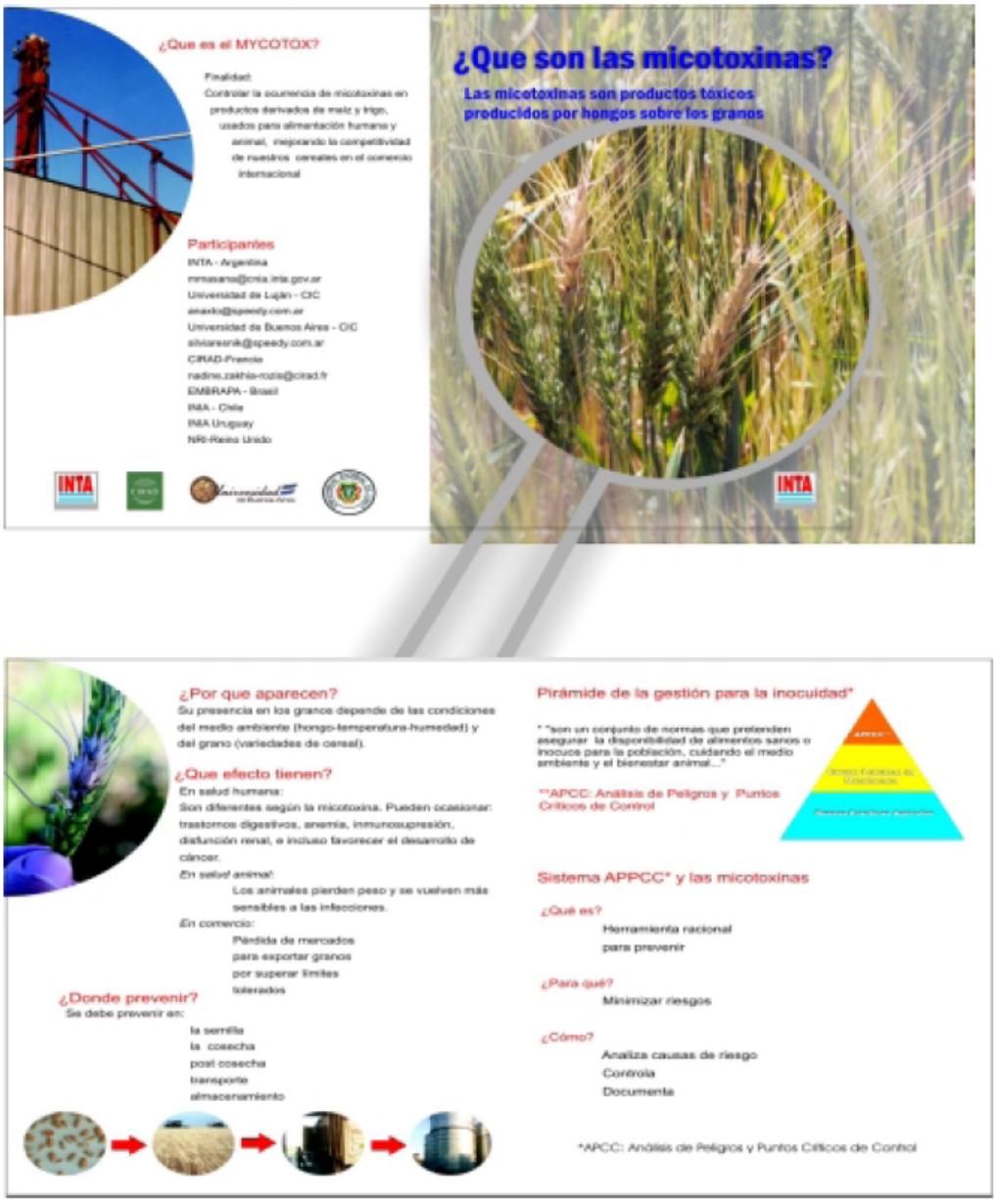
The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries

ANNEXES

Papers and publications

Dissemination documents

Workshop Brochure



¿Que es el MYCOTOX?

Finalidad:
Controlar la ocurrencia de micotoxinas en productos derivados de maíz y trigo, usados para alimentación humana y animal, mejorando la competitividad de nuestros cereales en el comercio internacional

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INIA - Chile
INIA Uruguay
NRI-Reino Unido

¿Que son las micotoxinas?

Las micotoxinas son productos tóxicos producidos por hongos sobre los granos

¿Por que aparecen?
Su presencia en los granos depende de las condiciones del medio ambiente (hongo-temperatura-humedad) y del grano (variedades de cereal).

¿Que efecto tienen?
En salud humana:
Son diferentes según la micotoxina. Pueden ocasionar: trastornos digestivos, anemia, inmunosupresión, disfunción renal, e incluso favorecer el desarrollo de cáncer.
En salud animal:
Los animales pierden peso y se vuelven más sensibles a las infecciones.
En comercio:
Pérdida de mercados para exportar granos por superar límites tolerados

¿Donde prevenir?
Se debe prevenir en:
la semilla
la cosecha
post cosecha
transporte
almacenamiento

Pirámide de la gestión para la inocuidad*

* "son un conjunto de normas que pretenden asegurar la disponibilidad de alimentos sanos e inocuos para la población, cuidando el medio ambiente y el bienestar animal..."

**APCC: Análisis de Peligros y Puntos Críticos de Control

Sistema APPCC* y las micotoxinas

¿Qué es?
Herramienta racional para prevenir

¿Para qué?
Minimizar riesgos

¿Cómo?
Analiza causas de riesgo
Controla
Documenta

*APCC: Análisis de Peligros y Puntos Críticos de Control

Newspaper Article in La Arena

LLEGÓ INVESTIGADOR EUROPEO

Reunión por proyecto de cooperación internacional

GENERAL PICO (Agencia) – Martin Nagler, un investigador de la Unión Europea, se reunió ayer en esta ciudad con autoridades del Inta y de Molinos Don Antonio, visitando además acopiadores y productores, para constatar el estado de avance de un proyecto de cooperación internacional destinado a establecer un sistema de gestión de calidad en cereales.

Nagler, que es investigador del Instituto de Recursos Naturales de Inglaterra fue presentado durante una conferencia de prensa que se realizó en la sede de la Sociedad Rural y que contó con la presencia de Luis Farinella de la firma Don Antonio, Marcelo Masara del Inta Castelar, Daniel Iglesias del Inta Anguil y Rubén Bogino del Inta Pico.

El coordinador zonal del programa Daniel Iglesias señaló en primer término que "éste es un proyecto de cooperación internacional entre la Unión Europea y los países del Cono Sur, en el que participan Brasil, Uruguay, Argentina y Chile y está destinado a establecer un sistema de gestión de calidad en cereales, en este caso en trigo y maíz".

"Argentina, Chile y Uruguay trabajan sobre el trigo y Brasil con el maíz –explicó–. El proyecto está dividido en módulos y cada país tiene la misión de cumplir con uno. Precisamente uno de los módulos es establecer todos los puntos críticos en la molienda, otro es mejorar los métodos de detección o elaborar un manual de gestión de calidad".

Precisó luego que "Argentina coordina el módulo de elaborar un sistema de gestión de calidad para producir trigo libre de microtoxinas y es coordinado por el director del Centro de Investigación del Inta Castelar y participan además, las universidades de Buenos Aires y de Luján, mientras que en el ámbito local, lo hacen el Molino Don Antonio, sus proveedores y la agencia del Inta de General Pico".

"La idea es cuantificar cuál es la cadena de valor de la harina del molino local, investigar cuáles serían los puntos críticos de control en la cadena y establecer cómo se podría construir un sistema de gestión de la calidad para producir harina de calidad y en este caso específico libre de microtoxinas, porque éste es un requerimiento que en los próximos años se va a acentuar aún más, ya que son sustancias que afectan la salud humana".

Luis Farinella de Molinos Don Antonio, Marcelo Masara del Inta Castelar, el investigador Martin Nagler y Daniel Iglesias del Inta Anguil.

Reiteró que "este estudio va a permitir saber cuáles son los puntos críticos en toda la cadena de acuerdo al manejo que se haga".

Luego Masara reveló que "las visitas que se hacen de la Unión Europea son anuales y son para ver cuál es el estado de avance del proyecto. En Montevideo dentro de una semana habrá una reunión global de todos los participantes para discutir los avances en cada uno de los países y lo que viene a ver Nagler es un poco cuál es ese estado de avance".

"Nagler ha estado reunido con nosotros, con la gente del Inta de la zona, viendo los avances específicos del proyecto y ha visitado acopiadores y productores para tener una mirada propia de la situación".

LA ARENA

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Mayo 2003 Número 233

Argentina desarrollará un sistema de gestión de calidad para el Cono Sur

Las micotoxinas son producidas por hongos que infectan al maíz y el trigo, y pueden tener serios efectos carcinogénicos, mutagénicos, e inmunosupresivos en animales o personas que consumen alimentos contaminados. Estas sustancias ocasionan **significativas pérdidas** en el ganado y la industria alimenticia. Se estima que sólo en Canadá y EE.UU. causan mermas anuales por US$ **5.000 millones**.

Este año comenzaron las actividades del Proyecto **"Desarrollo de un sistema de gestión de la calidad de los alimentos, para el control de las micotoxinas en la cadena de producción y procesamiento de cereales, en el Cono Sur de Latinoamérica"**, que cuenta con financiación de la Unión Europea. Participan las **Universidades de Luján y Buenos Aires**, el **INTA** y sus equivalentes: el **EMBRAPA** brasileño, los **INIA de Chile** y **Uruguay**, y el National Research Institute, del Reino Unido, coordinados desde Francia por el **Centre de Coopération Internationale en Recherche Agronomique pour le Développement** (CIRAD).

El objetivo central del proyecto es controlar la aparición de micotoxinas en derivados de **maíz y trigo, destinados a la alimentación humana y animal**, mejorando la **competitividad** de nuestros cereales en el comercio internacional. Se apunta al desarrollo de técnicas innovadoras en la **detección de las micotoxinas**, y al desarrollo de un sistema de monitoreo de su ocurrencia en la cadena. El **Instituto de Alimentos de INTA Castelar** es el responsable de crear un **sistema de gestión de la calidad** en toda la cadena, **en cada país participante**.

Informes: Ing. Agr. Ricardo Rodriguez, dircia@cnia.inta.gov.ar; Marcelo Masana, mmasana@cnia.inta.gov.ar y Norma Pensel npensel@cnia.inta.gov.ar, Instituto de Tecnología de Alimentos del INTA Castelar

INCO: International Scientific Cooperation Projects (1998-2002)

Contract number: ICA4-CT-2002-10043

FINAL REPORT

Start date: 01 January 2003 Duration: 45 months

Title:

The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries

Completed Catalogue Page

The MYCOTOX project (http://mycotox.cirad.fr), (ref ICA4-CT-2002-10043), entitled "The Development of a Food Quality Management System for the Control of Mycotoxins in Cereal Production and Processing Chains in Latin America South Cone Countries" started at the beginning of 2003. It involved partners from France, UK, Argentina, Brazil, Chile and Uruguay. The overall objective of the project was to improve the competitiveness of domestically and internationally traded cereals by controlling the occurrence of mycotoxins in maize and wheat products used as human food and animal feed. The project was based on a multidisciplinary approach, including analytical, technological, socio-economic components in order to ensure, jointly with all stakeholders, quality and safety throughout maize and wheat whole chains.

Abstract prepared at the beginning of the project

Food Safety and quality is a major research topic due to the increasing concern of consumers with public health related issues, along with the stronger sanitary standards set by the EU for agriproduct importation. Mycotoxin contamination of food and feed stuffs is among the top priority issues regarding human and animal safety, along with the economic losses they are responsible for. Although several mycotoxin surveys have been performed on cereals in the Latin America South Cone countries, the surveillance data are incomplete and widely dispersed. The control of mycotoxins is currently pursued mainly through quality control and regulatory procedures. However, the analytical methods (mainly chromatographic) currently used for mycotoxin determination need sophisticated equipment and trained analysts, which makes them unsuitable for routine on-field assessment. There is an urgent need for other accurate, simple and cost-effective techniques. There is also an urgent need for a systematic/proactive, cost-effective approach towards the control of mycotoxins throughout the agri-food chains. The proposed project will address the methodological and analytical issues associated with the establishment of a Food Quality Management System (FQMS), for controlling mycotoxins in the cereal chains in Latin America South Cone countries. The main expected project output consists of an efficient FQMS taking into account the socio-economic context along with the organisational and technological capabilities of the chain stakeholders, focusing on farmers, cooperatives and SMEs.

Results achieved

The results and outputs achieved by the project in order to meet the goal and objectives stated above are:

The analytical chromatographic methods used in the Southern Cone partner laboratories for mycotoxin determination were standardised and harmonised. Reference materials were prepared for the project's use and their use might be extended to the South Cone region. An analytical network was set up for facilitating support to trade and regulatory bodies. Alternative techniques were tested as tools for semi-quantitative measurement or bulk screening. The human exposure to ochratoxin A was assessed in Argentina and Chile, this pioneer data will help the decisions for health policies. Cereal processing steps were evaluated in terms of impact on the mycotoxin distribution in the resultant fractions, this resulted in technical recommendations and corrective actions to be put in place by the cereal industry.

The project has used specific case-studies to pioneer the application of a number of methodologies and approaches to the control of mycotoxins in cereal chains in the Southern Cone. The application of HACCP (Hazard Analysis and Critical Control Points), which was initially focusing on technical aspects at lab level, to the whole agri-food chain including stakeholders, and the full integration of socio-economic and technical inputs, has been successfully demonstrated and can therefore be recommended for use throughout the Southern Cone for the control of mycotoxins (and by extension to other types of contaminants) in all cereals and cereal products (and by extension to other types of commodities).

The project succeeded in establishing multidisciplinary HACCP teams for field work, the generated technical and socio-economic data were fully integrated and put together to develop HACCP plans and Good Agricultural Practices, resulting in a methodological architecture and concrete recommendations for implementing an efficient Food Quality Management System adapted to each country case.

Contract number : ICA.-CT-2002-10043 **Duration: 2003-2006**

Data sheet for final report

(to be completed by the co-ordinator for the whole project)

1. Dissemination activities	Published	Submitted
Number of communications in conferences	30	-
Number of communications in other media (internet, video, …)	26	-
Number of publications in refereed journals *(+ 12 under preparation)*	10	4
Number of articles/books	6	-
Number of other publications		
•1 Posters in scientific events	42	-
•2 Dissemination documents	11	-
•3 Articles in newspapers	12	-
•4 Dissemination conferences	11	-
•5 Sectorial meetings with stakeholders	17	-
•6 Participation in workshops on mycotoxins	6	-

2. Training	
Number of PhDs	8
Number of MScs	5
Number of visiting scientists	1
Number of exchanges of scientists (stay longer than 3 months)	5
Training courses delivered to food professionals	3

3. Achieved results	
Number of patent applications *(3 published + 3 under preparation)*	6
Number of patents granted	-
Number of companies created	1
Number of new prototypes/products developed	1
Number of new tests/methods developed	8
Number of new norms/standards developed	-
Number of new softwares/codes developed	-
Number of production processes	-
Number of new services	-
Number of licenses issued	-

4. Industrial aspects

Industrial contacts	yes	X	no
Financial contribution by industry	yes	X	no
Industrial partners : - Large	yes	X	no
- SME	yes	x	no

5. Comments

Other achievements (use separate page if necessary)

Relationships and collaborations were made with:

- The European Cluster on mycotoxins.

- The French Consortium on mycotoxins through the RARE Fusariotoxin project funded by the French Ministry for Research.

- The French National Institute for Agricultural Research (INRA) in Montpellier, Bordeaux and Rennes. This scientific collaboration was carried out through the joint supervision of the Chilean PhD, Ms Gisela Rios, from the University of Concepción, Chile (partner 11) (within the framework of WP 3).

- The MYCO-GLOBE Project (FP6, Specific Support Action): *"Integration of Mycotoxin and Toxigenic Fungi Research for Food Safety in Global System"*, through participation of MYCOTOX general coordinator in the steering committee of MYCO-GLOBE project, and strong participation of the MYCOTOX consortium in the MYCO-GLOBE conference on mycotoxins held in Argentina (15-17 March 2006).

- The CEREFER Project (FP5, INCO-DEV Programme): *"Meeting Consumer Requirements for Cereal-Based Fermented Foods with Improved Nutritional and Sanitary Quality and Shelf-Life in Africa"*, through participation of the MYCOTOX general coordinator (as member of the scientific committee and speaker) in the CEREFER conference held in Spain (6-8 September 2004).

- Participation of the MYCOTOX partners involved in analytical aspects in the Latin American electronic forum on mycotoxins "Micotoxinfo" (micotoxinfo@toxi.scu.sld.cu)

- Participation of 7 project members in the FAO training (in Spanish) (September 2003) on the specific application of HACCP method to mycotoxin prevention and control.

- The MYCOTOX general coordination acted as an interface for initiating contacts between some partners of the MYCOTOX consortium and the French enterprise (ECCLOR S.A.) interested in collaborations in South America upon cereal storage and OTA management within the silos.