
Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT:
Covering period from 1 January to 31 December 2003

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

Keywords:
Mycotoxins, Latin America, HACCP, Risk Management, Analytical Measurements

Project homepage:
For the first project year, the Intranet site "Quick Place" was implemented as an internal tool for information exchange with a restricted access to the project partners and associate institutions. All meetings reports and presentations were uploaded there. However, due to some software limitations and after one year-experience, this tool shows to be not enough user-friendly. We are moving towards the implementation of an Internet site with outside access to everybody and some restricted access areas dedicated to the project outputs and confidential issues. This will allow us on one hand to ensure a better diffusion of the project (general presentation, objectives, partners, activities) to the scientific community over the world and on the other hand, to keep a confidential space for the project partners, advances and outputs.

Coordinators:
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Summary
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The MYCOTOX project (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 involves partners from France, UK, Argentina, Brazil, Chile and Uruguay. The overall objective of the project is 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 involves 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 activities achieved in 2003 were in concordance with the initial proposal planning and the deliverables were issued as expected in the contract technical annex. Some delays occurred at the project start mainly for administrative arrangements and fund transfers.

Among the technical and analytical activities performed in 2003 was the standardisation and harmonisation of the existing chromatographic techniques for mycotoxin determination. This was achieved through the implementation of interlaboratory works between the partner laboratories for mycotoxin (aflatoxin, zearalenone, deoxynivalenol and fumonisins) determination on FAPAS reference materials, i.e. standard contaminated matrices, which allowed to evaluate and compare the lab performances and to put in place corrective actions when needed for improving the recovery percentages and Z score values. On the other hand, the preparation of homogeneous samples of naturally contaminated maize was initiated by one accredited partner laboratory with the aim to prepare internal reference materials that will serve for further interlaboratory works within the project.

As the conventional testing for mycotoxins require expensive, sophisticated equipment (e.g. high performance liquid chromatography, thin layer chromatography) and trained analysts, the access to these procedures is beyond the means of smaller players within the commodity system. Alternative techniques were then prospected for potential use as cost-effective, sensitive and simple analytical tools for mycotoxin determination in wheat and maize and derived products. A deep literature review was done on the chemiluminescent and bioluminescent techniques. A study was initiated on the use of a natural luminescent potential within a category of bacteria as an indirect means for mycotoxin measurement, the emitted light being inhibited by the presence of mycotoxins. Results are expected for the first semester of 2004. On the other hand, preliminary trials were carried out using the Near Infrared Reflectance Spectroscopy for the spectra acquisition on wheat and derived fractions. It was shown that this technique is potentially interesting as a screening tool for high numbers of commodity bulks. A higher number of samples is still to be analysed for improving the spectra significance.

To evaluate the risk assessment of human exposure to Ochratoxin A (OTA), blood samples were collected from volunteers and questionnaires were filled to survey the diet of those blood donors. The method for OTA determination in blood was standardized and will be applied to the collected blood samples. In addition,
commodities with high risk of human exposure to OTA were identified, such as wine and cereals. The method for OTA determination in wine was standardised and applied to 85 national and imported wines. The measured OTA contents will allow calculation of the level of human exposure in Argentina and Chile through wine consumption. The method for OTA determination in wheat is under development.

The milling procedures might be critical points for the grain quality and safety. A precise and accurate determination of deoxynivalenol (DON) in wheat and derived products is essential to assess the extent of human exposure to this mycotoxin. However, the DON contamination in grains shows a heterogeneous distribution. The wheat and maize milling diagrams, either dry or wet, were reported and samples were collected throughout the milling process. The method for DON determination in wheat and derived milling fractions was standardised and implemented. The statistical analysis of the contamination distribution for DON in wheat and derived fractions gave valuable data for improving the sampling plan and reducing the total variability. The method for fumonisin determination in maize and derived milling fractions is under development.

Conventional methods of food safety management depend heavily upon end-product testing followed by the segregation of acceptable and unacceptable product. The chosen approach in the project was to adapt the HACCP concept for use throughout the whole agrifood chain. HACCP is a multidisciplinary, proactive approach that focuses upon the control of the process, and by extension, of the whole agrichain, at specific critical points, rather than focusing only upon the finished product. Within the project activities performed in 2003, HACCP multidisciplinary teams were assembled in Argentina, Brazil, Chile and Uruguay, composed of the involved partner institutions together with representatives of key players from the private sector. A representative of each team followed a specific training on the application of HACCP for mycotoxin prevention and control. Confidentiality agreements were elaborated and formalized with the private enterprises participating in the project.

Information on the occurrence of mycotoxins in wheat and maize were collected, gathered and collated in the 4 countries partners. Those data were carefully examined and the priority mycotoxin-commodity combinations were selected in each country. The Commodity Flow Diagram (CFD) for each selected combination was then constructed, including the product flow, the environmental and agro-climatic conditions, the involved key players and the formal or informal relationships between them. The CFDs are under validation for describing at which steps the mycotoxin hazard originates or concentrations increase to unacceptable levels. On the other hand, methods were discussed and shared among the project partners for adapting and using harmonised tools to ensure a thorough understanding of the socio-economic, cultural and institutional issues. This understanding is essential for the further steps of proposing and validating appropriate control measures adapted to the local context of each country.

Three meetings were held during 2003 and the project partners participated in many scientific events. Various documents were issued such as scientific peer-reviewed papers (1 published and 2 submitted), oral presentations in conferences (5), posters (8), dissemination flyers (2). In addition, training was a strong component of the project’s strategy, in terms of PhD thesis (2 PhD students performed their last year
within the project frame, 1 PhD will start in 2004), and short term trainings (4).

We should highlight the enthusiasm and willingness of all partners to join their efforts for tackling the project challenge. This allowed a fluid and smooth cooperation between the project partners in 2003, along with permanent exchanges of technical and scientific information, methods, bibliographic references, relevant websites and scientific events.

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Scientific annual report
Scientific Annual Report

We should precise that in spite of the contract signature in January 2003, the project activities were effectively launched by the end-February 2003 after the kick-off meeting. This was due to i) some preliminary and essential administrative arrangements for project coordination and management logistics and ii) the unavailability of Latin American partners in the period January-February as it is annual holiday period in the Southern Cone region. Accordingly, this report actually summarises the activities and outputs performed in the period March 2003-March 2004.

Content

The scientific activities and results carried out during the reporting period are synthetised and presented below by tasks (within the corresponding work package) along the lines of the technical annexe of the contract. For more technical details, see the individual reports of the partners involved in the corresponding task.

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

(Leadership: Dr Tania Barreto Correa, EMBRAPA, Partner 4)

Objective of WP1: 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.

To meet the objective of WP1, the activities carried out are related to i) implementation of laboratory 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 chromatographic techniques for mycotoxin determination and ii) prospecting of alternative analytical techniques with a focus on their sensitivity and ease-to-use as on-field routine tools for mycotoxin determination.

We should precise that during 2003, two additional laboratories (partners 9 & 10 - Argentina) accepted to take part in the WP1 activities, even if not initially involved in this work package. Their involvement, mainly in the interlaboratory works, is very useful for the project because i) the increase of interlaboratory participants will improve the statistical significance and representativity of lab performances and ii) the partners 9 & 10 laboratories will perform the mycotoxin analysis on the samples which will be on-field collected in Argentina throughout the whole cereal chains in the frame of WP 4 & 5. This latter point is very positive for the project running because it contributes to the formalisation of close relationships and collaborations between the “analytical laboratories” and “field specialists” in Argentina, which was already done in the other three South Cone countries (Brazil, Chile and Uruguay). We should point out that this was a successful achievement which surely contributes to reach the project’s goal.
Task 1: Inventory of the analytical chromatographic methods currently used by the WP1 partners for mycotoxin determination in wheat and maize

A questionnaire was elaborated by the WP1 leader (EMBRAPA-partner 4) and distributed to all WP1 partners in order to collect information on the analytical chromatographic methods they are currently using for mycotoxin determination in wheat and maize. An inventory of all technical information related to the extraction, clean-up, analysis and mycotoxin calculation as applied in all WP1 laboratory partners was done. Based on this survey, the partner 4 elaborated a sheet for harmonising the mycotoxin result reporting and ensuring that all appropriate information will be taken into account for evaluating and comparing the laboratory and method performances (for further details, see the individual reports of the concerned partners).

On the other hand, the WP1 partners implemented and/or adjusted the chromatographic methods when needed for further use during the interlaboratory works. For instance, before starting MYCOTOX project, the partner 4 was using Thin Layer Chromatography (TLC) for aflatoxin and zearalenone determination. In 2003, the HPLC method was implemented for zearalenone determination in maize as a more effective analytical tool. This implementation was effective further to literature review and preliminary trials for selection of the best method and experimental conditions.

Task 2: Elaboration of protocols for harmonization of the analytical procedures and diffusion to all WP1 partners

Given that different chromatographic methods were used by WP1 partners for mycotoxin determination and with the aim to evaluate and compare the performances of labs, it was essential that all involved partners follow the same standardized and harmonized procedures for sampling and result reporting and statistical analysis. According to the experience of MAA (partner 5) as accredited laboratory in Brazil, this partner elaborated protocols for intralaboratory and interlaboratory controls and for sampling guidance and diffused them to all WP1 partners. Those protocols are considered as the guidelines within the project for sampling, result reporting and data analysis. Details on the protocols are given in the individual report of partner 5.

Task 3: Implementation of an interlaboratory work between all WP1 partners on 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 WP1. The following reference materials were purchased: i) maize with zearalenone (T 2209), ii) maize with fumonisins B1&B2 (T 2208 then T 2211), iii) maize with aflatoxins B1&B2&Total (T 0446 then T0453) and iv) wheat flour with DON (T 2210). This latter was purchased and sent to the partners by the end of 2003 because it was not available on stock when the other materials were initially ordered.

An interlaboratory work was put in place among the WP1 partners through the application of their respective analytical methods (and according to the protocol elaborated by partner 5 and mentioned in Task 2) for mycotoxin determination in the first three reference FAPAS materials. The partners 9 & 10 will determine mycotoxin
content on the same FAPAS materials in 2004 as they received the materials later than the other partners initially involved in WP1.

The results were reported according to the protocols elaborated in Task 2 and the percentage of recovery and the standard deviation were calculated for each partner and for each FAPAS material. The recovery percentages for all laboratories are within the satisfactory ranges of the European Committee for Standardisation (1999). All partners obtained satisfactory values within the FAPAS range but some experiments are to be repeated in 2004 because of some unsatisfactory Z score values.

In addition, 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.

This first serie of interlaboratory works was very useful for setting a network among the WP1 partner laboratories. Corrective actions were identified and will be implemented for improving the lab performances. This lab network will have a key role in supporting the activities of WP 4&5 through the analysis of samples collected on-field throughout the whole cereal chains and flow diagrams.

**OUTPUT related to Tasks 1 to 3:** The deliverable D4 entitled “Standardised and validated analytical chromatographic methods applicable by all partner laboratories for mycotoxin determination in wheat and maize” was achieved in due course as planned in the contract technical annex.

**Task 4: Preparation of internal regional reference materials (naturally contaminated & spiked samples) for proficiency test rounds among WP1 partners**

Another challenge raised within the WP1 was the elaboration of internal homogeneous reference materials, i.e. i) blank or mycotoxin-free materials and ii) naturally contaminated wheat and maize (by grain mixing in adequate proportions). Those reference materials are to be used for interlaboratory works among the WP1 partners and by extension might be used within the Latin American continent. Accepting this challenge can answer the need expressed by many scientists at the IV Latin American Congress of Mycotoxicology (Cuba, September 2003) for available reference materials in the region but less expensive than those marketed at the international level such as FAPAS ones. We should precise that those internal reference materials will be first used within the MYCOTOX frame but should be later certified by international and regional scientific authorities not involved in the project for further use as regional reference in the Latin American continent.

The partner 5 (MAA) initiated in 2003 the preparation or homogeneous maize reference samples, either blank or naturally contaminated with aflatoxin, zearalenone and fumonisin. Those materials will be then sent by partner 5 to all partners involved in WP1 for the next interlaboratory work.
Task 5: Literature review on the Bioluminescence and Chemiluminescence techniques as innovative tools and prospects for mycotoxin determination

The sensitivity, speed and convenience of chemiluminescent (CL) and bioluminescent (BL) immunoassays have led to a diverse range of applications for these technology, mainly in the clinical laboratory, pharmaceutical industry and food testing. Chemiluminescence is based on the light emitted when a luminescent chemical substrate moves from the excited to initial state, in the presence of hydrogen peroxide. Chemiluminescence is used for detection or amplification of the antibody-antigen reaction within immunoenzymatic assays (ELISA). Bioluminescence is based on the use of intrinsic adenosine triphosphate (ATP) of microorganisms and its reaction with firefly luciferase to produce an amount of luminescence that may be directly related to the microbial count and can be measured with a suitable luminometer.

At the stage of proposal writing, preliminary works carried out by CIRAD (partner 1) on the application of chemiluminescence for aflatoxin determination in groundnut and yam chips indicated an interesting potential for this innovative investigation, especially as a rapid and easy-to-use on-field routine measurement technique. However, those works showed that chemiluminescence was a good detection technique complementary to available ELISA kits but was not able to be used as a direct measurement for aflatoxin determination. This might then increase the unit cost of mycotoxin analysis and is not suitable for analysing high numbers of samples within the MYCOTOX project. On the other hand, the comparison of aflatoxin measurements through chemiluminescence and HPLC was not convincing because of low reproducibility and difficulty for stabilising the luminescent signals.

In order to optimise the project resources and to take benefit from the above mentioned advances, we undertook a deep literature review on the existing applications of chemiluminescence and bioluminescence techniques with a focus on mycotoxin determination (this led to a paper submitted to “Luminescence” journal for publication, see Papers and Publications section below). Bioluminescence was shown to be suitable for bacterial determination and not for molds, because of the difficulty for ATP extraction from molds. We decided then to reorient our investigations towards the use of “toxicity tests” which are based on the natural ability (through specific “lux” genes) of some bacteria to emit light when one specific aldehyde internal to their metabolism is oxidized. The emitted light is measured with a luminometer and is proportional to the bacterial growth. In presence of inhibitory molecules such as antibiotics, antimicrobial agents or even mycotoxins, the light emission is lower because the bacterial activity is reduced. An indirect way for mycotoxin determination might be then the mycotoxin concentration for which the bacterial light emission decreases to the half. Experiments were planned for testing this innovative investigation. Two bacteria (Vibrio fischeri and Vibrio harveyi) and aflatoxin were chosen for the first trials which started at the beginning of 2004.

Task 6: Preliminary work on the application of Near Infrared Reflectance Spectroscopy (NIRS) technique for mycotoxin determination

A preliminary work was carried out by CIRAD (partner 1) and LATU (partner 12) for DON determination in wheat and derived milling fractions by using the Near Infrared Reflectance Spectroscopy (NIRS). This technique is based on the relationship between the chemical composition of the organic matter and its absorption of infrared
wavelengths. The NIRS technique is fast and inexpensive but it 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.

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. The NIRS prediction model seems to be significant and allows distinguishing the samples with a high DON content from those with a low DON content. A more detailed study should be conducted on samples with low contamination.

This preliminary study confirms the interest for pursuing the investigation on NIRS potential for mycotoxin determination. However, it is essential to analyse a higher number of samples. To this end, all samples to be collected in 2004 within the field activities of WP 4&5 and from WP1 & 3 will be sent to the partner 1 for NIRS analysis.

Task 7: Evaluation of the Toximet mini-column as a potential on-field tool for mycotoxin determination

This task is a part of both WP1 (for analytical aspects) and WP5 (as a potential control measure throughout the commodity whole agrichain). The Toximet is a simple, inexpensive, robust, user-friendly and efficient procedure for accurately detecting and measuring poisonous compounds in foods. It is planned that the procedure will approach the efficiency of highly expensive quantitative methods, such as high performance liquid chromatography, but at a fraction of the cost. The Toximet approach will include novel equipment, consumables and methods that can be applied by semi-skilled operators in a wide variety of environments, throughout the global food chain. The Toximet procedure is under development by NRI (partner 2) and will be likely evaluated in the frame of MYCOTOX project by the end of 2004.
**Work Package 2: Risk Assessment of Human Exposure to Ochratoxin A**
(Leadership: Dr Ana Pacin, University of Lujan, 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 WP2, 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: Selection and standardisation of a methodology for OTA determination in 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) for standardizing the method, i.e. extraction, OTA detection and confirmation and improving the recovery values and method reproducibility.

**Task 9: Implementation of the selected and standardized method for OTA determination in blood samples in Latin America South Cone Laboratories**
Exchanges between the involved partners allowed to fully standardize the method which is currently implemented in the labs of partners 9 & 10 (Argentina) and partner 11 (Chile). The partner 11 moved towards using the same Ochraprep immuno-affinity columns than Argentinean partners 9 and 10.

**OUTPUT related to Tasks 8 and 9:** The deliverable D5 entitled “A standardised methodology for OTA determination in blood samples implemented in Latin America South Cone Laboratories” was achieved in due course as planned in the contract technical annex.

**Task 10: Blood sampling in Argentina and Chile**
200 blood samples were collected from a hospital (Centro Regional de Hemoterapia, Mar del Plata, Buenos Aires, Argentina). In Chile, blood samples are still to be collected. Three representative locations were selected and the authorization for blood sampling was obtained from the Health Department authorities. The standardised method is then to be applied for OTA determination in those blood samples.

**Task 11: Survey on the diet of the blood donors**
A questionnaire was elaborated by the University of Lujan (partner 10) on the global diet in Argentina, including the different types of consumed foodstuffs, the ingested quantities and the frequency of ingestion. The questionnaire was filled by the donors of blood samples mentioned in Task 10 with the aim of identifying any potential correlation between the most frequently commodities they ingest and the OTA
content in blood. The questionnaire was transferred to partner 11 in Chile for the same purpose.

**Task 12: Selection of commodities with high risk of human exposure to OTA**

Significant contamination of wine has been reported by many authors in different countries (see references in the individual annual reports of partner 10). The Codex Alimentarius Commission reported in 1998 that wine, especially red wine, is the second major source of human exposure to OTA, cereals being the first one. Due to 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. It is essential to note that OTA determination in both wine and cereals is the preliminary action for the next planned activities related to risk characterization for OTA in the South Cone countries.

**Task 13: Standardisation and implementation of a methodology for OTA determination in wine**

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 is in agreement with previous reports showing a low toxigenic capacity for those fungi isolated from grapes grown in the region.

**Task 14: Calculation of the level of human exposure to OTA through wine consumption**

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.

We should note that there are a few literature on the exposure to OTA through wine consumption in Argentina and Chile which make the works performed within WP2 pioneer and essential for a further risk characterization for human exposure to OTA in those countries.

**Task 15: Standardization and validation of the methodology for OTA determination in wheat**

A HPTLC methodology is under standardisation in Chile (partner 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. Permanent exchanges between Chile (partner 11) and Argentina (partners 9 & 10) will allow the further validation and implementation of the same method in those laboratories.
Work Package 3: Evaluation of Milling Procedures as Potential CCPs
(Leadership: Dr Silvia Resnik, University of Buenos Aires, Partner 9)

Objective of WP3: 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.

A precise and accurate determination of DON contamination in wheat and wheat 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.

Task 16: Reporting of the wheat and maize milling diagrams in Argentina and Chile
As first step of the WP3, the diagrams of wheat and maize milling in Argentina and Chile were reported and fully analysed. It is interesting to point out that the industrial milling processes are different in the two countries. In Chile, only dry milling is usually practiced, either for wheat or maize. In Argentina, wet milling is 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 is needed in grains and all derived milling fractions, i.e. flour, starch, gluten, bran and germ.

Task 17: Standardisation of the methodology for deoxynivalenol (DON) determination in wheat and derived milling fractions
In Argentina, the gas chromatography method with electron capture detection (GC-ECD) was standardised and validated for DON determination in wheat and derived milling fractions. For optimising the project financial resources, the work was divided into two parts. The extraction and clean up of analytical samples were carried out at the University of Lujan (partner 10) according to the slightly modified method of Truckess 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) 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. The partner 11 will take benefit of this new analytical tool for implementing the method used by the Argentinean partners 9 & 10 and using it in parallel to the HPTLC one for result confirmation and validation.

Task 18: Design of an efficient sampling plan for wheat milling in Argentina and Chile and DON determination on the samples
Procedures for wheat sampling 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. 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. 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) and their DON content was determined.

**Task 19: Statistical analysis of the contamination distribution for DON in wheat and derived milling fractions**

An ANOVA statistical test was conducted on the data obtained in Argentina from DON determination in wheat and derived milling fractions. This showed differences among the samples probably caused by the sampling variability. The mean squares within the samples may 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 distribution of DON contamination 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 contamination in wheat and derived milling fractions will help for improving 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 will be used in 2004 by the partner 11 in Chile.

**OUTPUT related to Tasks 16 to 19:** The deliverable D1 entitled “Standardised procedures for wheat and maize sampling during milling processes in the South Cone region” was achieved in due course as planned in the contract technical annex.

**Task 20: Standardisation of the methodology for fumonisin determination in maize and derived milling fractions**

The method for fumonisin determination in maize and derived milling fractions was standardised and validated in the labs of Argentinean partners 9 and 10. 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 then determined on subsamples according to the standard AOAC method.

**Task 21: Determination of fumonisins in maize and derived milling fractions**

Preliminary experiments were carried out in Argentina for fumonisin determination in 100 maize subsamples. The distribution of fumonisins in maize showed a great asymmetry. The work needs further development and is to be repeated along the different technological steps of wet maize milling to allow estimation of the variability.
and distribution of fumonisin contamination in the whole grain and the derived milling fractions. Chile is not concerned by maize wet milling and a few fumonisin contamination has been reported in Chilean maize. However, according to the results to be obtained, the Argentinean partners might propose, if convenient, to enlarge the study to other mycotoxins that might appear on maize in the other South Cone partner countries.

**Work Package 4: Hazard Analysis of Mycotoxins**

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

**Objectives of WP4:** 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 22:** Establishment of multidisciplinary HACCP (Hazard Analysis and Critical Control Point) teams

HACCP multidisciplinary Teams were assembled in Brazil, Uruguay, Argentina and Chile, composed of representatives of the involved project partner institutions together with representatives of non-project partners (universities for instance) and key players from the commercial sector. 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).

**Task 23:** 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.

**Task 24:** Decision making regarding the need for further information on the occurrence of mycotoxins

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. After careful examination of the data collected, no additional surveillance data was considered to be required at this stage.
**OUTPUT related to Tasks 22 to 24:** The deliverable D2 entitled “A report documenting mycotoxin surveillance data, both from the literature and from surveillance studies conducted by this project” was achieved in due course as planned in the contract technical annex.

**Task 25: Completion of preliminary Hazard Analysis and identification/selection of mycotoxin-commodity combinations**

The bibliographic and collated data for each country were carefully examined and the following commodity-mycotoxin(s) combinations were selected for further study:

- Argentina: deoxynivalenol & zearalenone in wheat (flour production)
- Brazil: aflatoxins, ochratoxin A, fumonisins, deoxynivalenol & zearalenone in corn (poultry feed production)
- Chile: deoxynivalenol & zearalenone in wheat (flour production)
- Uruguay: deoxynivalenol & zearalenone in wheat (flour production)

**Task 26: Following of a specific training on the application of HACCP method to mycotoxin control**

A representative of each multidisciplinary team per country partner followed a FAO training in Spanish on the HACCP method and its specific application to mycotoxin prevention and control. The direct benefits of this training were: i) a harmonization of the HACCP glossary and methodological tools among the project partners, ii) a share of Latin American experiences and case studies on HACCP application to mycotoxins, and iii) the access to the same manuals and methodological documents.

**OUTPUT related to Tasks 25 and 26:** The deliverable D3 entitled “A report describing the hazard analyses conducted and justifying the commodity-mycotoxin combination selected for further study” was achieved in due course as planned in the contract technical annex.

**Task 27: Making contacts with key players in the Commodity System (producers to consumers). This task is also valid for Work Package 5 and is closely related to Task**

All partners have made contact with key players in their respective commodity systems. A confidentiality agreement was prepared and validated by all partners for use with the commercial sector actors. Some commercial players (in Brazil for instance) agreed to receive the trainees supervised by the project partners for carrying out joint activities on the CFD (Commodity Flow Diagram) and HACCP application to the whole agrichain. However, it should be noted that the sensitivity of some of key players, regarding the mycotoxin contamination of their products, has delayed their effective commitment to participate in the CFD construction and validation.

**Task 28: Construction of the Commodity Flow Diagram (CFD) for selected mycotoxin-commodity combination(s)**

All partners have constructed a preliminary CFD for the relevant component of their commodity system. The CFDs are currently being refined in consultation with key players in the respective commodity systems.
Task 29: Verification of the CFDs for selected mycotoxin-commodity combination(s)
This task is partially completed. It has been delayed by the reluctance of some key players to become involved in an examination of the occurrence of mycotoxins in their commodity system. It is planned that the verification of the CFDs will now be completed, by all the partners, by mid-April 2004.

OUTPUT related to Tasks 27 to 29: The deliverable D6 entitled “A report documenting the verified commodity flow diagrams for each commodity-mycotoxin combination in each of the selected countries” which was expected at month 12 in the contract technical annex is to be achieved by the end of April 2004

Work Package 5: Identification and Validation of Mycotoxin Control Measures
(Leadership: Prof Ray Coker, NRI, partner 2)

Objectives of WP5: 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 30: Identification of key players in the selected CFDs. This task is closely related to Task of WP4
Key players have been identified and their participation in the project has been discussed. Private sector representatives are now effectively involved in the project activities in Uruguay and Chile. The confirmation of their involvement was effective by the end-February 2004 in Brazil and by end-March 2004 in Argentina.

Task 31: Sharing, discussion and adaptation of existing participatory methods for socio-economic issues related to the introduction of mycotoxin control measures
A workshop has been held at the University of Campinas, Brazil (project associate institution, see section Management Annual Report, paragraph Organisation of the collaboration) involving socio-economists from each of the HACCP Teams and an outside adviser from the University of São Paulo, Brazil (see section Management Annual Report, paragraph Meetings). Methods for the collection, presentation and interpretation of data were discussed and shared, 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).

The outputs of WP5 as initially stated in the contract technical annex are expected for 2004.
**Work Package 6: Development of a Food Quality Management System**

(Leadership: Dr Ricardo Rodriguez, INTA, Partner 8)

**Objective of WP6:** 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.

According to the timetable presented in the contract technical annex, the WP6 activities should start in year 2 (2004). Indeed, those activities are based on the first results and outputs from WP 4&5 which are presently in progress (validation of the CFD in each country, testing and validation of potential control measures at the identified critical control points of the CFD, etc). Results of WP6 will be reported in the second scientific annual report (2004).

**Contribution of participants**

**Work Package 1 (led by the partner 4)**
The partners 1, 4, 5, 11 and 12 were involved in WP1. The partners 4, 5, 11 and 12 worked on the chromatographic methods for mycotoxin determination and carried out interlaboratory works on FAPAS reference materials. The partner 5 was in charge of elaborating the adequate protocols for interlaboratory works and guidance for further sampling. The partners 9 & 10 will join the interlaboratory work in 2004 even if not initially involved in WP1. The partner 1 was in charge of prospecting alternative techniques such as luminescence and NIRS (this latter in collaboration with the partner 12).

**Work Package 2 (led by the partner 10)**
The partners 9, 10 and 11 were involved in WP2. The partners 9&10 were in charge of standardizing the method for OTA determination in blood and transferred it to partner 11. The partner 10 elaborated questionnaires for surveying the diet of blood donors. The three partners worked on the method standardization for OTA determination in cereals (still under development).

**Work Package 3 (led by the partner 9)**
The partners 1, 9, 10 and 11 were involved in WP3. The partner 9 designed a sampling plan for collecting wheat and derived milling fractions. The partners 9&10 standardised the method for DON determination in wheat and carried out the adequate statistical analysis for expressing the DON distribution within the wheat and derived fractions. The partner 11 used in 2003 a different analytical protocol for DON determination in wheat but will use in 2004 the same method as partners 9&10 for confirmation. The partners 9&10 initiated experiments on fumonisin determination in maize and derived milling fractions. The partners 1 & 11 planned to carry out a joint fundamental study (in 2004) on the influence of grain structural properties and processing steps on the distribution of mycelium and DON in wheat and derived milling fractions.

**Work Package 4&5 (led by the partner 2)**
The partners 1, 2, 4, 6, 7 and 8 were involved in WP 4&5. The Brazilian Universities of Campinas and Maringa were associate institutions to the project and participated in WP 4&5. The partners 4, 6, 7 and 8 selected the priority mycotoxin-commodity
combinations, they identified the cereal chain key players and constituted multidisciplinary teams for HACCP application throughout the whole chains. A representative at least of the partners 4, 6, 7 & 8 followed a training on the HACCP method. The partners 1 & 2 participated in the elaboration of the commodity flow diagrams and in the discussion of socio-economic tools to be used. The partner 2 pursued the development of the Toximet procedure for further validation as a potential control measure. The partner 1 also brought support for formalizing the relationships with the private sector through meetings, project presentation and elaboration of confidentiality agreements.

Problems

For WP 1
The unavailability of FAPAS reference material T 2210 (wheat flour with DON) in the first semester of 2003 delayed the material order and delivery to the concerned partners until the end of the year. The interlaboratory works will be performed using this material in 2004.

Some partners had unsatisfactory Z score values for aflatoxin and zearalenone determination in maize. However, the technical problems were identified and corrective actions put in place. Some experiments will be repeated in 2004.

For WP 2
The major problem encountered in the frame of WP2 was the difficulty of the University of Lujan (partner 10) for acquiring immuno-affinity Ochraprep columns (needed for subsequent use in OTA determination). Indeed, with the help of Dr Monica Olsen, we could get a special price offer for column purchase from Biopharm company. With the aim of optimising the financial resources and project running, the columns were ordered in Europe by the project general coordinator and sent to Argentina by the end of 2003. However, due to high complications for material clearance from the local customs, the materials are still not delivered to the partner lab. We are awaiting for the help of Biopharm company for solving this problem.

For WP 3
Some administrative changes occurred within the El Globo industrial mill in Chile, which induced additional constraints for wheat sampling according to the sampling plan designed by partner 11. In addition, it was too difficult to find naturally contaminated wheat with DON in Chile (which is indeed a good thing for Chilean consumers). Spiked wheat samples should then be considered for the forthcoming WP3 activities in Chile.

For WP 4&5
No major technical problems have been encountered, to date. The problem which has been experienced was related to the sensitivity of some key players in the selected commodity systems against the likely implications of the mycotoxic contamination of their products (i.e. wheat flour and corn-based poultry feed). This has delayed the addition of commercial members to the HACCP teams, which in turn, has delayed the verification of the CFDs.
Publications and papers

1) Publications in peer-reviewed scientific journals


2) Oral presentations in conferences and congresses


3) Posters presented in congresses


**4) Diffusion and dissemination documents**

*These documents were elaborated with the logistic support of PROCISUR (Partner 3) for translation to Spanish and edition.*


- 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.
Outline plans for next year

The planned activities for the project’s year 2 (2004) are the following:

For Work Package 1

The interlaboratory work will be performed on the T2210 FAPAS (wheat flour with DON) material received by partners by the end of 2003. Besides, two additional laboratories (partners 9 & 10) will join the interlaboratory work and analyse the same FAPAS materials than the other labs initially involved in WP1.

Some experiments for aflatoxin and zearalenone determination in maize will be repeated in 2004 in order to improve the performances of the concerned laboratories (partners 4 & 11). In addition, the implementation of analytical methods will be pursued by partner 4 when needed (for zearalenone and fumonisins in maize for instance).

A proficiency test round will be implemented among the laboratories using the homogeneous naturally contaminated maize samples prepared by the partner 5.

Sampling protocols will be shared between the WP1 and 4&5 partners for optimising both field sample collection and sample analysis at lab.

Continuation of the work on alternative techniques for mycotoxin determination, i.e. i) Toximet procedure development, ii) NIRS spectra on all samples which will be analysed by chromatographic methods in 2004 within the project (either from WP1, 3 and 4&5) and iii) measurement of bacterial luminescence inhibition by mycotoxins.

For Work Package 2

i) Application of the standardized (in 2003) method for OTA determination in 200 collected blood samples collected in Argentina and those which will be collected in Chile. In parallel, the questionnaires surveying the blood donor diet will be exploited and potential correlations will be pointed out between OTA values and the donor diet.

ii) Development and implementation of a HPTLC-based method for OTA determination in cereals both in Argentina and Chile. As an alternative faster technique for on-field measurements, it will be interesting to test, as proposed by Dr Monica Olsen, the Lateral Flow Device (developed in the frame of the EC-funded OTA PREV project QLKI-CT-1999-00433) as a potential rapid tool for on-field OTA detection.

iii) The calculation of OTA intake will initiate on the basis of the population diets in Argentina and Chile. This will help for risk characterization for OTA in the Latin America South Cone countries, planned in the third year of the project.
For Work Package 3
- In Chile, the wheat sampling planned for 2004 will be repeated at two different periods, one at the start of the year where the industrial silos are only filled with the national crop harvest and the second when wheat starts to be imported for fulfilling the country’s needs. On the other hand, the gas chromatography will be implemented in 2004 at the University of Concepción (partner 11 – Chile) for DON determination in wheat and derived milling fractions.

- In Argentina, it is planned by partners 9 and 10 for 2004 to i) reproduce the maize wet milling at the laboratory scale and determine fumonisins at all process stages. This will allow to propose improvements that could be fed back to the industry, and ii) to try different sieve mesh size for maize cleaning before silo storage.

- Within the frame of WP3, a fundamental study is planned for 2004 by CIRAD (partner 1) in relation with Argentina (partners 9 & 10) and Chile (partner 11) in order to better understand the influence of both structural grain properties and processing steps on the distribution of mycelium and DON contamination in wheat and derived milling fractions. Preliminary experiments will be made (April to July 2004) by a trainee in Montpellier (France), in close collaboration with the French INRA specialised lab on mycotoxins. Afterwards, the work will be pursued by a PhD student (Gisela Rios) from the University of Concepción (Chile – partner 11) who got a Chilean scholarship and will come to France for PhD work, under the co-supervision of partners 1 and 11.

For Work Package 4
To determine at which step(s) in the CFD the mycotoxin hazard originates or increases to an unacceptable level, requiring control, preliminary action are currently undertaken on the design of sampling & surveillance procedures. Arrangements are in place for the analysis of the samples of wheat & corn generated during the above mentioned sampling and surveillance and for the statistical analysis and result interpretation (Deliverable D14 expected in 2004). We should point out that the sample analysis will be performed in each country by the “lab” partners involved in the project WP 1&2&3, which strengthens the relationships between the project partners and allows for a real joint strategy for mycotoxin prevention and control, at the national and regional levels in Latin America South Cone.

It is important to point out the effective relationships among the project partners and the real linkages between the “analytical” work packages (WP 1 & 2 & 3) and the “field” ones (WP 4 & 5) in each country, which will facilitate the mycotoxin determination in the wheat & corn sampled during the commodity system study (CFD validation, identification of CCPs, potential control measures, etc).

For Work Package 5
There are three types of planned activities:

i) The application and adaptation of participatory appraisal methods used to obtain a thorough understanding of those socio-economic, cultural and institutional issues associated with the introduction of control measures at Critical Control Points (Deliverable D9 expected in 2004).
ii) The identification of the Critical Control Points (CCPs) in each selected mycotoxin-commodity combination.

iii) The development, evaluation and validation of control measures that will prevent, eliminate, or reduce mycotoxin content to an acceptable level, when applied to a specific step in the CFD, and according to the specific socio-economic and institutional context in each country (Deliverable D13 expected in 2004).

For Work Package 6
Once the Critical Control Points (CCPs) are identified and confirmed in the Commodity System, and the potential measures are developed and validated, the activities which are planned within WP6 for 2004 are related to the establishment of a global HACCP plan including good practices, corrective actions and monitoring procedures in each country, according to its specific socio-economic and institutional context (Deliverable D15 expected in 2004).

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT:
Covering period from 1 January to 31 December 2003

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 annual report
Organisation of the collaboration

- The rules of project running and management as initially stated in the proposal submitted to the EC were again discussed and validated by the whole consortium members at the start of the project. This contributed to a fluid and smooth cooperation between the partners in 2003. We should highlight the enthusiasm and willingness of all partners to join their efforts for tackling the project challenge, and specifically among the Latin American partners through a strong regional cooperation.

- Another positive point about the cooperation between partners was the permanent exchange 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). Information has also been regularly exchanged between the partners, the work package leaders and the general coordination. Electronic discussions raised for instance on some general issues which are essential for the whole project, such as sampling (at lab or in field), sample preparation before analysis, integration of socio-economic aspects and chain actor organization, etc.

- An important step 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 include agronomists, socio-economists, HACCP specialists and analysts, which enables 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.

- Seven project members (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 are numerous: i) harmonization of the HACCP glossary and methodological tools among the partners, ii) sharing 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.

- As the private sector is one of the wheat and maize chain actors, it was essential to make contacts with the enterprises concerned by mycotoxins and to involve them in the activities related to the whole chains. All contacted enterprises expressed their worries about the impact of mycotoxin grain contamination and the need for an integrated quality management system throughout the whole grain chain. All showed high interest for collaborating with the MYCOTOX project. In this sense, linkages are now tied with the private sector (mills, poultry farms, agropoles) for
joint work in the frame of “field” activities of WP 4 & 5. However, we should point out that those linkages took longer than planned for contractualisation because of the sensitivity of the enterprises to the likely implications of their product contamination. A specific confidentiality agreement was elaborated and proposed by the general project coordination and validated by all partners 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). It is essential to mention that the further diffusion and result dissemination (initially planned for the project third year) should be closely performed with the professional organisations and bodies involved in wheat and maize chains in each South Cone country partner.

- Scientific linkages were initiated with Brazilian institutions who are not direct partners of MYCOTOX project but who can bring 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).

- In 2003, two scientists from CIRAD-partner 1 (Dr C. Brabet and Dr G. Henry) were outposted to Brazil. By the end of 2003, Dr G. Henry moved to Argentina and is presently based at INTA (partner 8) offices. This allowed to strengthen the cooperation and enabled permanent interaction with the South Cone partners. On the other hand, Dr Tania Barreto Correa (EMBRAPA-partner 4) retired from her institution in October 2003 but kept 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 taking over the leadership of the Work Package 1.

Meetings

1) **Kick-off meeting held in Montevideo (Uruguay), 17 to 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 to 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 held 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 WP2 in 2003 but taking the opportunity of Argentina WP 4&5 meeting for discussing the WP2 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 to 26 September 2003.

Benefiting from a regional scientific event, the IV Latin american 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. A flyer (in spanish) was also distributed to the participants in the congress for presenting the MYCOTOX project (see list of publications and papers in the scientific annual report). 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.
Outline of meetings planned for year 2 (2004)
The second general annual project meeting is planned for mid year, probably within the two first weeks of June 2004. Dates are still to be fixed.

A second regional meeting for WP 4&5 is planned in year 2. Dates will be fixed according to the advances of current work (CFD application and HACCP diagnosis in field) and to the advice of WP 4&5 leader.

Additional regional meetings will be organised if there is any need expressed from the partners or the leader of the corresponding work package.

Exchanges
- Training is one of the main supports of the project. In this sense, the following students participated in the activities under the supervision of the partners:

1) Rodolfo Osório de Oliveira, Institute of Economics, University of Campinas (Brazil), March to July 2003.

2) 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.

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

4) 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.

5) Leticia Broggi, University of Buenos Aires (Argentina), PhD student under the supervision of Dr Silvia Resnik. Topic: Potential contamination by molds and mycotoxins of the agricultural products in the Entre Rios province and influence of regional milling processes (“Productos agrícolas de la provincia de Entre Ríos: contaminación potencial por hongos y micotoxinas e influencia de los procesos de molienda regional”).

6) Maria Margarita Samar, University of Buenos Aires (Argentina), PhD student under the supervision of Dr Silvia Resnik. Topic: Influence of some steps of wheat processing on the tricothecene persistence or modification (“Persistencia y/o transformacion de tricotoxenos durante algunas etapas del procesamiento de trigo”).
The last year of these two PhDs was supported by MYCOTOX project and the thesis were defended in 2003.

- Within the frame of WP 4&5&6 and the constitution of the multidisciplinary teams in each Southern Cone country, the partner 6 (INIA Uruguay) expressed the need for contracting a socio economist to participate in the “field” activities through socio economic studies and diagnosis of the whole cereal chains. The general coordinator transmitted this request to the EC. Further to the agreement of the former EC scientific project officer, Dr Gerasimos Apostolatos, the partner 6 could contract Dr Gonzalo Gutierrez by reallocating budget from the Travel and Durable Equipment categories to the Subcontracting/External Services one.

- An exchange is foreseen in year 2 (2004) between Chile and France through the co-supervision of a PhD within the frame of WP3. The candidate is Gisela Rios from the University of Concepción (Chile) (partner 11). Benefiting from a Chilean scholarship, G. Rios will come to France in September 2004 for lab work. **Topic:** Influence of structural grain properties and processing steps on the mycelium and mycotoxin distribution in grains and outcoming fractions.

**Problems**

- Because of local administrative rules and constraints, the fund 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 concerned partners and in accordance with the above mentioned difficulties. Fund transfers to Argentina are now achieved. 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. This agreement is still under signature. This procedure delayed the effective fund transfer to those two partners. However, in order to allow their activities running in good conditions, the general coordinator (partner 1) advanced the needed money for the expenses of partners 4&5 in 2003. This advance will be reimbursed to the general coordinator by deducting the advanced amounts from the next fund transfers to partners 4 & 5.

- With the aim of optimising financial resources and project running, we could get, with the help of Dr Monica Olsen, a special price offer for purchasing Ochraprep immuno affinity columns. Those lab materials were ordered in Europe (Biopharm company) by the general coordinator and sent to the University of Lujan (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 Argentinian customs, the materials are still not delivered to the Argentinian partner. We are awaiting for the help of Biopharm and the partner 10 for solving this problem.
Partner 01 - CIRAD

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
FIRST ANNUAL REPORT

The activities performed by CIRAD (partner 1) in 2003 within the frame of MYCOTOX project are detailed below.

General coordination

Task 1: Fund transfer
As general coordinator of the project, the partner 1 proceeded in 2003 to the fund transfer according to the first EC advance and planned budget for each partner. Some problems and delays were encountered with Brazil and Argentina because of the local administrative rules and constraints. We did the best for managing this situation, in concertation with the concerned partners. Fund transfers to Argentina are now achieved.

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 institutions (partners 4 & 5) and the FUNARBE Brazilian foundation committed to manage the financial resources of partners 4 & 5. This agreement is still under signature. This procedure delayed the effective fund transfer to those two partners. However, in order to allow their activities running in good conditions, the general coordinator (partner 1) advanced the needed money for the expenses of partners 4 & 5 in 2003. This advance will be reimbursed to the general coordinator by deducting the advanced amounts from the next fund transfers to partners 4 & 5.

Task 2: Organisation and participation in the project meetings
In collaboration with the partners, the general coordinator organised and participated in three meetings during 2003.

- Kick-off meeting held in Montevideo (Uruguay), 17 to 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.

- Mid-year progress meeting specific for WP 4&5 held in Buenos Aires (Argentina), 20 to 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 held 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 WP2 in 2003 but taking the opportunity of Argentina WP 4&5 meeting for discussing the WP2 activities allowed optimising the financial resources and kill two birds with one stone.

- Informal meetings for WP 1, 3, 4&5 held in la Habana (Cuba), 22 to 26 September 2003.

Benefiting from a regional scientific event, the IV Latin american 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. A flyer (in Spanish) was also distributed to the participants in the congress for presenting the MYCOTOX project (see list of publications and papers in the scientific annual report). 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.

- 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.

Task 3: Regional coordination in the Southern Cone region
In 2003, two scientists from CIRAD (Dr C. Brabet and Dr G. Henry) were outposted to Brazil. By the end of 2003, Dr G. Henry moved to Argentina and is presently based at
INTA (partner 8) offices. This allowed a close regional coordination and an institutional support for various administrative and financial issues and bottlenecks. All teams were regularly visited in the 4 countries.

**Task 4: Support for 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. Reference FAPAS materials, i.e. standard contaminated matrices with a known mycotoxin concentration, were ordered, by the general project coordinator, in UK (a special discount was obtained for the grouped order) and sent to the partners involved in WP1. In addition, Ochrarep imuno affinity columns were ordered by the general coordinator at Biopharm company (UK) for partners 10 and 11. We could get a special price offer through the help of Dr Monica Olsen. The columns were well delivered to the University of Concepción (partner 11) in Chile. Some complications were faced for good clearance from the Argentinean customs and the columns are still not delivered to the University of Luján (partner 10). Actions are undertaken in collaboration with Biopharm and the partner 10 for solving this problem.

**Task 5: Formalization of confidentiality agreements with the private sector**

As the private sector is one of the wheat and maize chain actors, it was essential to make contacts with the enterprises concerned by mycotoxins and to involve them in the activities related to the whole chains. All contacted enterprises expressed their worries about the impact of mycotoxin grain contamination and the need for an integrated quality management system throughout the whole grain chain. All showed high interest for collaborating with the MYCOTOX project. In this sense, linkages are now tied with the private sector (mills, poultry farms, agropoles) for joint work in the frame of WP 4&5. However, we should point out that those linkages took longer than planned for contractualisation because of the sensitivity of the enterprises to the likely implications of their product contamination. A specific confidentiality agreement was elaborated and proposed by the general project coordination and validated by all partners 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). It is essential to mention that the further diffusion and result dissemination (initially planned for the project third year) should be closely performed with the professional organisations and bodies involved in wheat and maize chains in each South Cone country partner.

**Task 6: Extension of the project’s partnership to associate institutions**

Scientific linkages were initiated with Brazilian institutions who are not direct partners of MYCOTOX project but who can bring 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.
Task 7: Implementation of an project Intranet site
For the first project year, the Intranet site “Quick Place” was implemented as an internal tool for information exchange with a restricted access to the project partners and associate institutions. All meetings reports and presentations were uploaded there. However, due to some software limitations and after one year-experience, this tool shows to be not enough friendly. We are moving towards the implementation of an Internet site with outside global access and some restricted access areas dedicated to the project outputs and confidential issues. This will allow us on one hand to ensure a better diffusion of the project (general presentation, objectives, partners, activities) to the scientific community over the world and on the other hand, to keep a confidential space for the project partners, advances and outputs.

Participation in Work Package 1

Task 8: Literature review on the Bioluminescence and Chemiluminescence techniques as innovative tools and prospects for mycotoxin determination
The sensitivity, speed and convenience of chemiluminescent (CL) and bioluminescent (BL) immunoassays have led to a diverse range of applications for these technology, mainly in the clinical laboratory, pharmaceutical industry and food testing. Chemiluminescence is based on the light emitted when a luminescent chemical substrate moves from the excited to initial state, in the presence of hydrogen peroxide. Chemiluminescence is used for detection or amplification of the antibody-antigen reaction within immunoenzymatic assays (ELISA). Bioluminescence is based on the use of intrinsic adenosine triphosphate (ATP) of microorganisms and its reaction with firefly luciferase to produce an amount of luminescence that may be directly related to the microbial count and can be measured with a suitable luminometer.

At the stage of proposal writing, preliminary works carried out by CIRAD on the application of chemiluminescence for aflatoxin determination in groundnut and yam chips indicated an interesting potential for this innovative investigation, especially as a rapid and easy-to-use on-field routine measurement technique. However, those works showed that chemiluminescence was a good detection technique complementary to available ELISA kits but was not able to be used as a direct measurement for aflatoxin determination. This might then increase the unit cost of mycotoxin analysis and is not suitable for analysing high numbers of samples within the MYCOTOX project. On the other hand, the comparison of aflatoxin measurements through chemiluminescence and HPLC was not convincing because of low reproducibility and difficulty for stabilising the luminescent signals.

In order to optimise the project resources and to take benefit from the above mentioned advances, we undertook a deep literature review on the existing applications of chemiluminescence and bioluminescence techniques with a focus on mycotoxin determination (**this led to a paper submitted to “Luminescence” journal for publication, see Papers and Publications section below**). Bioluminescence was shown to be suitable for bacterial determination and not for molds, because of the difficulty for ATP extraction from molds. We decided then to reorient our investigations towards the use of “toxicity tests” which are based on the natural ability (through specific “lux” genes) of some bacteria to emit light when one specific
aldehyde internal to their metabolism is oxidized. The emitted light is measured with a luminometer and is proportional to the bacterial growth. In presence of inhibitory molecules such as antibiotics, antimicrobial agents or even mycotoxins, the light emission is lower because the bacterial activity is reduced. An indirect way for mycotoxin determination might be then the mycotoxin concentration for which the bacterial light emission decreases to the half. Experiments were planned for testing this innovative investigation. Two bacteria (Vibrio fisheri and Vibrio harveyi) and aflatoxin were chosen for the first trials which started at the beginning of 2004.

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

A preliminary work was carried out by CIRAD (partner 1) and LATU (partner 12) for DON determination in wheat and derived milling fractions by using the Near Infrared Reflectance Spectroscopy (NIRS). This technique is based on the relationship between the chemical composition of the organic matter and its absorption of infrared wavelengths. The NIRS technique is fast and inexpensive but it 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.

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, mean of 2355 ppb and standard deviation 2373 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).

DON content is highly variable, which is a good thing for calibration. 3 groups very well identified on a spectral basis (figure 1). The groups correspond to 1) Flour, 2) “wheat” and 3) Bran + by-products

The difference between groups is high so the calibration should preferably be performed on separate groups in a first step. However the number of samples in each group does not allow such an analysis, so only the global calibration can be done.
Figure 1: Position of samples on the 3 first principal components.

Calibrations were performed on wavelengths between 1100 and 2500nm. Visible wavelengths were not used in computation because their use introduced variability not linked to explicative capacity. The best mathematical data pre-treatment were second derivative, SNV and detrend procedures.

<table>
<thead>
<tr>
<th>Math Treatment: 2, 5, 5, 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of variables: 172</td>
</tr>
<tr>
<td>Scatter Corr.: SNV and Detrend</td>
</tr>
<tr>
<td>Downweight outliers: No</td>
</tr>
<tr>
<td>Constituent: DONppb</td>
</tr>
<tr>
<td>Number of samples: 24</td>
</tr>
<tr>
<td>Mean: 2355.208</td>
</tr>
<tr>
<td>Range: 413.00 - 11322.00</td>
</tr>
<tr>
<td>Std Dev.: 2373.216</td>
</tr>
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</table>

<table>
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<tr>
<th>SEC</th>
<th>RSQ</th>
<th>F</th>
<th>SECV</th>
<th>1-VR</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>968.9</td>
<td>0.833</td>
<td>20.94</td>
<td>1593.2</td>
<td>0.542</td>
<td>1.49</td>
</tr>
</tbody>
</table>

SEC = standard error of calibration, RSQ = R-square, SECV = standard error of cross-validation, RPD = ratio performance to deviation (= SD/SECV)
The calibration was limited to 24 samples which is not sufficient for a real feasibility study. The statistics presented are only indicative because of the probable overfitting of the model. However the first comments could be:

- There is a significative NIR prediction model derived from this dataset. The RPD value greater than 1 means that the model contains valuable information. However the low number of sample leads to a poor SECV (standard error of the cross validation, estimating quality of prediction) even if $R^2$ is acceptable ($R^2=0.83$)
- As seen on figure 2 the agreement allows to distinguish high samples from low samples, but not within low samples. A more precise calibration should be done on low content samples only.
- As we have 3 subgroups, it is not possible to determine at this stage if the prediction model detects really a trace of contamination, or only the membership to one of the category (which happen to have different mean DON content)

This preliminary study confirmed the interest for pursuing the investigation on NIRS potential for mycotoxin determination. However, it is essential to analyse a higher number of samples. To this end, all samples to be collected in 2004 within the field activities of WP 4&5 and from WP1&3 will be sent to the partner 1 for NIRS analysis.

**Participation in Work Package 3**

*Task 10: Elaboration of a work plan for studying the influence of grain structural properties and milling steps on the mycotoxin distribution*  
The partner 1 has on-going collaboration with the French INRA research institution specialised in cereals and currently working on mycotoxins. INRA has a milling pilot plant (in Montpellier, France) with the capacity of 150 kg of wheat per hour or 50 kg
of maize per hour. Initially, and in concertation with the partner 9 (leader of the WP3), we discussed different possibilities for the CIRAD contribution to WP3. Using the pilot plant, it was interesting to compare either different milling process parameters using the same raw material (grain) or to compare different raw materials (coming from different production places) using the same milling process. The problem encountered was the difficulty for getting contaminated grains coming from Argentina, because of the current international regulations that prohibit the transfer of contaminated material. We reoriented then the investigation focus towards i) studying the influence of grain structural properties on the distribution of mycotoxin contamination in the whole grain and ii) conducting experiments using the pilot plant for milling grains produced in France and analysing samples for mycotoxin determination, both by chromatographic and NIRS methods. A work plan was then elaborated and started in 2004 with a preliminary work (conducted by a French trainee) that will be followed by a PhD (a scientist coming from Chile in France in September 2004, see the outline plans for next year). The data to be generated will give useful inputs for further actions to be undertaken at the industrial milling scale in Argentina and Chile.

Participation in Work Packages 4 & 5

Task 11: Support for the multidisciplinary HACCP teams
The formation and organization of the 4 project multidisciplinary HACCP teams (one per country) was assisted. The working plans for WP 4&5 in the 4 countries were co-designed and discussed. Potential economic theories and instruments, pertinent to the project objectives, were proposed and co-analysed with partners.

Task 12: Collection and collation of data from literature
A literature review on mycotoxin occurrence in Brazilian maize and wheat was performed in collaboration with the HACCP team in Brazil. 60 bibliographic references were collected and analysed. This allowed to select the area (Parana state), the commodity-mycotoxin combinations (aflatoxins, fumonisins and zearalenone in maize) and the agrisystem of maize production for poultry feeding.

Task 13: Making contact with key players in the commodity system
All teams were visited in the 4 countries of the Southern Cone, and field trips were conducted to identify, discuss and negotiate collaboration with pertinent stakeholders from private and public sectors. These field trips also served to better understand the constraints and opportunities of the wheat and maize agrisystems in the 4 countries. Participation in the contractualisation of confidentiality agreements with the private sectors.

Task 14: Co-construction of the Commodity Flow Diagrams (CFD) in Brazil and Argentina
The partner 1 participated in the preliminary commodity flow diagrams of the selected agrisystems in Brazil and Argentina. The CFDs are currently being refined in consultation with key players in the respective commodity systems.
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.

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

Publications and papers

Publications in peer-reviewed scientific journals

Oral presentations in conferences and congresses

Diffusion and dissemination documents (These documents were elaborated with the logistic support of PROCISUR (Partner 3) for translation to Spanish and edition).
- 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 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.

Outline plans for next year

- Continuation of the work on alternative techniques for mycotoxin determination, i.e. i) Toximet procedure development, ii) NIRS spectra on all samples which will be analysed by chromatographic methods in 2004 within the project (either from WP1, 3 and 4&5) and iii) measurement of bacterial luminescence inhibition by mycotoxins.

- Within the frame of WP3, a fundamental study is planned for 2004 by CIRAD (partner 1) in relation with Argentina (partners 9 & 10) and Chile (partner 11) in order to better understand the influence of both structural grain properties and processing steps on the distribution of mycelium and DON contamination in wheat and derived milling fractions. Preliminary experiments will be made (April to July 2004) by a trainee in Montpellier (France), in close collaboration with the French INRA specialised lab on mycotoxins. Afterwards, the work will be pursued by a PhD student (Gisela Rios) from the University of Concepción (Chile – partner 11) who got a Chilean scholarship and will come to France for PhD work, under the co-supervision of partners 1 and 11.

- Participation in the next steps of WP 4&5, i.e. i) the Commodity Flow Diagram validation through field trips with the HACCP teams in Brazil (Dr C. Brabet) and Argentina (Dr G. Henry) and the identification of critical control points, ii) exploitation of the questionnaires and data obtained through socio economic studies on the agrichain organization, and iii) proposition of potential control measures for mycotoxin contamination throughout the cereal whole chain.
Partner 02 - NRI

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
**FIRST ANNUAL REPORT**

**WORK PACKAGES NUMBER 4 & 5:**
- **HAZARD ANALYSIS OF MYCOTOXINS (24 FEBRUARY 03 – 31 DECEMBER 03)**
- **IDENTIFICATION & VALIDATION OF MYCOTOXIN CONTROL MEASURES (24 FEBRUARY 03 – 30 JUNE 04)**

<table>
<thead>
<tr>
<th>Deliverable</th>
<th>NRI Inputs</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Work Package Leadership</strong></td>
<td></td>
</tr>
</tbody>
</table>
| To create an enabling environment that will encourage the timely production of deliverables by the project partners | • Close collaboration was maintained with each partner country in order to facilitate the assembly of appropriate teams and co-operation within and between the teams  
• A project work-plan was agreed with each team and a progress review table was established to assist with the timely & concise reporting of progress  
• The project teams were closely monitored in order to strongly encourage the timely production of deliverables  
• Technical advice was provided on a variety of issues including: (a) the design of surveillance & sampling procedures; (b) the HACCP approach to food safety management, including (c) the production of Commodity Flow Diagrams, CFDs and (d) Hazard Analysis  
• Field visits were undertaken to Argentina, Uruguay, Brazil and Chile in order to advise the HACCP teams on the construction of CFDs and the performance of Hazard Analysis  
• A Progress Meeting was organised and held in Buenos Aires (at INTA, Argentina) in order to review progress and to agree the next steps with the project partners |
<table>
<thead>
<tr>
<th>Objective:</th>
<th>Deliverable D2 (30 June 2003): A report documenting mycotoxin surveillance data, both from the literature and from surveillance studies conducted by this project</th>
<th>Collaboration with project partners in order to: (a) assemble HACCP teams; (b) gather information on the occurrence of mycotoxins in wheat and corn from a variety of published &amp; unpublished sources; and, (c) summarise and collate the collected data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deliverable D3 (31 August 2003): Report describing the hazard analyses conducted, justifying the commodity-mycotoxin combination selected for further study</td>
<td>In collaboration with partners, the bibliographic and collated data for each country were carefully examined and commodity-mycotoxin(s) combinations were selected for further study</td>
<td></td>
</tr>
<tr>
<td><strong>Objective:</strong></td>
<td><strong>To construct &amp; verify the respective Commodity Flow Diagrams (CFDs)</strong></td>
<td></td>
</tr>
<tr>
<td><strong>(1 August 2003 – 31 December 2003)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Deliverable D6 (31 December 2003): Report documenting the verified CFDs for each commodity-mycotoxin combination in each of the selected countries</td>
<td>All partners were assisted in the construction of a preliminary CFD for the relevant component of their commodity system</td>
<td></td>
</tr>
<tr>
<td><strong>To determine at which step(s) in the CFD the mycotoxin hazard originates or increases to an unacceptable level, requiring control</strong></td>
<td></td>
<td></td>
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<tr>
<td><strong>(3 November 2003 – 1 April 2004)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Deliverable D14 (1 April 2004, or within 3 months of harvest): Report describing at which steps in the CFD the mycotoxin hazard originates, or at which steps concentrations increase to unacceptable levels</td>
<td>Partners were advised on the selection of appropriate procedures so that preliminary action could be undertaken on the design of sampling &amp; surveillance procedures</td>
<td></td>
</tr>
<tr>
<td><strong>Objective:</strong></td>
<td><strong>To evaluate the socio-economic, cultural and institutional issues associated with the introduction of control measures at CCPs</strong></td>
<td></td>
</tr>
<tr>
<td>Deliverable D9 (30 June 04): 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 &amp; opportunities affecting the implementation of proposed mycotoxin control measures</td>
<td>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 &amp; institutional issues associated with the introduction of control measures at Critical Control Points (CCPs) were discussed with partners</td>
<td></td>
</tr>
</tbody>
</table>

The development of a simple, inexpensive Toximet procedure for the quantitative determination of mycotoxins has continued.
Partner 03 - PROCISUR

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
FIRST ANNUAL REPORT

1- Kick-Off Meeting - 17 to 19 February 2003 - Montevideo, Uruguay

PROCISUR was responsible for the logistic of the meeting held at NH Columbia Hotel, 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
- secretarial support during the meetings

2- On May 2003, in Montevideo, PROCISUR Technical Assistant in Programming and Management, Eng.Cecilia Gianoni, representing PROCISUR Executive Secretary, met with Dr. Guy Henry, CIRAD Coordinator for WP 4&5, in order to: check actions taken until the moment; be informed of the incorporation of new specialists with economic profile to the work teams; and coordinate the first meeting of Work Programmes 4&5 to be held in Buenos Aires, Argentina, on August 2003.

3- First Co-ordination Meeting of Work Programmes 4 & 5 - 20 to 22 August 2003 INTA - Buenos Aires, Argentina

Eng. Cecilia Gianoni, representing PROCISUR, participated of the meeting where progress achieved by WP 4 & 5 partners to that date were submitted. Socio-economic and thecnical aspects were integrated to the project and future actions to be developed were defined.

4- Mycotoxin brochure

PROCISUR helped with the Spanish translation, edited and printed the project brochures that were distributed among the participants of the Micotoxins Seminar held in Cuba, on the month of September.

5- During 2003 PROCISUR has promoted the activities of the project through Procisur Online, both on the section "PROCISUR Informa" as well as in "Projects with external financing" (www.procisur.org.uy).
Contract number: ICA4-CT-2002-10043

Partner 04 - EMBRAPA

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
FIRST ANNUAL REPORT

Embrapa was involved in the WP1 and WP4 & 5 activities of the Mycotox project, and is the leader of the workpackage WP1.
This report presents the activities performed by the Embrapa within the framework of the WP1 and WP4 in 2003, as well as the outline plans for 2004.

WP1: “Development and standardisation of effective analytical tools for mycotoxin determination in cereals and by-products”

WP4: “Hazard analysis of mycotoxins”

Participation in WP 1

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

Two principal axes of scientific activities were led by the EMBRAPA within the framework of the WP1:
- the implementation and validation of analytical chromatographic methods for mycotoxin determination in corn, in its laboratory;
- as the coordinator of the WP1, the inventory of the analytical chromatographic methods currently used by the different WP1 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.

➢ Implementation of HPLC method for zearalenone determination in corn

Before initiating the Mycotox project, TLC methods for aflatoxin and zearalenone determination were already implemented and used by the mycotoxin laboratory of the EMBRAPA. In 2003, HPLC method was implemented for zearalenone determination in corn as a more effective analytical tool.

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, fumonisins and aflatoxin. Based on this review, nine analytical chromatographic methods for zearalenone determination were selected (Table 1). Six HPLC mobile phase/wave-length combinations were first tested (Table 2) using Sigma zearalenone standard, then two clean-up methods (Romer column and immunoaffinity column) for zearalenone determination in corn.
Results
About 300 bibliographic references were collected (Appendix 5). This literature review will be also used in 2004 for the implementation of an analytical chromatographic method for fumonisin determination in corn. The best results were obtained with the mobile phase/wave-length combination 6 (best ration signal/noise), and the Romer column (higher recovery). The method of the Diário Oficial da União (DOU), 2000, Brazil was implemented at the EMBRAPA. This method which is also used by the MAA, presented the best performance among the different tested methods. These preliminary tests will be completed in 2004, and statistical analysis performed.

➢ Participation in FAPAS proficiency tests

The 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.

➢ Description of the analytical chromatographic methods currently used by the WP1 partners for mycotoxin determination in wheat and maize

Material and methods
As the coordinator of the WP1, EMBRAPA distributed the form in Figure 1 to each WP1 partner in order to collect information on the analytical chromatographic methods they are currently using for mycotoxin determination in wheat and maize.

Results
The table 3 summarizes these methods. Based on this survey, the EMBRAPA elaborated a sheet for reporting mycotoxin results (Figure 2), with the aim to harmonize result presentation and to include all the appropriate information to evaluate the laboratory and method performance.
Table 1: Selected analytical chromatographic methods for zearalenone determination

<table>
<thead>
<tr>
<th>Extraction</th>
<th>Clean up</th>
<th>Mobil phase</th>
<th>Detection and Quantification</th>
<th>Wave-length</th>
<th>Bibliographic Reference</th>
</tr>
</thead>
</table>
Table 2: HPLC mobile phase/wave-length combinations tested for zearalenone determination in corn

<table>
<thead>
<tr>
<th>Mobil phase</th>
<th>Wave-length</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 AcCN: H₂O MeOH (46:46:8)</td>
<td>Em:440nm Ex:274nm</td>
</tr>
<tr>
<td>2 AcCN: H₂O MeOH (46:46:8)</td>
<td>Em:418nm Ex:236nm</td>
</tr>
<tr>
<td>3 AcCN: H₂O MeOH (35:43:22)</td>
<td>Em:440nm Ex:274nm</td>
</tr>
<tr>
<td>4 AcCN: H₂O MeOH (35:43:22)</td>
<td>Em:418nm Ex:236nm</td>
</tr>
<tr>
<td>5 MeOH: H₂O (80:20)</td>
<td>Em:465nm Ex:280nm</td>
</tr>
<tr>
<td>6 MeOH: H₂O (80:20)</td>
<td>Em:440nm Ex:274nm</td>
</tr>
</tbody>
</table>

Infrastructure and Analytical Procedures for Mycotoxin Determination

| Laboratory:                          |
| Sample:                              |
| Analysis (mycotoxin):                |
| Reference:                           |
| Sample weight:                       |
| Subsample weight:                    |
| Extraction procedure (aqueous slurry or not): |
| Clean-up:                            |
| Determination:                       |
| Equipments (Mills, HPLC, Fluorimeter, etc..): |

Figure 1: Form distributed by the EMBRAPA to the WP1 partners for a description of the analytical chromatographic methods currently used for mycotoxin determination in wheat and corn.
Table 3: Analytical chromatographic methods currently used by the WP1 partners for mycotoxin determination in wheat and maize

<table>
<thead>
<tr>
<th>Institution</th>
<th>Mycotoxin</th>
<th>Matrix</th>
<th>Sample weight</th>
<th>Subsample weight</th>
<th>Extraction procedure</th>
<th>Clean-up</th>
<th>Determination</th>
<th>Method reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMBRAPA</td>
<td>AF</td>
<td>Wheat and corn</td>
<td>3Kg</td>
<td>50g</td>
<td>Blend with MeOH and KCl sol</td>
<td>Liq-liq extraction</td>
<td>TLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41</td>
</tr>
<tr>
<td></td>
<td>ZEA</td>
<td>Wheat and corn</td>
<td>3Kg</td>
<td>25g</td>
<td>Shaker with AcCN and H2O</td>
<td>ROMER column</td>
<td>HPLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41</td>
</tr>
<tr>
<td>MAA</td>
<td>AF</td>
<td>Wheat and corn</td>
<td>3Kg</td>
<td>50g</td>
<td>Shaker with H2O and CHCl3</td>
<td>SPE column (florisil and C-18)</td>
<td>TLC or HPLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>Wheat and corn</td>
<td>3Kg</td>
<td>50g</td>
<td>Blend with MeOH and KCl sol</td>
<td>Liq-liq extraction</td>
<td>TLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41</td>
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<td>Wheat and corn</td>
<td>3Kg</td>
<td>25g</td>
<td>Shaker with AcCN and H2O</td>
<td>ROMER column</td>
<td>HPLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41</td>
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<tr>
<td>ZEA</td>
<td>Wheat and corn</td>
<td>3Kg</td>
<td>50g</td>
<td></td>
<td>Blend with MeOH and KCl sol</td>
<td>Liq-liq extraction</td>
<td>TLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>Wheat and corn</td>
<td>3Kg</td>
<td>50g</td>
<td>Shaker with H2O, MeOH and NaCl</td>
<td>LC-Si SPE tubes 1000 mg</td>
<td>TLC or HPLC</td>
<td>DOU 2000 – 993.17</td>
</tr>
<tr>
<td></td>
<td>ZEA</td>
<td>Wheat, corn and their</td>
<td>1Kg</td>
<td>25g</td>
<td>Shaker with MeOH, H2O and NaCl</td>
<td>Liq-liq extraction</td>
<td>TLC</td>
<td>AOAC 2000 – 970.45</td>
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<tr>
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<td>Wheat, corn and their</td>
<td>1Kg</td>
<td>25g</td>
<td>Shaker with AcCN and H2O</td>
<td>SPE column</td>
<td>TLC or HPLC</td>
<td>AOAC 2000 - 986.17</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>Wheat, corn and their</td>
<td>1Kg</td>
<td>25g</td>
<td>Shaker with MeOH and H2O</td>
<td>SPE column</td>
<td>TLC or HPLC</td>
<td>AOAC 2000 – 993.17</td>
</tr>
<tr>
<td></td>
<td>FUM</td>
<td>Wheat, corn and their</td>
<td>1 Kg</td>
<td>10 g</td>
<td>Shaker with MeOH and H2O</td>
<td>LC-Si SPE tubes 100 mg</td>
<td>HPLC</td>
<td>AOAC, 2000 – 995.15</td>
</tr>
<tr>
<td>LATU</td>
<td>ZEA</td>
<td>Wheat, corn and their</td>
<td>1Kg</td>
<td>25g</td>
<td>Shaker with AcCN and H2O</td>
<td>SPE column</td>
<td>TLC</td>
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<td>Wheat, corn and their</td>
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<td>TLC or HPLC</td>
<td>AOAC 2000, Seção 1, n° 62, p. 35-41</td>
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<td>1Kg</td>
<td>25g</td>
<td>Shaker with AcCN and H2O</td>
<td>SPE column</td>
<td>TLC or HPLC</td>
<td>AOAC 2000, Seção 1, n° 62, p. 35-41</td>
</tr>
<tr>
<td></td>
<td>ZEA</td>
<td>Wheat, corn and their</td>
<td>1Kg</td>
<td>25g</td>
<td>Shaker with AcCN and H2O</td>
<td>SPE column</td>
<td>TLC or HPLC</td>
<td>AOAC 2000, Seção 1, n° 62, p. 35-41</td>
</tr>
<tr>
<td>UdeC</td>
<td>AF</td>
<td>Corn</td>
<td>1.5 Kg</td>
<td>25 g</td>
<td>Blender with AcCN and H2O</td>
<td>ROMER column</td>
<td>HPTLC</td>
<td>AOAC, 2000 – 994.08</td>
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<tr>
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<td>ZEA</td>
<td>Corn</td>
<td>1.5 Kg</td>
<td>25 g</td>
<td>Blender with AcCN and H2O</td>
<td>ROMER column</td>
<td>HPTLC</td>
<td>AOAC, 2000, Seção 1, n° 62, p. 35-41</td>
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<td>Trichothecenes</td>
<td>Wheat</td>
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<td>shaker with AcCN and H2O</td>
<td>ROMER column</td>
<td>CGL and ECD</td>
<td>JAOAC International, 86, No. 3, 2003</td>
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<td>25 or 50 g</td>
<td>shaker with AcCN and H2O</td>
<td>ROMER column</td>
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<tr>
<td>UnLu</td>
<td>AF</td>
<td>Wheat</td>
<td>3Kg</td>
<td>25 or 50 g</td>
<td>Blender with AcCN and H2O</td>
<td>ROMER or Trilogy column</td>
<td>HPLC and TLC</td>
<td>AOAC 975.36 Metodo ROMER LABS No. MY 8402S</td>
</tr>
<tr>
<td></td>
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<td>25 or 50 g</td>
<td>Blender with AcCN and H2O</td>
<td>ROMER or Trilogy column</td>
<td>HPLC and TLC</td>
<td>AOAC 975.36 Metodo ROMER LABS No. MY 8402S</td>
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<td>Wheat</td>
<td>3Kg</td>
<td>25 or 50 g</td>
<td>shaker with H2O and MeOH</td>
<td>SAX or Baker Amino</td>
<td>HPLC</td>
<td>AOAC 995.15 Frist avtion 1995</td>
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<tr>
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<td>5Kg</td>
<td>25 or 50 g</td>
<td>shaker with H2O and MeOH</td>
<td>SAX or Baker Amino</td>
<td>HPLC</td>
<td>AOAC 995.15 Frist avtion 1995</td>
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<td>HPLC and TLC</td>
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<td>25 or 50 g</td>
<td>Blender with AcCN and H2O</td>
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<td>TLC</td>
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<td>25 or 50 g</td>
<td>Blender with AcCN and H2O</td>
<td>ROMER or Trilogy column</td>
<td>TLC</td>
<td>AOAC 986.7, clean up with ROMER or Trilogy column</td>
</tr>
<tr>
<td></td>
<td>OTA</td>
<td>Wheat</td>
<td>3Kg</td>
<td>25 or 50 g</td>
<td>Blender with AcCN and H2O</td>
<td>Ochrapped or Vicam columns</td>
<td>HPLC</td>
<td>AOAC 991.44 with Ochrapped or Vicam columns</td>
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<td>25 or 50 g</td>
<td>Blender with AcCN and H2O</td>
<td>Ochrapped or Vicam columns</td>
<td>HPLC</td>
<td>AOAC 991.44 with Ochrapped or Vicam columns</td>
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### Report Resulting Sheets

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<tr>
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<td>✓</td>
</tr>
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<td>DON</td>
<td>✓</td>
</tr>
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</tr>
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<td>50g</td>
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</tr>
<tr>
<td>Blend with MeOH and KCl sol</td>
<td>✓</td>
</tr>
<tr>
<td>shaker with NaCl, H2O and MeOH</td>
<td>✓</td>
</tr>
<tr>
<td>shaker with NaCl, H2O and MeOH</td>
<td>✓</td>
</tr>
<tr>
<td>shaker with AcCN and H2O</td>
<td>✓</td>
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<tr>
<td>shaker with AcCN and H2O</td>
<td>✓</td>
</tr>
<tr>
<td>Blender with AcCN and H2O</td>
<td>✓</td>
</tr>
<tr>
<td>shaker with H2O and MeOH</td>
<td>✓</td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Clean-up</th>
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</thead>
<tbody>
<tr>
<td>Liq-liq extration</td>
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</tr>
<tr>
<td>SPE column (florisil and C-18)</td>
<td>✓</td>
</tr>
<tr>
<td>LC-Si SPE tubes 1000mg</td>
<td>✓</td>
</tr>
<tr>
<td>Immunoafinity column</td>
<td>✓</td>
</tr>
<tr>
<td>ROMER column</td>
<td>✓</td>
</tr>
<tr>
<td>ROMER or Trilogy column</td>
<td>✓</td>
</tr>
<tr>
<td>SAX or Baker Amino</td>
<td>✓</td>
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<tr>
<td>Ochrapped or Vicam columns</td>
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<td>HPLC</td>
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</tr>
<tr>
<td>TLC</td>
<td>✓</td>
</tr>
<tr>
<td>CGL and ECD</td>
<td>✓</td>
</tr>
</tbody>
</table>

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<th>TLC data:</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Plate:</td>
<td></td>
</tr>
<tr>
<td>Mobile phase</td>
<td></td>
</tr>
<tr>
<td>Spotted vol.</td>
<td></td>
</tr>
<tr>
<td>Cromaovisor</td>
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</tr>
<tr>
<td>Densitometer</td>
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<th>HPLC data:</th>
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</thead>
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<tr>
<td>Flow</td>
<td></td>
</tr>
<tr>
<td>gradient</td>
<td></td>
</tr>
<tr>
<td>Injection vol.</td>
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</table>

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<th>CGL and ECD data:</th>
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</tr>
</thead>
<tbody>
<tr>
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</tr>
<tr>
<td>Gas</td>
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</tr>
<tr>
<td>Flow</td>
<td></td>
</tr>
<tr>
<td>Gradient Temp</td>
<td></td>
</tr>
<tr>
<td>Injection vol.</td>
<td></td>
</tr>
</tbody>
</table>

| Analyst name     |  |
| Reference        |            |

| Percentage recovery |  |
| Data               |            |
| Deviation standard ( ) |       |
| Corrected Data     |            |

---

**Figure 2:** Mycotoxin report resulting sheets
Interlaboratory works

In order to evaluate the performance of the analytical chromatographic methods currently used by the WP1 partners for mycotoxin determination in corn and wheat, a first serie of interlaboratory works was organized by the EMBRAPA and performed by the different WP1 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).

Material and methods
The table 4 describes the FAPAS materials used by the WP1 partners, and the number of repetitions that they performed for each of them. The analytical chromatographic methods are given in table 5. Intralaboratory controls were performed by all the WP1 partners in order to determine the percentage of recovery. The results were reported according to the Report Resulting Sheets elaborated by the EMBRAPA and the MAA.

Results
The results are summarised in table 7. The recovery percentages obtained by the MAA, EMBRAPA and LATU laboratories are within the acceptable range of the CEN, 1999, as a preliminary condition for mycotoxin result reporting. The mycotoxin determined average values, corrected by the recovery, are within the FAPAS satisfactory ranges for all of these laboratories, except for the EMBRAPA, AFB2.

In the case of the EMBRAPA, the AFB2 determined value is 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 recently changed. Indeed, the mycotoxin laboratory of the EMBRAPA demonstrated good performance with higher recovery percentage (more than 90 %) in FAPAS rounds using the same methodology. EMBRAPA planned to repeat these FAPAS material analysis as only one repetition was carried out.

The mycotoxin average values determined by the UdeC, which were not corrected by the recovery, are within the FAPAS satisfactory ranges, except for AFB1 and AFB2. The recovery obtained for these two aflatoxins, 55 and 52 % respectively, is very low, and even under the acceptable range of the CEN, 1999 (70-110 %) for the AFB1. The UdeC already started to repeat these analyses in order to identify the critical points in the procedure they used, and to apply corrective measures. They also initiated intralaboratory controls for determining the recovery percentage of ZEA and confirming the satisfactory result they obtained for this mycotoxin.

Even if all the results of this first serie of interlaboratory works are not within the FAPAS acceptable ranges, they could be considered as satisfactory for the Mycotox laboratory network. Indeed, they made it possible to achieve the initial goal, which was to evaluate and identify the problems and corrective actions to be implemented in order to improve the performance of the WP1 laboratories.
**Table 4:** Repetitions performed by the WP1 partners for each FAPAS material used in the interlaboratory works

<table>
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<th>Mycotoxin / FAPAS reference number</th>
<th>Institution</th>
<th>EMBRAPA</th>
<th>MAA</th>
<th>LATU</th>
<th>UdeC</th>
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<td>10</td>
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<td>Fumonisin B1, B2 / T2208</td>
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<td>na</td>
<td>na</td>
<td>3</td>
<td>na</td>
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<td>Zearalenone / T2209</td>
<td></td>
<td>1</td>
<td>6</td>
<td>2</td>
<td>2</td>
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</table>

*na: not analysed*

---

**Table 5:** Analytical chromatographic methods used by the WP1 partners for mycotoxin determination in FAPAS materials.

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<tr>
<th>Institution</th>
<th>Mycotoxin</th>
<th>Subsample weight</th>
<th>Extration procedure</th>
<th>Clean-up</th>
<th>Determination</th>
<th>Reference</th>
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<td>EMBRAPA</td>
<td>AF</td>
<td>50g</td>
<td>Blend with MeOH and KCl sol</td>
<td>Liq-liq extraction</td>
<td>TLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41</td>
</tr>
<tr>
<td></td>
<td>ZEA</td>
<td>25g</td>
<td>shaker with AcCN and H2O</td>
<td>ROMER column</td>
<td>HPLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41</td>
</tr>
<tr>
<td></td>
<td>MAA</td>
<td>AF 50g</td>
<td>Shaker with H2O, MeOH and NaCl</td>
<td>Immunoafinity column</td>
<td>TLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41 and POP 055, 2002 - Ed. 02, Rev. 01</td>
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<td>25g</td>
<td>Shaker with AcCN, H2O and celite</td>
<td>ROMER column</td>
<td>HPLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41 and POP 041, 2001 - Ed. 03, Rev. 02</td>
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<td>LATU</td>
<td>ZEA</td>
<td>25g</td>
<td>Shaker with NaCl, H2O and MeOH</td>
<td>Liq-liq extraction</td>
<td>TLC</td>
<td>AOAC, 2000 – 970.45</td>
</tr>
<tr>
<td></td>
<td>AF</td>
<td>25g</td>
<td>Shaker with MeOH and H2O</td>
<td>LC-Si SPE tubes 1000 mg</td>
<td>TLC</td>
<td>AOAC, 2000 – 993.17</td>
</tr>
<tr>
<td></td>
<td>FUM</td>
<td>10 g</td>
<td>Shaker with H2O and MeOH</td>
<td>LC-Si SPE tubes 100 mg</td>
<td>HPLC</td>
<td>AOAC, 2000 – 995.15</td>
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<tr>
<td></td>
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<td>AF 25 g</td>
<td>Blender with AcCN and H2O</td>
<td>Romer column</td>
<td>HPTLC</td>
<td>AOAC, 2000 – 949.08</td>
</tr>
<tr>
<td></td>
<td>ZEA</td>
<td>25 g</td>
<td>Blender with AcCN and H2O</td>
<td>Romer column</td>
<td>HPTLC</td>
<td>DOU 2000, Seção 1, n° 62, p. 35-41</td>
</tr>
<tr>
<td>Mycotoxin</td>
<td>FAPAS assigned value (μg/kg)</td>
<td>FAPAS satisfactory range (μg/kg)</td>
<td>LATU</td>
<td>UdeC</td>
<td>MAA</td>
<td>EMBRAPA</td>
</tr>
<tr>
<td>-----------</td>
<td>-----------------------------</td>
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<td>------</td>
<td>-----</td>
<td>-----</td>
<td>---------</td>
</tr>
<tr>
<td></td>
<td>Recovery (%)</td>
<td>Determined average value* (μg/kg)</td>
<td>SD</td>
<td>Recovery (%)</td>
<td>Determined average value** (μg/kg)</td>
<td>SD</td>
</tr>
<tr>
<td>AF B1</td>
<td>6.78</td>
<td>3.8-9.76</td>
<td>94</td>
<td>5.9</td>
<td>0.9</td>
<td>55</td>
</tr>
<tr>
<td>AF B2</td>
<td>1.66</td>
<td>0.93-2.39</td>
<td>89</td>
<td>1.8</td>
<td>0.3</td>
<td>52</td>
</tr>
<tr>
<td>Total AF</td>
<td>8.66</td>
<td>4.85-12.46</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ZEA</td>
<td>228</td>
<td>137-319</td>
<td>85</td>
<td>290</td>
<td>16.5</td>
<td>-</td>
</tr>
<tr>
<td>FB1</td>
<td>879.1</td>
<td>432.1-1326.1</td>
<td>94</td>
<td>447.1</td>
<td>38.0</td>
<td>-</td>
</tr>
<tr>
<td>FB2</td>
<td>305.9</td>
<td>150.4-461.3</td>
<td>89</td>
<td>268.9</td>
<td>15.6</td>
<td>-</td>
</tr>
</tbody>
</table>

* Value corrected by the recovery
** Value not corrected by the recovery
*** No repetition

Table 6: Results of the interlaboratory works performed by the WP1 partners using FAPAS materials of known mycotoxin contamination
Participation in congresses and Mycotox meetings

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

- WP1 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.
  Meeting with the Mycotox Brazilian team: Eugenia Vargas, O. Freitas-Silva., M. de L.M de Souza, T.B.S Correia, C. Brabet

  Presentation of one oral communication (see Publications).

- 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.

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

- WP4 &5 Brazilian team meeting with the industry Frango Canção, December, Maringá, PR, Brazil, in order to define the partnership with the private sector: Otniel Freitas-Silva

Publications


Participation in WP 4&5

EMBRAPA contribution in the workpackage WP4 consists 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-ctaa.
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 synthesized and organized 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. This combination is:
- Corn selected as commodity
- Aflatoxins, fumonisins and zearalenone identified as the most important toxins. OTA to be still confirmed.
- 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 confirmed.
  Industry Abatedouro de Aves Canção - Gonçalvez & Tortola LTDA: Poultry slaughter-house, Maringa, PR and feed ration plant, Indianapolis, PR.

OUTLINE PLANS FOR 2004

WP1 ACTIVITIES

- Implementation of the corrective actions by EMBRAPA according to the results of the first series of FAPAS interlaboratory works.

- Implementation and validation of mycotoxin analytical methods: FUM at the EMBRAPA

- Final tests for implementation and validation of the HPLC method for ZEA determination at the EMBRAPA

- Participation in the one round of proficiency test between labs from WP1, 4 and 5 using homogeneous corn sample naturally contaminated.

- Participation in the determination of the sample collection, preparation and sending methods for the WP 4&5.
- Present a conference or Poster at the Brazilian Corn Congress, focusing on the validation of analytical method for Zearalenone determination

- Prepare the documentation for the accreditation of the CTAA mycotoxin laboratory by the MAA and the Rio metrology.

**WP4 & 5 ACTIVITIES**

- Construction and verification of the CFD for the technical aspects

- Corn sample collection along the selected supply chain

- Mycotoxins analysis

- Data analysis and reporting
FAPAS interlaboratory works: EMBRAPA results

Report Resulting Sheets: Zearalenone

Laboratory: Embrapa –CTAA

Toxin analysed:
- AFL
- FUM
- ZEA
- DON
- OTA

Sample:
- Wheat
- X: corn
- Wheat products
- Corn products
- Others

Sample weight:
- 5Kg
- 9Kg
- 1Kg

Subsample:
- X: 25g
- 50g

Extraction procedure:
- Shaker with H2O and CHCl3
- Blend with MeOH and KCl sol
- Shaker with NaCl, H2O and MeOH
- Shaker with NaCl, H2O and MeOH
- X: Shaker with ACN and H2O
- Shaker with ACN and MeOH
- Blender with ACN and H2O
- Shaker with H2O and MeOH

Clean-up:
- Liq-liq extraction
- SPE column (florisil and C-18)
- LC-Si SPE tubes 1000mg
- Immunoaffinity column
- XROMER column
- ROMER or Trilogy column
- SAX or Baker Amino
- Ochrapped or Vicam columns

Determination:
- X: HPLC
- TLC
- CGL and ECD

TLC data:
- Plate:
- Mobile fase:
- Spotted vol.
- Chromatovisor:
- Densitometer

Column:
- X-Terra RP-18 5 m (4.8x250mm)
- Mobile fase: MeOH 80% flow 0,5mL/min. gradient
- Injection vol. 50 L

CGL and ECD data:
- Column:
- Gas:
- Flow
- Gradient Temp
- Injection vol.

Reference:
- DOU 2000, Seção 1, n° 62, p. 35-41

Percentage recovery:
- 120 %

Data:
- 241,25 μg/kg

Corrected Data:
- 289,5 μg/kg
### Report Resulting Sheets: Aflatoxins

- **Laboratory**: Embrapa Agroindústria de Alimentos _CTAA_

- **Toxin analysed**: AFL, FUM, ZEA, DON, OTA

- **Sample**: wheat, corn, wheat products, corn products, others

- **Sample weight**: 5Kg, 3Kg, 1Kg

- **Subsample**: 25g, 50g

- **Extraction procedure**: shaker with H2O and CHCl3, Blend with MeOH and KCl sol, shaker with NaCl, H2O and MeOH, shaker with NaCl, H2O and MeOH, shaker with ACN and H2O, shaker with ACN and MeOH, Blender with ACN and H2O, shaker with H2O and MeOH

- **Clean-up**: Liq-liq extraction, SPE column (florisil and C-18), LC-Si SPE tubes 1000mg, immunoaffinity column, ROMER column, ROMER or Trilogy column, SAX or Baker Amino, Ochrapped or Vicam columns

- **Determination**: HPLC, TLC, CGL and ECD

- **TLC datas**: Plate: TLC aluminium sheets silica gel 60 (Merck 1.05553), Mobile fase: éter etílico anidro: metanol: água (96:3:1) e acetona-clorofórmio (1+9), Spoted vol.: 10 L, X Cormatovisor, Densitometer

- **HPLC datas**: Column: Mobile fase: flow, gradient, Injection vol.

- **CGL and ECD Column**: Gas: flow, Gradient Temp, Injection vol.

- **Reference**: DOU 2000, Seção 1, n° 62, p. 35-41

- **Percentage recovery**: B1=97%, B2=77%

- **Data**: B1=4.99μg/kg, B2=1.04μg/kg

- **Corrected Data**: B1=4.84μg/kg, B2=0.80 μg/kg
Contract number: ICA4-CT-2002-10043

Partner 05 - MAA

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
FIRST ANNUAL REPORT

Participation in WP1

OBJECTIVE: To report concisely the activities developed by the LACQSA/LAV-MG, laboratory of the Ministry of Agriculture, Livestock and Supply.

MATERIAL AND METHODS:

a) Fapas samples
b) Maize samples
c) Aflatoxin and zearalenone methods of analysis

RESULTS:


b) Identification and preparation of homogeneous corn sample (reference material) blank for aflatoxins, zearalenone and fumonisins, and preliminary analyses for assessment of contamination.

c) Identification and start of preparation of homogeneous corn sample (reference material) naturally contaminated with aflatoxins, zearalenone and fumonisins;

d) Analysis of corn samples acquired from FAPAS for determination of aflatoxin and zearalenone;

e) Participation in the Encounter with Directors of Frango Canção to discuss the activities related to the FLD for maize productions destined to poultry production (Study case) for the Mycotox Project - Work Package 4 and 5 (WP 4 and 5), in Maringá, Paraná, on 10th December, 2003

PARTICIPATION IN MEETINGS AND CONGRESS

a) Participation in the Pre-congress “La Aplicación de los principios de HACCP en la prevención y control de micotoxinas” – Food Quality Service and Standards, Food Direction and Nutrition, FAO, Rome, administered by Dr. Maya Pineiro, on 22 and 23 September 2003 in Havana, Cuba;

b) Participation in the Encounter for the Mycotox Project - Work Package 1 (WP1), on 24 and 25 September 2003 in Havana, Cuba;


d) Working out of report of the travel to Cuba;
PLANS FOR 2004

a) To participate in the WP4 and WP5 carrying out zearalenone analysis. The participation will depend upon the budget available to LACQSA;

b) To participate in the construction of CFD diagrams in WP4 and WP5 – Frango Canção. The participation will depend upon the budget available to LACQSA;

c) To finalize the homogeneous maize sample preparation (blank and naturally contaminated) to aflatoxin, zearalenone and fumonisins;

d) To implement one round of proficiency test between labs from WP1, 4 and 5 using homogeneous naturally contaminated maize samples;

e) To distribute samples for the intralaboratory control of the laboratories taking part in the other WPs (see detail in the protocols).

f) To receive the proficiency test and internal quality control results from the labs and elaborate the final report.
Protocol elaborated by MAA for sampling plan

1) INTRODUCTION

Due to a non-homogeneous distribution of mycotoxins in the samples, special attention is indispensable to the sampling procedure. It is important that the sampling procedure be clearly defined, be unique, to be followed by all those who will collect samples related to the Project **Mycotox ICA 4-CT-2002-10043**. Attainment of significant monitoring data demands a collection of representative samples from lots of samples carefully selected (lots representative of well defined regions – country or region inside a country).

2) OBJECTIVES

To provide uniformity of the sampling procedure to be utilised for collection of samples that shall be analysed at the Project **Mycotox ICA 4-CT-2002-10043** so that the results from monitoring may be correctly obtained and utilised.

3) PROCEDURES

The information regarding collection of samples should include number, origin, (city, state, country), type of sample, size of sample, data on the collection (year, month, season), sampling points, sub-sampling scheme, treatment of the samples after collection (mixture, storage, grinding) and other relevant information. The sampling procedure to be used shall be discussed between the Laboratories participating in the WP1. This procedure should be in accordance with some plan already published and accepted internationally (plans established by FAO, Codex Alimentarius, USDA, European Union or suggested by some committee like JECFA) for which the target critical level (4 or 20 μg/kg of, for example, total aflatoxin), the risks to the producer and consumer and variability expected for each stage of sampling (sampling, sub-sampling and analysis) are established. Also, aspects like capability of the Laboratory in terms of infrastructure and equipment needed for processing and storage of samples should be considered (equipment for grinding, homogenisation or cold chamber).

*Note 1: The procedures for preparation of samples (sub-sampling) can only be defined vis-à-vis the capability of the laboratories to adopt a certain procedure.*

*Note 2: We emphasise the necessity of establishing a logistics for the chains to be surveyed to enable determining the sampling points.*

*Note 3: We emphasise the importance of reporting any deviations from the methods as proposed by the Protocols of Report of Results, intralaboratory control and interlaboratory control (production of homogeneous materials to perform the analyses), as the laboratories may adopt different methods and the variability of the analytical process needs to be known.*
4) REFERENCES


FAO- Plano de Amostragem para Análise de Aflatoxinas em Milho e Amendoins. FAO Alimento e Nutrição, Boletim 55, 1993


JECFA – JECFA sampling working paper 2, elaborated by Coker & Whitaker, Revised Draft 1, February, 14th, 2001.


MAA Ministério da Agricultura, Pecuária e Abastecimento, Procedimento Operacional Padrao: Determinação de aflatoxinas B₁, B₂, G₁ e G₂ por cromatografia líquida de alta eficiência e em camada delgada, POP 055 (ed 02, rev 01) publicação interna do LACQSA/LAV-MG de 29/05/2002, 22 p.


Protocol elaborated by MAA for intralaboratory control

1) INTRODUCTION

Intralaboratory control combined with the use of validated methods is an essential tool for assurance of reliability of the data generated and should make part of the routine procedures of the laboratories.

Intralaboratory control can be performed using control materials: certified reference materials, in-house homogeneous materials, artificially contaminated samples and blank samples. This control can be blind (the analyst does not know the mycotoxin contamination in the control sample) or non-blind to the analyst, and should be performed at each batch of samples. The determination of the level of contamination in the control sample should be utilised as a criterion of acceptability or rejection for the analytical results obtained in a given batch of samples, and this criterion should be established and defined for each laboratory and for each mycotoxin.

The Project, by means of the WP, shall establish agreeing criteria between the participant laboratories and those, which will perform all the analyses related to implementation of the Project MYCOTOX so that the data generated, can be correctly utilised and interpreted.

2) OBJECTIVES

To assure that the analytical results from the Laboratories participating in the Project Mycotox ICA 4-CT-2002-10043 are obtained within a criterion of acceptability, ensuring the quality and reliability of the analytical data generated and contributing to harmonisation of the analytical procedures.

Specific objectives
- To produce homogeneous test samples both blank and/or naturally contaminated;
- To establish intralaboratory control at each batch of samples, by means of recovery tests, non-blind, with either artificially contaminated or naturally contaminated samples, by using control charts (based on average of standard deviation from at least 10 analysis of the same sample);
- To establish acceptability criteria for release of the results obtained in the batch, based either on the recovery values obtained or on the control chart;

3) MATERIAL AND METHODS

3.1 Material

Test samples of corn and wheat, either homogeneous, artificially and/or naturally contaminated with the mycotoxin of interest, i.e. total aflatoxins (B1, B2, G1 and G2), ochratoxin A, zearalenone, fumonisin B1, B2 and deoxynivalenol.

3.2 Methods

The test samples should be analysed together with the samples coming from the various actions of the Project. The sample identified as blank sample should be artificially contaminated with standard solution of the mycotoxin of interest, in a combination of matrix/toxin, and shall be utilised as bench control. The results of
analysis can only be utilised by the Project in case they meet the criteria described in Table 1. Non-observation of these criteria determines that the laboratory should analyse the cause of the non-conformity, provide correction of the non-conformity and analyse again the samples related to that specific control (as shown in Figure 1).

Figure 1: Internal quality control scheme (LACQSA 2001b)
### Table 1: Criteria of acceptability of recovery results (CEN 1999)

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Level (μg/kg)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁ and/or Total</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>50 a 120</td>
<td></td>
</tr>
<tr>
<td>1 – 10</td>
<td>70 a 110</td>
<td></td>
</tr>
<tr>
<td>&gt; 10</td>
<td>80 a 110</td>
<td></td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 1</td>
<td>50 a 120</td>
<td></td>
</tr>
<tr>
<td>1 – 10</td>
<td>70 a 110</td>
<td></td>
</tr>
<tr>
<td>Fumonisin B₁ ou B₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 500</td>
<td>50 a 120</td>
<td></td>
</tr>
<tr>
<td>500 - 5000</td>
<td>70 a 110</td>
<td></td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td></td>
<td></td>
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<tr>
<td>&gt; 100</td>
<td>70 a 110</td>
<td></td>
</tr>
<tr>
<td>Zearalenone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 100</td>
<td>70 a 110</td>
<td></td>
</tr>
</tbody>
</table>

It is strongly recommended that the naturally contaminated test samples produced by each participant laboratory be utilised, at each batch of samples analysed in the scope of the project and that the results should be reported in the form of control charts- \( x \pm s, 2s, 3s \) as shown in Figure 2.
### Test sample code: 04NC002  
Matrix:  
Assigned value (Xm): 4.99 μg/kg  
S (standard deviation): 0.58 μg/kg  
SOP (ed/rev): 039 (03/02)  
Mycotoxin: Ochratoxin A  
Year: 2004

| Control no  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|-------------|---|---|---|---|---|---|---|---|---|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Date (month/day) | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 | 01 |
| Analyst | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Result (μg/kg) | 4, | 5, | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| +3s = 6.73 Warning limit | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| +2s = 6.15 Action limit | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| + s = 5.57 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Assigned value = 4.99 x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| - s = 4.41 x | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Action limit - 2s = 3.83 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Warning limit - 3s = 3.25 | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

**Notes:**

**Figure 2:** Control chart – reference material
4) RESULT REPORTING
The results of recovery (R%) should be reported together with the results of the samples related to that control, for each combination of matrix/toxin in the worksheets/registration forms of recovery listed in Annex 1: Recovery – Intralaboratory Control – Non-blind samples.
The analytical results of the monitoring shall be reported corrected and not corrected by recovery control.
At the end of each 03 months the laboratories should report to the WP1 the results of intralaboratory control, both by mail and electronically, for purposes of scoring on tables and statistical analysis.
The data displayed on tables shall be forwarded to the Project Co-ordination, who shall make them available on the “Quick Place” to all participants.

5) CONCLUSION
The data obtained by the intralaboratory control will be used to:
- Help the laboratories enhance the performance of their methods;
- Provide sustainability to the analytical results generates by the Project;
- Help at interpretation of the analysis data made by the various WPs;
- Help at definition of a general report of methods’ performance.

6) REFERENCES
FAPAS - FOOD ANALYSIS PERFORMANCE ASSESSMENT SCHEME. Protocol for the FAPAS. Organisation and Analysis of Data, CSL. 5th ed., April 1997
HORWITZ W., ALBERT R., NESHEIM S.; 1993; Reliability of Mycotoxin Assays – An Update; Journal of AOAC International; 76; 3.

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.


Recoveries Aflatoxins B₁, B₂, G₁ e G₂ - Internal Quality Control - Non blind samples

<table>
<thead>
<tr>
<th>Analysed samples (code)</th>
<th>Date of Analysis Start/end</th>
<th>Registration blank/matrix</th>
<th>Contamination level (μg/kg)</th>
<th>Determined contamination level (μg/kg)</th>
<th>Recovery (%)</th>
<th>Laboratory</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>B₁</td>
<td>B₂</td>
<td>G₁</td>
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<td></td>
<td></td>
<td>B₁</td>
<td>B₂</td>
<td>G₁</td>
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<td></td>
<td>B₁</td>
<td>B₂</td>
<td>G₁</td>
<td>G₂</td>
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</tbody>
</table>
### Recoveries Fumonisinas B₁ e B₂ – Internal Quality Control - Non blind samples

<table>
<thead>
<tr>
<th>Analysed samples (code)</th>
<th>Date of Analysis</th>
<th>Working standard solution concentration (µg/mL)</th>
<th>Contamination level (µg/g)</th>
<th>Determined contamination level (µg/g)</th>
<th>Registration nº Blank</th>
<th>Recovery (%)</th>
<th>Laboratory</th>
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</table>
## Recoveries Deoxynivalenol - Internal Quality Control - Non blind samples

<table>
<thead>
<tr>
<th>Analysed samples (code)</th>
<th>Date of Analysis</th>
<th>Working standard solution concentration (μg/mL)</th>
<th>Nível de contaminação teórico (μg/kg)</th>
<th>Determined contamination level (μg/kg)</th>
<th>Registration nº blank</th>
<th>Recovery (%)</th>
<th>Laboratory</th>
</tr>
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<tbody>
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<tr>
<th>Analysed samples (code)</th>
<th>Date of Analysis</th>
<th>Working standard solution concentration (μg/mL)</th>
<th>Contamination Level (μg/kg)</th>
<th>Determined contamination level (μg/kg)</th>
<th>Registration n°. blank</th>
<th>Recovery (%)</th>
<th>Laboratório</th>
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<td>Analysed samples (code)</td>
<td>Date of Analysis</td>
<td>Working standard solution concentration (μg/mL)</td>
<td>Contamination Level (μg/kg)</td>
<td>Determined contamination level (μg/kg)</td>
<td>Registration nº. blank</td>
<td>Recovery (%)</td>
<td>Laboratório</td>
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<td>Start</td>
<td>End</td>
<td>Zearalenone</td>
<td>Zearalenone</td>
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</table>
Protocol elaborated by MAA for interlaboratory control

1) INTRODUCTION
Interlaboratory comparisons are made with various purposes, among which, the ones more closely related to Project MYCOTOX are: to determine and monitor the performance of laboratories (proficiency test), to identify non-conformities at the execution of methods with consequent performance of corrective actions, to provide additional reliability to the data generated and to the users of such data and to identify differences between laboratories (ISO Guide 43-1, 1997).
Participation in proficiency tests provides an objective and continuous assessment of the capability of the laboratory in producing exact and reliable results (Gilbert, 2002).

2) OBJECTIVES
To contribute to enhancement of analytical performance of the laboratories participating in the Project Mycotox ICA 4-CT-2002-10043 at analysis of mycotoxins (aflatoxin B₁, B₂, G₁ and G₂, zearalenone, fumonisins B₁, B₂ and/or deoxinivalenol) in samples of corn and/or wheat, aiming at improvement of the quality and reliability of the analytical data generated and harmonisation of the analytical procedures.

Specific objectives
- To provide assessment and enhancement of the methods on the part of the Laboratories;
- To provide comparison of the data obtained by the Laboratories participating in the Project with data of other laboratories;
- To offer the availability of homogeneous samples for purposes of intra and inter-laboratory control;
- To determine the characteristics of the methods utilised by the laboratories as a support to the actions of the monitoring process predicted in other WPs.
- To make available an inventory of the performance of the analytical methods used by the Laboratories of the South Cone.

3) MATERIAL AND METHODS
3.1. Material
- “Surplus test material” samples acquired from FAPAS, of contamination known by the Laboratories, in a combination of matrix x toxin of interest;
- Samples from the Proficiency Test organised by FAPAS, or “Surplus test material” samples acquired from FAPAS in a combination of matrix x toxin of interest, and centralised in an institution of WP1;
- Homogeneous test samples of matrix and mycotoxin of interest, prepared by the LACQSA/LAV-MG in accordance with international protocols for proficiency tests and collaborative studies;
3.2. Methods

3.2.1 Phase 1 – FAPAS samples of known contamination
The samples shall be utilised by the Laboratories for internal assessment of applicability and adequacy of the methods.

3.2.2. Phase 2 – Participation in proficiency test organised by FAPAS
The laboratories shall participate in the rounds of proficiency test organised by FAPAS for the matrices and toxins of interest. The results obtained shall be evaluated and, any questionable or unsatisfactory results shall be treated as non-conformity (Figure 1) and the procedures shall be reviewed.

3.2.3. Phase 3 – Homogeneous test samples
The test samples shall be prepared, and their homogeneity and most probable value of contamination shall be statistically estimated, according to Horwitz (1995), Thompson and Wood (1993), ISO/IEC 43 (1997), Thompson (2000) and FAPAS (2002) and Vargas et al. (2001). The homogeneity of the samples produced shall also be evaluated by another laboratory, not participant in the Project. These samples shall be forwarded to the Laboratories and shall make part in the laboratory control performed at each batch of samples analysed at routine. The sample concentration will not be known to the Laboratory (blind test).
3.2.4. Data Treatment

The results referring to the samples of Phase 1 shall be evaluated by the Laboratories in terms of recovery and standard deviation, by considering the certified value and comparing with the values already stipulated at the validation of the method. In case the results do not match, the Laboratory shall provide the necessary alterations and adjustments in the methods, taking into consideration the criteria of performance according to the Protocol of Intralaboratory Control.

The results of Phase 2 forwarded by FAPAS shall be evaluated by the Laboratories. When these would show “questionable” or “unsatisfactory”, they should be treated as non-conformities (the report and analysis of causes shall be registered; corrective actions shall be proposed; implementation of the actions and elimination of the non-conformity shall be verified, according to Figure 1).

---

**Figure 1: Flow chart of treatment of FAPAS Results**

---

- **FAPAS**
  - Samples, analysis record
  - Questionnaire sheets

- **LACQSA**
  - If Iz-score > 2
  - Non-conforming work
  - Analysis of causes
  - Corrective Actions
  - Implementation of corrective actions
  - ELIMINATION OF NON-CONFORMITY
  - Non-conformity not eliminated

- **Examples of Checking**
  - Level of contamination is above the LD of the method
  - Calculation and report of results
  - Standard solution
  - Recovery of control sample of the batch
  - Analytical procedures
  - Equipment utilized at critical stages
The z-score function can be shown in the form of chart, and is customarily utilised as a control graph, being calculated by the following equation:

\[ z = \frac{X - \bar{X}}{\sigma} \]

Where:
- \( X \) is the value of the contamination determined by the Laboratory;
- \( \bar{X} \) is the value that best represents the true measure of aflatoxin in the sample (according to evaluation in the sample homogeneity tests);
- \( \sigma \) is the standard deviation of the value that best represents the true measure of aflatoxin, being the standard deviation (\( \sigma \)) calculated as \( b \bar{X} \), where;

\[ b = \frac{\% \text{RSD}_{R}}{100} \]

For concentrations of the analyte <120 µg/kg, \( \% \text{RSD}_{R} \) is obtained from the modified form of Horwitz equation, where \( \% \text{RSD}_{R} = 22 \) and for concentrations of the analyte >120 µg/kg, \( \% \text{RSD}_{R} = 2 \left(1 - 0.5 \log_{10} X\right) \)

The interpretation of the z-score is made as follows:

<table>
<thead>
<tr>
<th>Ranges</th>
<th>The Laboratory result shall be considered as:</th>
</tr>
</thead>
<tbody>
<tr>
<td>If (</td>
<td>z</td>
</tr>
<tr>
<td>If ( 2 &lt;</td>
<td>z</td>
</tr>
<tr>
<td>If (</td>
<td>z</td>
</tr>
</tbody>
</table>

Note 1: In case Phase 2 is organised by a WP1 laboratory, the value of FAPAS sample (not the robust average) should be used to evaluate FAPAS results.

The statistical evaluation of the analytical results obtained for the homogeneous sample test reached in **Phase 3** by each laboratory and for each aflatoxin shall be performed using control charts as shown in Figure 2. The parameters “average” and “standard deviation” to be considered initially should be the ones defined by the Laboratory that produced the homogeneous material. The variability of the results shall be estimated as predicted by modification of Horwitz equation (\( 2 \times \text{RSD}_{R} \)) where the target \( \text{RSD}_{R} \) (22%) should be the one established by Thompson (2000), and shall be expressed in the control charts in form of bars.

At the end of the analyses, the data generated by all Laboratories shall be compiled and the values of average and standard deviation contamination shall be calculated, excluding the “outliers”. The methods will be compared and the level of agreement between them will be established, i.e. the reproducibility of the South Cone laboratories' works and also repeatability at each laboratory.
4) RESULT REPORTING

All data obtained by the Laboratories and their evaluations shall be sent electronically to the WP1 Co-ordination for compilation and statistical analysis, according to the forms shown in item 6) of Annexes.

After statistical evaluation of the data, a report containing results from all participant laboratories shall be written. Each laboratory will receive a code number which shall be kept confidential. The results shall be presented in the form of z-score and control tables and charts.

5) REFERENCES

FAPAS - FOOD ANALYSIS PERFORMANCE ASSESSMENT SCHEME. Protocol for the FAPAS. Organisation and Analysis of Data, CSL. 5th ed., April 1997

HORWITZ W., ALBERT R., NESHEIM S.; 1993; Reliability of Mycotoxin Assays – An Update; Journal of AOAC International; 76; 3.


GILBERT, J. Quality Control Measures for Mycotoxin Laboratories, FDA Workshop on Mycotoxins, 22-26, July, 2002, Maryland, USA.


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.


Form – Results of FAPAS samples –Phase 1

RESULTS OF FAPAS SAMPLES (MATRIX/MYCOTOXIN)

Laboratory: .................................................................................................................................
Date: ..........................................................................
Material code:
Mycotoxin:
Matrix:
Date of analysis: _____ / _____ / ______
Analyst: Sign:

Results:

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Result (μg/kg)</th>
<th>Recovery (%)</th>
<th>Result corrected by recovery (μg/kg)</th>
<th>Assigned value (μg/kg)</th>
<th>Interpretation</th>
</tr>
</thead>
</table>
### RESULTS OF PROFICIENCY TESTING

Laboratory: ..........................................................

Date: ..........................................................

Material code: ..........................................

**Mycotoxin:**

**Matrix:**

**Date of analysis:** _____ / _____ / _____

**Analyst:** ..............................................

**Sign:** ..............................................

#### Results

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Result (μg/kg)</th>
<th>Recovery (%)</th>
<th>Result corrected by the recovery (μg/kg)</th>
<th>Assigned value (μg/kg)</th>
<th>z-score</th>
<th>Corrective action</th>
</tr>
</thead>
</table>
Form – analytical methodology – Phases 1, 2 and 3

ANALYTICAL WORK QUESTIONNAIRE

1) Reference method

2) Sample weight (g)

<table>
<thead>
<tr>
<th>&lt;25</th>
<th>≥25 to &lt;50</th>
<th>≥50</th>
<th>Others</th>
</tr>
</thead>
<tbody>
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</tbody>
</table>

3) Extraction solvent

<table>
<thead>
<tr>
<th>Water</th>
<th>Acetonitrile</th>
<th>Dichloromethane</th>
<th>Others (specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Acetone</td>
<td>Chloroform</td>
<td></td>
</tr>
</tbody>
</table>

Volume of extraction solvent (mL)

4) Extraction procedure

<table>
<thead>
<tr>
<th>Blend with solvent</th>
<th>Shake with solvent</th>
<th>Add NaCl</th>
<th>Others</th>
</tr>
</thead>
<tbody>
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</table>

5) Sample work up

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<thead>
<tr>
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<th>Centrifuge</th>
<th>Decant</th>
<th>Dilute</th>
<th>Evaporate</th>
<th>pH Adjustment</th>
<th>Others</th>
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</thead>
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</table>

6) Clean-up

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<tr>
<th>Immunoaffinity column</th>
<th>Solid phase</th>
<th>Liquid-liquid partition</th>
<th>Others</th>
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</thead>
<tbody>
<tr>
<td>Brand: Florisil</td>
<td>Silica</td>
<td>alumina</td>
<td>Romer</td>
</tr>
<tr>
<td>Precipitation with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cupric sulphate</td>
<td>Ammonium sulphate</td>
<td>Celite</td>
<td>Others</td>
</tr>
</tbody>
</table>


7) Determination and quantification

Final test sample in the final extract (g):
Solvent of dissolution the final extract:
Volume of solvent to dissolve the final extract (µL):

<table>
<thead>
<tr>
<th>HPLC</th>
<th>TLC</th>
<th>Others (specify)</th>
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</thead>
<tbody>
<tr>
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<tr>
<td>Injection volume (µL)</td>
<td>Spotted volume (µL)</td>
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</tr>
<tr>
<td>Column packing and size</td>
<td>TLC brand and size</td>
<td></td>
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<tr>
<td>Column Temperature (°C)</td>
<td>One or bi directional</td>
<td></td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>Visual or densitometer quantification</td>
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</tr>
<tr>
<td>Detector</td>
<td>λ (nm)</td>
<td></td>
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<tr>
<td>λ_{ex} and λ_{em} (nm)</td>
<td>Mobile phase</td>
<td></td>
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<tr>
<td>Mobile phase</td>
<td>Derivatization</td>
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<tr>
<td>Derivatization solvent</td>
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<td></td>
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<tr>
<td>Derivatization condition</td>
<td></td>
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</tbody>
</table>

8) Standard Brand

<table>
<thead>
<tr>
<th>Sigma</th>
<th>Rhône Poulenc</th>
<th>Fluka</th>
<th>Others</th>
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</table>

9) Concentration of standard work solution (µg/mL):

10) Standard calibration curve

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Concentration (µg/mL) or mass of aflatoxins used (ng) to quantify the samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard 1</td>
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</tbody>
</table>

11) Method quantification limit (µg/kg):

12) Additional comments and/or observations
# RESULTS OF HOMOGENEOUS TEST SAMPLES

**Laboratory:** .................................................................

**Date:** .................................................................

**Material code:** .........................................................

**Matrix:** .................................................................

**Analyst:** .................................................................

**Sign:** .................................................................

## Results

<table>
<thead>
<tr>
<th>Date of analysis</th>
<th>Contamination level (μg/kg)</th>
<th>Recovery (%)</th>
<th>Result corrected by the recovery (μg/kg)</th>
<th>Corrective action</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Afla B₁</td>
<td>Afla B₂</td>
<td>Afla G₁</td>
<td>Afla G₂</td>
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Protocol elaborated by MAA for result reporting

1. INTRODUCTION
The Project, by means of the WP1 shall establish criteria for report of results for all laboratories that will perform analyses related to implementation of the MYCOTOX Project so that the data generated may be correctly utilised and interpreted.

Therefore, to prevent contradictory situations at interpretation of the results generated by the MYCOTOX Project, it is important to have proper criteria established for the results to be interpreted in a clear, complete and harmonised way.

2. OBJECTIVES
To ensure that the analytical results of the Laboratories participating in the Mycotox Project ICA 4-CT-2002-10043 are reported in a proper and harmonised way.

3. DISPLAY OF RESULTS
3.1. Regarding the criteria for display of analysis results
The results shall only be reported if the outcomes of intralaboratory control meet the criteria established as acceptable according to the Protocol of Intralaboratory Control.

The analysis results by liquid and gaseous chromatography shall be considered when the correlation coefficient (r²) for the calibration curve is greater than 0.99. The results of densitometric analysis shall be considered, preferably in regard of those obtained by visual analysis, when the correlation coefficient (r²) for the calibration curve is greater than 0.9. When this it is not possible, the results of visual analysis shall be considered (average of readings of two analysts, at least).

The monitoring data should be reported by referencing the method utilised, such as the their Limits of Detection (LOD) and Limits of Quantification (LOQ).

The analytical results should be reported after corrected, and should not be corrected by the recovery of the control, which should be indicated, such as an estimation of the uncertainty of measurement, in terms of relative standard deviation (RSD).

Considering that the limits of legislations for mycotoxins, presently in force, are expressed with one whole numeral, all laboratories shall express their analysis results with one decimal figure.

3.2 Regarding the minimum content of the terms of emission of results
The results of analysis should be reported as per the Model described.

3.3 Regarding treatment of results
For purposes of statistical analysis by the WPs, the ND results (non detected) or smaller than the limits of detection (LOD) of the methods shall be considered as being half the limit of detection of the method reported. For results between the LOD and the limit of quantification (LOQ), the value found should be utilised. If only the
LOD has been determined, the results smaller than the LOQ should be considered as half the LOQ.

The WPs shall report the results including the following data: total samples and contaminated samples, in terms of number and percentage, maximum level determined, range of concentrations detected, average contamination (including samples with ND results), average contamination of positive samples, median of positive samples.

Example of Table for display of the data compiled.

<table>
<thead>
<tr>
<th>Mycotoxins</th>
<th>Number of Samples per range of contamination (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ND</td>
</tr>
<tr>
<td>Aflatoxin B₁</td>
<td></td>
</tr>
</tbody>
</table>

4. REFERENCES


ANALYSIS REPORT

Nº 001 / 2003

Laboratory sample code:

Customer identification

Costumer: Procissur Project
Address:
City:
State:
Country:
ZIP code:
Phone:
Fac-simile:

Product identification

Product:
Product register n°:
Sample n°:
Batch size (kg or metric ton.)
Batch No
Incremental size
Crop:
Location of storage:
Storage condition:
Origen (City/State/Country):
Destination:
Transportation:
Producer/Storekeeper/Dealer/Importer/Exporter/Packer:
Date of collection:
Year/Month/Estação da coleta:
Sampling points:
Subsampling points:
Subsampling procedure (aleatória ou dirigida):
Collect placed (city/state):
Sample size received by Laboratory (kg):
Analysis (es) requested
Date of receipt of sample
Date of analysis
Date of emission
## Analysis results

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Results (µg/kg)</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Aflatoxin B₂</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Aflatoxin G₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aflatoxin G₂</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zearalenone</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Ochratoxin A</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Fumonisin B₁</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Fumonisin B₂</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>Deoxynivalenol</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

*Procisur method reference.
Nd: not detected.

Signatures:

____________________________________  ____________________________________
Analyst Name                              Technical Manager Name
RESULTS OF FAPAS SAMPLES - AFLATOXINS and ZEARALENONE

Laboratory: LACQSA/LAV-MG
Date: 19/02/2004
Material code: 04-46 e 22-09
Mycotoxin: aflatoxins B₁, B₂, G₁ e G₂ and zearalenone
Matrix: maize
Date of analysis: 23, October 2003
Analyst: Eliene Alves dos Santos  Signature:
### Results:

<table>
<thead>
<tr>
<th>Sample n° FAPAS/LACQSA</th>
<th>Mycotoxin</th>
<th>Result (μg/kg)</th>
<th>Recovery (%)</th>
<th>Result corrected by the recovery (μg/kg)</th>
<th>Assigned value (μg/kg)</th>
<th>Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>04-46/03AC001</td>
<td>Aflatoxin B₁</td>
<td>6.8</td>
<td>85.7</td>
<td>7.9</td>
<td>6.78</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>Aflatoxin B₂</td>
<td>1.3</td>
<td>88.9</td>
<td>1.5</td>
<td>1.66</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>Afla total</td>
<td>8.1</td>
<td>87.3</td>
<td>9.3</td>
<td>8.66</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>04-46/03AC002</td>
<td>Aflatoxin B₁</td>
<td>7.2</td>
<td>85.7</td>
<td>8.4</td>
<td>6.78</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>Aflatoxin B₂</td>
<td>1.2</td>
<td>88.9</td>
<td>1.3</td>
<td>1.66</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>Afla total</td>
<td>8.4</td>
<td>87.3</td>
<td>9.6</td>
<td>8.66</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>04-46/03AC003</td>
<td>Aflatoxin B₁</td>
<td>6.8</td>
<td>85.7</td>
<td>7.9</td>
<td>6.78</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>Aflatoxin B₂</td>
<td>1.2</td>
<td>88.9</td>
<td>1.3</td>
<td>1.66</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>Afla total</td>
<td>8.0</td>
<td>87.3</td>
<td>9.2</td>
<td>8.66</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>04-46/03AC004</td>
<td>Aflatoxin B₁</td>
<td>7.2</td>
<td>85.7</td>
<td>8.1</td>
<td>6.78</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>Aflatoxin B₂</td>
<td>1.2</td>
<td>88.9</td>
<td>1.3</td>
<td>1.66</td>
<td>Satisfactory</td>
</tr>
<tr>
<td></td>
<td>Afla total</td>
<td>8.2</td>
<td>87.3</td>
<td>9.4</td>
<td>8.66</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22-09/03AC005</td>
<td>Zearalenone</td>
<td>153.9</td>
<td>80.7</td>
<td>190.7</td>
<td>228</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22-09/03AC006</td>
<td>Zearalenone</td>
<td>155.4</td>
<td>80.7</td>
<td>192.6</td>
<td>228</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22-09/03AC007</td>
<td>Zearalenone</td>
<td>148.3</td>
<td>80.7</td>
<td>183.8</td>
<td>228</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22-09/03AC008</td>
<td>Zearalenone</td>
<td>149.9</td>
<td>80.7</td>
<td>185.7</td>
<td>228</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22-09/03AC009</td>
<td>Zearalenone</td>
<td>151.8</td>
<td>80.7</td>
<td>188.1</td>
<td>228</td>
<td>Satisfactory</td>
</tr>
<tr>
<td>22-09/03AC010</td>
<td>Zearalenone</td>
<td>151.3</td>
<td>80.7</td>
<td>187.5</td>
<td>228</td>
<td>Satisfactory</td>
</tr>
</tbody>
</table>
## ANALYTICAL WORK QUESTIONNAIRE

1) **Reference method:** For aflatoxins analysis
   DOU 2000 e POP 055 (ed02, rev 01)

2) **Sample weight (g):**
   - <25
   - ≥25 to <50
   - ≥50
   - Others

3) **Extraction solvent:**
   - **Water**
   - Acetonitrile
   - Dichloromethane
   - Others (specify)
   - **Methanol**
   - Acetone
   - Chloroform
   - Volume of extraction solvent (mL):

4) **Extraction procedure:**
   - Blend with solvent
   - Shake with solvent
   - Add NaCl
   - Others

5) **Sample work up:**
   - Filter
   - Centrifuge
   - Decant
   - Dilute
   - Evaporate
   - pH Adjustment
   - Others

6) **Clean-up:**
   - Immunoadfinity column
   - Solid phase
   - Liquid-liquid partition
   - Others
   - Brand: VICAM
   - Silica
   - Florisil
   - alumina
   - Romer
   - Precipitation with
   - Cupric sulphate
   - Ammonium sulphate
   - Celite
   - Others
7) **Determination and quantification:**
Final test sample in the final extract (g): 1,667g
Solvent of dissolution the final extract: **Toluene: acetonitrile (9:1, v/v)**
Volume of solvent to dissolve the final extract (µL): **100**

<table>
<thead>
<tr>
<th>HPLC</th>
<th>TLC</th>
<th>Others (specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume (µL)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Column packing and size</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Column Temperature (^0C)</td>
<td>-</td>
<td>Merck 10x20cm</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>-</td>
<td>one</td>
</tr>
<tr>
<td>Detector</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>( \lambda_{ex} ) and ( \lambda_{em} ) (nm)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Derivatization solvent</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Derivatization condition</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

| Spotted volume (µL)         | x                             | -                |
| TLC brand and size          | Merck 10x20cm                 | -                |
| One or bi directional       | one                           | -                |
| Visual or densitometer quantification | visual | - |
| \( \lambda \) (nm)         | -                             | -                |
| Mobile phase                | Eter:methanol: water (96:3:1, v/v/v) | - |
| Derivatization              | -                             | -                |

8) **Standard Brand:**

<table>
<thead>
<tr>
<th>Sigma</th>
<th>Rhône Poulenc</th>
<th>Fluka</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9) **Concentration of standard work solution (µg/mL):**
Afla B₁:1,0312; afla B₂:0,4573; afla G₁:1,0221; afla G₂:0,5075
10) **Standard calibration curve:**

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Concentration (µg/mL) or mass of mycotoxin used (ng) to quantify the samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard 1</td>
</tr>
<tr>
<td>Afla B₁</td>
<td>0.1088</td>
</tr>
<tr>
<td>Afla B₂</td>
<td>0.0483</td>
</tr>
<tr>
<td>Afla G₁</td>
<td>0.1079</td>
</tr>
<tr>
<td>Afla G₂</td>
<td>0.0536</td>
</tr>
</tbody>
</table>

11) **Method quantification limit (µg/kg):**

Afla B₁: 0.4; afla B₂: 0.2; afla G₁: 0.3; afla G₂: 0.2

12) **Additional comments and/or observations**
ANALYTICAL WORK QUESTIONNAIRE

1) Reference method:
For zearalenone analysis - DOU 2000 e POP 041 (ed03, rev 02)

2) Sample weight (g):

<table>
<thead>
<tr>
<th></th>
<th>&lt;25</th>
<th>≥25 to &lt;50</th>
<th>≥50</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

3) Extraction solvent:

<table>
<thead>
<tr>
<th>Water</th>
<th>Acetonitrile</th>
<th>Dichloromethane</th>
<th>Others (specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>Acetone</td>
<td>Chloroform</td>
<td></td>
</tr>
</tbody>
</table>

Volume of extraction solvent (mL):

4) Extraction procedure:

<table>
<thead>
<tr>
<th>Blend with solvent</th>
<th>Shake with solvent</th>
<th>Add NaCl</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td></td>
<td></td>
<td>Add celite</td>
</tr>
</tbody>
</table>

5) Sample work up:

<table>
<thead>
<tr>
<th>Filter</th>
<th>Centrifuge</th>
<th>Decant</th>
<th>Dilute</th>
<th>Evaporate</th>
<th>pH Adjustment</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6) Clean-up:

<table>
<thead>
<tr>
<th>Immunoaffinity column</th>
<th>Solid phase</th>
<th>Liquid-liquid partition</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>Silica</td>
<td>Florisil</td>
<td>alumina</td>
</tr>
<tr>
<td>Precipitation with</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cupric sulphate</td>
<td>Ammonium sulphate</td>
<td>Celite</td>
<td></td>
</tr>
</tbody>
</table>

7) **Determination and quantification:**

Final test sample in the final extract (g): **0.62g**

Solvent of dissolution the final extract: **Methanol:water (8:2, v/v)**

Volume of solvent to dissolve the final extract (µL): **300**

<table>
<thead>
<tr>
<th>HPLC</th>
<th>TLC</th>
<th>Others (specify)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection volume (µL)</td>
<td>20</td>
<td>Spotted volume (µL)</td>
</tr>
<tr>
<td>Column packing and size</td>
<td>250x4,6mm</td>
<td>TLC brand and size</td>
</tr>
<tr>
<td>Column Temperature (°C)</td>
<td>Room temperature</td>
<td>One or bi directional</td>
</tr>
<tr>
<td>Flow rate (mL/min)</td>
<td>0.5</td>
<td>Visual or densitometer quantification</td>
</tr>
<tr>
<td>Detector</td>
<td>Fluorescence</td>
<td>λ (nm)</td>
</tr>
<tr>
<td>λ&lt;sub&gt;ex&lt;/sub&gt; and λ&lt;sub&gt;em&lt;/sub&gt; (nm)</td>
<td>280 and 465</td>
<td>Mobile phase</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Methanol:water (8:2, v/v)</td>
<td>Derivatization</td>
</tr>
</tbody>
</table>

8) **Standard Brand:**

<table>
<thead>
<tr>
<th>Sigma</th>
<th>Rhône Poulenc</th>
<th>Fluka</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9) **Concentration of standard work solution (µg/mL):** **19.8732**

10) **Standard calibration curve:**

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Concentration (µg/mL) or mass of mycotoxin used (ng) to quantify the samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Standard 1</td>
</tr>
<tr>
<td>ZEA</td>
<td>1.5899</td>
</tr>
</tbody>
</table>

11) **Method quantification limit (µg/kg):** **3.6**

12) **Additional comments and/or observations**
Partner 06 - INIAGR-PV

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
# FIRST ANNUAL REPORT

## PARTICIPATION IN WORK PACKAGES 4&5

Mycotoxin-commodity combination: Deoxynivalenol-wheat

### HACCP TEAMS, RESOURCES & ISSUES

<table>
<thead>
<tr>
<th>Team members</th>
<th>Institution</th>
<th>Disciplines/Skills/Knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cecilia Gianoni</td>
<td>Procisur</td>
<td>Eng. Agrichain organization</td>
</tr>
<tr>
<td>Silvina Stewart</td>
<td>INIA</td>
<td>Phytopathologist/knowledge of field diseases</td>
</tr>
<tr>
<td>Gonzalo Gutierrez</td>
<td>UdelaR</td>
<td>Socio-economist/knowledge wheat chain</td>
</tr>
<tr>
<td>Jacqueline Cea</td>
<td>LATU</td>
<td>Analyst/Knowledge in HACCP</td>
</tr>
</tbody>
</table>

### Other Resources

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Disciplines/Skills/Knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jorge Escudero</td>
<td>Copagran (cooperative)</td>
<td>Wheat accumulator</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Issues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium is a disease that develops under rainy weather</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weather has not been favourable for Fusarium so far, so we will probably have a year with little or no DON problems</td>
</tr>
</tbody>
</table>

## PROGRESS

<table>
<thead>
<tr>
<th>Milestones/Deliverable</th>
<th>Progress</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Work Package 4, Hazard Analysis of Mycotoxins</strong> (24 February 03 – 31 December 03):</td>
<td></td>
</tr>
<tr>
<td><strong>To identify cereal commodities-mycotoxin combinations that present an unacceptable risk to human health or present a constraint to trade</strong> (24 February 03 – 31 August 03)</td>
<td></td>
</tr>
<tr>
<td>1. Establishment of multidisciplinary HACCP Team (including recruitment of team members from non-project partners)</td>
<td>Complete</td>
</tr>
<tr>
<td>2. Data from literature collected &amp; collated (from learned journals, reports etc) on the occurrence of mycotoxins in wheat &amp; corn in HACCP Team’s country</td>
<td>Complete</td>
</tr>
<tr>
<td>3. Data from other sources collected &amp; collated from (e.g producers, manufacturers, processors, wholesalers, consumers etc) on the occurrence of mycotoxins in wheat &amp; corn in HACCP Team’s country</td>
<td>Data collected from many private laboratories, from Baker's Industrial Centre, from Ministry of Public Health and from local administrations</td>
</tr>
<tr>
<td>4. Decision made regarding the need for further information on the occurrence of mycotoxins</td>
<td>No further information required at this stage</td>
</tr>
<tr>
<td><strong>Deliverable D2 (30 June 2003):</strong> A report documenting mycotoxin occurrence data obtained from the literature and from information derived from trade sources</td>
<td>Report completed</td>
</tr>
<tr>
<td><strong>Contract number:</strong> ICA4-CT-2002-10043</td>
<td><strong>Individual partner annual reports</strong></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>---------------------------------------</td>
</tr>
</tbody>
</table>

| 5. Preliminary Hazard Analysis completed & mycotoxin/commodity combinations identified | Completed |
| 6. Attend first review meeting of WP 4&5 partners at INTA-Argentina (20 -22 August 03) | Meeting attended by Elisabete Salay, Catherine Brabet, Guy Henry & Otneil Freitas |

**Deliverable D3 (31 August 2003): Report describing the hazard analyses conducted, justifying the commodity-mycotoxin combination selected for further study**

| **To construct & verify the respective Commodity Flow Diagrams (CFDs)** |
| **(1 August 03 – 31 December 03)** |

| 7. Contact made with key players in the Commodity System (producers to consumers) | • We have contacted a key player Ing. Escudero from COPAGRAN (big Cooperative) who is the middle man between farmers and two very important mills. We have arranged to work with him. He is going to help us to pick out contrasting situations in the field, some favourable for Fusarium (ex: wheat sowned over contaminated stubble, with susceptible varieties etc) and some where Good Agricultural Practice (GAP) has been used. We are going to try to follow these crops from the field into the mill and survey the toxin throughout the whole process; trying to monitor the toxin in the field (before harvest), from the trucks (during harvest), and inside the mill throughout the milling process and in the flour.  
| • We have not been able to find any Fusarium contaminated fields, so we did not follow any crop from the farm to the mill. We only did some sampling in a situation where the farmer has stored the grain in a “silo bag” and we are going to monitor the bag over a period of time.  
| • We have contacted Molino Rio Uruguay (Biochemist Claudia Veira and Rodolfo Ratto Chief of Production) to make the commodity flow diagram in their mill, but this will only be possible after February 15th because they were on vacation. |

| 8. Commodity Flow Diagram (CFD) completed for selected mycotoxin-commodity combination(s) | We are planning to do produce a CFD beginning at the Molinos Rio Uruguay mill. They have a lot of information about DON and how it decreases during milling and are willing to share this information with us |
| 9. CFD verified, for selected mycotoxin-commodity combination(s) | Verification will be done during the last two weeks of February 2004 |

**Deliverable D6 (31 December 03): Report documenting the verified CFDs for each commodity-mycotoxin combination in each of the selected countries**
| Work Package 5, Identification & Validation of Mycotoxin Control Measure (24 February 03 – 30 June 05):  
To evaluate the socio-economic, cultural and institutional issues associated with the introduction of control measures at CCPs  
(24 February 03 – 30 June 04) |
<table>
<thead>
<tr>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>13. Key players in the CFD identified, for each mycotoxin-commodity combination</td>
</tr>
<tr>
<td>14. Participatory appraisal methods used to obtain a thorough understanding of those socio-economic, cultural &amp; institutional issues associated with the introduction of control measures at Critical Control Points (CCPs)</td>
</tr>
</tbody>
</table>

**Deliverable D9 (30 June 04):** 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

| 15. Attend second review meeting of WP 4&5 partners (Feb. 04; venue to be confirmed) | |
To develop and validate control measures that will prevent, eliminate, or reduce mycotoxin content to an acceptable level, when applied to a specific step in the CFD (January 04 – 31 December 04)

16. Identify potential CCPs in each selected mycotoxin-commodity combination

17. Identify potential Control Measures

- We think that the best way to avoid flour with high DON content would be to exclude grain with high concentrations of DON from the wheat-flour chain. This is difficult because there are no easy methods to predict DON content without doing an analysis. Today mills buy their grain using % FDK (Fusarium damage kernels) as an indicator (0.5% FDK is the acceptable level), but correlations with DON are very low. We are working on a test using visual determination of FDK, separating fusarium damage grain in two categories (white grains and pink grains) and we were able to increase this correlations from $r^2=0.55$ to $r^2=0.848$. We are very exited about these results, we have to validate this analysis with this years data to see if results keep on looking good.
- We have also used a denso-gravity table to separate Fusarium kernels from the rest (they are smaller and lighter) instead of the classical cleaning methods were screens are used to take out the smaller grains. We have obtain very good results with this kind of cleaning process using wheat with very high DON content from the previous harvest (9-37 ppm) and obtain grain that could be used to make flour if we only use the grain coming out from the first mouths (heavier grain). This type of denso-gravity table is not used commercially for wheat processing, it is usually used or found in seed processing facilities (not for grain).

18. Modified or novel control measures devised, if necessary

19. Validate simple, inexpensive methods for achieving segregation, including BCL and Toximet monitoring of a CCP

20. Control measures validated, at laboratory or pilot level

21. Validate CCPs

Deliverable D13 (31 December 04):
A series of reports documenting studies to develop and evaluate control measures, and studies to validate CCPs
Partner 07 – INIACL-CRIR

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
# FIRST ANNUAL REPORT

## PARTICIPATION IN WORK PACKAGES 4&5

### HACCP TEAMS, RESOURCES & ISSUES

<table>
<thead>
<tr>
<th>Team members</th>
<th>Institution</th>
<th>Disciplines/Skills/Knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alejandra Engler</td>
<td>National Institute of Agricultural Research INIA</td>
<td>Socioeconomist</td>
</tr>
<tr>
<td>Ricardo Madariaga</td>
<td>National Institute of Agricultural Research INIA</td>
<td>Wheat Phytopathologist</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Other Resources</th>
<th>Institution</th>
<th>Disciplines/Skills/Knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eric Von Baer</td>
<td>Baer Seed Company</td>
<td>Plant Breeder – Seed Producer</td>
</tr>
<tr>
<td>Ricardo Villegas</td>
<td>University of Concepción</td>
<td>Toxicologist – HACCP – Mill Management</td>
</tr>
<tr>
<td>Mario Vega Herrera</td>
<td>University of Concepción</td>
<td>Mycotoxicologist – Mycotoxins analysis</td>
</tr>
<tr>
<td>Marcelo Cadagan Molino Collico</td>
<td>PDP Coordinator</td>
<td>Agronomist Coordinator of Wheat Producers</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Issues</th>
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</thead>
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<table>
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## Issues

<table>
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<tr>
<th>Milestones/Deliverable</th>
</tr>
</thead>
<tbody>
<tr>
<td>Work Package 4, Hazard Analysis of Mycotoxins (24 February 03 – 31 December 03):</td>
</tr>
<tr>
<td>To identify cereal commodities-mycotoxin combinations that present an unacceptable risk to human health or present a constraint to trade (24 February 03 – 31 August 03)</td>
</tr>
</tbody>
</table>

1. Establishment of multidisciplinary HACCP Team (including recruitment of team members from non-project partners)  
   It is complete.

2. Data from literature collected & collated (from learned journals, reports etc) on the occurrence of mycotoxins in wheat & corn in HACCP Team’s country  
   No additional information to the report mailed on July 9 to Martin Nagler.

3. Data from other sources collected & collated from (e.g producers, manufacturers, processors, wholesalers, consumers etc) on the occurrence of mycotoxins in wheat & corn in HACCP Team’s country  
   We had negative results on the testing of DAS, DON, T2 and HT2 on 62 wheat samples harvested on the cycle 2002 – 2003, even though, some of them had high mould contamination and even some has Fusaria specie present. Testing for Fumonisins are currently under way. The most abundant
fungy on wheat kernel was *Alternaria* sp., a fungus involved on the Black Point Disease, and eventually a Mycotox producer (*Alternariol, Tenuazonic acid?). There is a major concern on farmers at the present 2003 – 2004 wheat harvest since the crops yield abundant Black Pointed Kernels and Mill Brokers are giving at least a 2% price reduction by this problem. Contacts has been made with a broker to provide samples to be tested for mycotoxins contamination.

The evaluation of wheat harvested during crop cycle 2002 – 2003 gave negative results, but only a few samples from the different movement that a wheat batch suffer inside the mill were tested. A meeting with the Mill partners will be scheduled on March 2004 to plan the adjustments to the sampling methodology used on the previous cycle. A survey questionnaire was applied to the PDP growers in a meeting where we show the Mycotox CIRAD-NRI-INIA’s-UNIVERSITY Project and discuss Mould and Mycotoxin contamination. They demonstrated enthusiasm in the topic and their willingness to further collaborate.

| Deliverable D2 (30 June 2003): A report documenting mycotoxin occurrence data obtained from the literature and from information derived from trade sources | No additional comments to the August 20, 2003 report delivered by hand, at the Buenos Aires INTA - Meeting. It could be add that Dr. Alejandra Engler attend an additional meeting held at Campinas Brasil, where Socioeconomists discussed economic issues of the Project. |
| Deliverable D3 (31 August 2003): Report describing the hazard analyses conducted, justifying the commodity-mycotoxin combination selected for further study | Representatives of HACCP Team attended meeting |

5. Preliminary Hazard Analysis completed & mycotoxin/commodity combinations identified

Wheat and deoxynivalenol & zearalenone selected as mycotoxin/commodity combination

6. Attended first review meeting of WP 4&5 partners at INTA-Argentina (20 -22 August 03)

Deliverable D6 (31 December 03): Report documenting the verified CFDs for each commodity-mycotoxin combination in each of the selected countries

To construct & verify the respective Commodity Flow Diagrams (CFDs) *(1 August 03 – 31 December 03)*

7. Contact made with key players in the Commodity System (producers to consumers)

8. Commodity Flow Diagram (CFD) completed for selected mycotoxin-commodity combination(s)

9. CFD verified, for selected mycotoxin-commodity combination(s)

Deliverable D6 (31 December 03): Report documenting the verified CFDs for each commodity-mycotoxin combination in each of the selected countries

| 7. Contact made with key players in the Commodity System (producers to consumers) | Partially complete |
| 8. Commodity Flow Diagram (CFD) completed for selected mycotoxin-commodity combination(s) | Partially complete |
| 9. CFD verified, for selected mycotoxin-commodity combination(s) | Partially complete |
| Deliverable D6 (31 December 03): Report documenting the verified CFDs for each commodity-mycotoxin combination in each of the selected countries | Partially complete |
To determine at which step(s) in the CFD the mycotoxin hazard originates or increases to an unacceptable level, requiring control  
(3 November 03 – 1 April 04)

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.</td>
<td>Design survey and arrange for sample collection</td>
</tr>
</tbody>
</table>
| 11.  | Arrange for laboratory analysis using validated methodology (WP1 D4)  
      | In progress |
| 12.  | Perform statistical analysis and interpret results |

**Deliverable D14 (1 April 2004, or within 3 months of harvest):**  
Report describing at which steps in the CFD the mycotoxin hazard originates, or at which steps concentrations increase to unacceptable levels

---

**Work Package 5, Identification & Validation of Mycotoxin Control Measure (24 February 03 – 30 June 05):**  
To evaluate the socio-economic, cultural and institutional issues associated with the introduction of control measures at CCPs  
(24 February 03 – 30 June 04)

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
</table>
| 13.  | Key players in the CFD identified, for each mycotoxin-commodity combination  
      | Completed |
| 14.  | Participatory appraisal methods used to obtain a thorough understanding of those socio-economic, cultural & institutional issues associated with the introduction of control measures at Critical Control Points (CCPs)  
      | In progress |

**Deliverable D9 (30 June 04):**  
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

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
</table>
| 15.  | Attend second review meeting of WP 4&5 partners (Feb. 04; venue to be confirmed)  
      | In progress |

To develop and validate control measures that will prevent, eliminate, or reduce mycotoxin content to an acceptable level, when applied to a specific step in the CFD  
(January 04 – 31 December 04)

<table>
<thead>
<tr>
<th>Step</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>16.</td>
<td>Identify potential CCPs in each selected mycotoxin-commodity combination</td>
</tr>
<tr>
<td>17.</td>
<td>Identify potential Control Measures</td>
</tr>
<tr>
<td>18.</td>
<td>Modified or novel control measures devised, if necessary</td>
</tr>
<tr>
<td>19.</td>
<td>Validate simple, inexpensive methods for achieving segregation, including BCL and Toximet monitoring of a CCP</td>
</tr>
<tr>
<td>20.</td>
<td>Control measures validated, at laboratory or pilot level</td>
</tr>
<tr>
<td>21.</td>
<td>Validate CCPs</td>
</tr>
</tbody>
</table>
Partner 08 – INTECA TA

Contract number: ICA4-CT-2002-10043

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PARTICIPATION IN WORK PACKAGES 4&5

HACCP TEAMS, RESOURCES & ISSUES

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<thead>
<tr>
<th>Team members</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Mphil. Marcelo Masana</td>
<td>INTA, CIA, ITA Castelar</td>
<td>HACCP specialist</td>
</tr>
<tr>
<td>Dra. Alejandra Ricca</td>
<td>INTA, CIA, ITA Castelar</td>
<td>Mycotoxin specialist</td>
</tr>
<tr>
<td>Dr. Daniel Iglesias</td>
<td>INTA, Anguil, Santa Rosa - La Pampa</td>
<td>Agricultural economist, sector specialist</td>
</tr>
<tr>
<td>Bioq. Alejo Farinella</td>
<td>Molino Don Antonio S.A.</td>
<td>Mill quality manager</td>
</tr>
<tr>
<td>Ing. Agr. Alejandro Navone</td>
<td>Molino Don Antonio S.A.</td>
<td>Mill agronomist</td>
</tr>
</tbody>
</table>

Other Resources

<table>
<thead>
<tr>
<th>Name</th>
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<th>Disciplines/Skills/Knowledge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prof. Dra. Silvia Resnik</td>
<td>FCEyN. UBA</td>
<td>Mycotoxin specialist and WP 3 coordinator</td>
</tr>
<tr>
<td>Dra Ana Pacin</td>
<td>UNLU</td>
<td>Mycotoxin specialist and WP 2 coordinator</td>
</tr>
<tr>
<td>Dr. Guy Henry</td>
<td>CIRAD</td>
<td>Agricultural economist, sector specialist</td>
</tr>
</tbody>
</table>

Issues

<table>
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</tr>
</thead>
<tbody>
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<td>Milestones/Deliverable</td>
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</tr>
<tr>
<td>4. Decision made regarding the need for further information on the occurrence of mycotoxins</td>
</tr>
<tr>
<td><strong>Deliverable D2 (30 June 2003):</strong></td>
</tr>
<tr>
<td>A report documenting mycotoxin occurrence data obtained from the literature and from information derived from trade sources</td>
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</table>
| **To construct & verify the respective Commodity Flow Diagrams (CFDs)**  
| **(1 August 03 – 31 December 03)** |
| 7. Contact made with key players in the Commodity System (producers to consumers) | Molino Don Antonio S.A., a wheat flour mill integrated at the raw material level and at the further processing level, has become project partner. In addition, an INTA sector specialist and agricultural economist, from the La Pampa region joined the project. Also, an INTA extension specialist joined the team. |
| 8. Commodity Flow Diagram (CFD) completed for selected mycotoxin-commodity combination(s) | Attached is a preliminary CFD draft. This was constructed with primary and secondary data. Currently, additional primary data collection is ongoing at the grain producer and assembly point levels. This new information will be collated before the end of April. |
| 9. CFD verified, for selected mycotoxin-commodity combination(s) | **Deliverable D6 (31 December 03):** Report documenting the verified CFDs for each commodity-mycotoxin combination in each of the selected countries |

| **To determine at which step(s) in the CFD the mycotoxin hazard originates or increases to an unacceptable level, requiring control**  
| **(3 November 03 – 1 April 04)** |
| 10. Design survey and arrange for sample collection | In progress |
| 11. Arrange for laboratory analysis using validated methodology (WP1 D4) | |
| 12. Perform statistical analysis and interpret results | |
| **Deliverable D14 (1 April 2004, or within 3 months of harvest):** Report describing at which steps in the CFD the mycotoxin hazard originates, or at which steps concentrations increase to unacceptable levels | |
| Work Package 5, Identification & Validation of Mycotoxin Control Measure (24 February 03 – 30 June 05):  
To evaluate the socio-economic, cultural and institutional issues associated with the introduction of control measures at CCPs  
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<tr>
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</tr>
<tr>
<td><strong>Deliverable D9 (30 June 04):</strong> 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 &amp; opportunities affecting the implementation of proposed mycotoxin control measures</td>
</tr>
<tr>
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*(January 04 – 31 December 04)* |
| 16. Identify potential CCPs in each selected mycotoxin-commodity combination |
| 17. Identify potential Control Measures |
| 18. Modified or novel control measures devised, if necessary |
| 19. Validate simple, inexpensive methods for achieving segregation, including BCL and Toximet monitoring of a CCP |
| 20. Control measures validated, at laboratory or pilot level |
| 21. Validate CCPs |
| **Deliverable D13 (31 December 04):** A series of reports documenting studies to develop and evaluate control measures, and studies to validate CCPs |
Partner 09 – UBAIR DCO

Contract number: ICA4-CT-2002-10043

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Task WP1 Harmonization and standardization of the analytical methods for mycotoxin analysis.
Control samples analysis for aflatoxins, zearalenone and fumonisins on corn and DON in wheat felt in the reference range provided by FAPAS.

Future task WP1
Samples provided by Brazil partners would be analysed at reception.

Task WP2 Risk assessment of human exposure to ochratoxin A
The methodology for Ochratoxin A was standardized in collaboration with the partners 10 and 11. Further to the extraction and clean-up step (see details in the individual report of partner 10), there is a confirmation step which is detailed here. Ochratoxin A standard solutions were derivatized by forming the methyl ester derivative of the mycotoxin. Slightly modifications of Grosso et al. (2003) procedure were made. A quantitative portion of the standard solutions were evaporated to dryness. The first step was to determine the derivatization optimal time. The table 1 shows examples of area response at two derivatization times at 60º C.

<table>
<thead>
<tr>
<th>Repetition</th>
<th>Time (minutes)</th>
<th>15</th>
<th>20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>AREA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>2496 2452</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td>2489 2400</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td>2497 2484</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td>2494,0</td>
<td>2445,3</td>
</tr>
<tr>
<td>Standard deviation</td>
<td></td>
<td>4,36</td>
<td>42,39</td>
</tr>
<tr>
<td>Median</td>
<td></td>
<td>2496,0</td>
<td>2452,0</td>
</tr>
<tr>
<td>Error %</td>
<td></td>
<td>0,17</td>
<td>1,73</td>
</tr>
</tbody>
</table>

Table 1: Influence of derivatization times on the area response

A greater area and less dispersion were obtained when heating lasted 15 min. instead of 20 min. The next objective was then to concentrate as much as possible the derivate to obtain better sensibility, small LOQ and LOD. At this moment the results obtained in the different approaches were:

a) re-suspended into 1000 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 ochratoxin A.

b) re-suspended into 1000 µl of a 12% methanolic solution of boron trifluoride (Baker C701-07). After 15 min heating at 60ºC, the derivatization extracts were dried and the methyl ester of OTA in evaporated extracts was re-suspended into 250 µl mobil phase and then analysed by HPLC with the same chromatographic conditions as for ochratoxin A.
c) re-suspended into 120, 200 or 500 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 ochratoxin A.

![Graph](image.png)

**Figure 1:** Relationship between OTA added and area response

A better relation between OTA added and areas were found when 100 µl of derivatized standard were injected following a) or c) procedures than b) procedure (figure 1). In this last procedure, the methyl ester of OTA area decreased and OTA peak increased. Retention time of the OTA methyl ester was 16.3 min. With c) procedure and 120µl of a 12% methanolic solution of boron trifluoride, detection and quantification limits expressed as OTA were 0.017 µg/l and 0.028 µg/l respectively.

**Future task WP2**

The methodology for OTA determination and confirmation in blood and pig samples is under standardization (CIM-UnLu, Concepcion University and UBA).

**TASK WP3 Evaluation of milling procedures as potential CCPs**

Grain contamination by fusariotoxins 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 mycotoxin. 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 developed in this period, together with CIM-UnLu (partner 10) focused on statistically analyzing the contamination occurring with DON in wheat and derived milling fractions and with fumonisins in maize. This allowed definition of the statistical
function associated with the contamination distribution and variability for both considered mycotoxins.

Schematic procedures for wheat and corn sampling were then tested and optimised in Argentina. These procedures are presently under testing and application by Chilean partners. The collected samples, either whole or coming fractions from wheat milling, such as flour, bran and gluten, were then analysed for DON. For maize, an assay was performed in Argentina for analysing the distribution of fumonisins in the grains.

**DON Analysis in Wheat and wheat fractions** were divided into two steps to optimise the project financial resources. The first step, i.e. the extraction and cleanup of analytical samples, were performed at CIM-UnLu (partner 10) and the second step, i.e. the derivatisation and gas chromatography quantification at UBA (partner 9).

The dried extract residues were derivatized as described by Croteau et al. (*J. Agric. Food Chem.*, 1994, 42, 928-934). Briefly, the catalyst solution (100 µL) was added to the dried extract and, after mixing on a Vortex mixer, 50 µL HFBA were added. The tube was placed in a heating block for 20 min. Excess derivatizing agent was destroyed with 1 mL sodium bicarbonate solution, and 400 µL toluene were added. After centrifuging, the upper organic layer was transferred to an auto sampler vial for GC analysis. The injection volume was 2 µL. The temperature program consisted of holding the temperature for 1 min at 80°C, and then increasing at 30°C/min from 80 to 160 °C, followed by an increase from 160 to 183°C at 1°C/min, and then 183-280°C at 12°C/min (with 5 min hold). Column head pressure was 12 psi. The temperatures of the injector and detector were 250 and 300°C, respectively. A standard curve was constructed each day (40–100 pg DON) to determine the response of electron capture detector to DON standard. The limit of both detection and quantification of DON was 11 and 18 µg/kg at signal-to-noise ratios of 3:1 and 5:1, respectively.

Test results on DON contamination of samples taken at regular intervals during the cleaning and milling of a single 13-ton lot of wheat and during the production of wheat starch and gluten were used to estimate the distribution function of DON contamination in wheat, wheat flour, bran, and gluten. Bulk sample (wheat sample weight = 3 kg, other products = 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 test sample. By use of variance, the error structure becomes:

\[ S^2_T = S^2_s + S^2_sp + S^2_a \]

Where \( S^2_T \) (total testing variance) is equal to the sum of the sampling (\( S^2_s \)), sample preparation (\( S^2_sp \)), and analytical (\( S^2_s \)) variance.

Samples of screening, unclean wheat, clean wheat, flour, and gluten of 5 different concentrations were selected to give a wide range of DON concentrations. Ten aliquots taken from each extract were analysed for the toxin by the gas chromatography (GC) method as described above.

An ANOVA statistical test was conducted on the data obtained from DON contamination in wheat, flour, bran and gluten; the results are shown in table 2. To
test the hypothesis that there is a location effect and the groups are in fact different, the $F$ ratio can be computed from the ratio of the mean squares among the samples and the mean squares within the samples. If the null hypothesis is correct, $F$ is expected to be below 1.62 with $p = 95\%$ (significance level), whereas $F$ indicates a location effect. Thus, it was concluded that there are differences among the samples, and they were probably caused by the sampling variability, as shown in table 2.

**Table 2. Results of the ANOVA statistical test**

<table>
<thead>
<tr>
<th>Product</th>
<th>Source of variation</th>
<th>Sum of squares (ppm$^2$)</th>
<th>Degree of freedom</th>
<th>Mean squares (ppm$^2$)</th>
<th>$F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>wheat ($&lt;c&gt;=1.928$ppm)</td>
<td>among the samples</td>
<td>15.39</td>
<td>23</td>
<td>0.669</td>
<td>3.56</td>
</tr>
<tr>
<td></td>
<td>within the samples</td>
<td>22.55</td>
<td>120</td>
<td>0.188</td>
<td>17.34</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>37.94</td>
<td>143</td>
<td>0.188</td>
<td>26.05</td>
</tr>
<tr>
<td>flour ($&lt;c&gt;=0.994$ppm)</td>
<td>among the samples</td>
<td>13.35</td>
<td>23</td>
<td>0.58</td>
<td>17.34</td>
</tr>
<tr>
<td></td>
<td>within the samples</td>
<td>4.02</td>
<td>120</td>
<td>0.0335</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>17.37</td>
<td>143</td>
<td>0.00144</td>
<td>26.05</td>
</tr>
<tr>
<td>bran ($&lt;c&gt;=4.68$ppm)</td>
<td>among the samples</td>
<td>251.9</td>
<td>23</td>
<td>10.95</td>
<td>26.05</td>
</tr>
<tr>
<td></td>
<td>within the samples</td>
<td>50.4</td>
<td>120</td>
<td>0.42</td>
<td>17.34</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>302.4</td>
<td>143</td>
<td>0.00144</td>
<td>26.05</td>
</tr>
<tr>
<td>gluten ($&lt;c&gt;=0.293$ppm)</td>
<td>among the samples</td>
<td>0.163</td>
<td>8</td>
<td>0.02</td>
<td>14.1</td>
</tr>
<tr>
<td></td>
<td>within the samples</td>
<td>0.062</td>
<td>43</td>
<td>0.00144</td>
<td>26.05</td>
</tr>
<tr>
<td></td>
<td>total</td>
<td>0.225</td>
<td>51</td>
<td>0.00144</td>
<td>26.05</td>
</tr>
</tbody>
</table>

The mean squares within the samples may be associated with the total variance: combined sampling, sample preparation (sample division in 6 sub samples, milling, taking the 25 g analytical sample, and cleanup) and the analytical variance (derivative reaction and GC quantification). The value 0.188 ppm$^2$ is in good agreement with the Whitaker et al. (*J. AOAC Int.*, 2000, 83, 1285-1292) prediction of a variance of 0.205 ppm$^2$ for wheat mean concentration of 1.928 ppm (calculated from the regression equation of Figure 2).

**Figure 2:** Full-log plot of analytical variance *versus* deoxynivalenol concentration. Each point is the mean of ten determinations
Figure 2 shows that the analytical variance was dependent on the concentration as $V = 0.002 \times c^{1.825}$. The repeatability of the GC method was evaluated by calculating the HORRAT value (15) for each analytical sample, i.e., HORRAT = [experimentally found relative standard deviation (RSD)] divided by RSD calculated from the Horwitz formula [RSDR, % = 2(1–0.5 log 10C) where C is the concentration expressed as a decimal fraction]. The chemical analytical method is deemed acceptable if HORRAT is approximately 1.0 (± 0.5) for inter-laboratory performance studies and ½ of this value for intra-laboratory ones. The HORRAT values obtained indicated acceptable precision of the method as shown in table 3.

<table>
<thead>
<tr>
<th>Analytical variance (ppm²)</th>
<th>Concentration (ppm)</th>
<th>RSDr predicted</th>
<th>RSDr observed</th>
<th>Horrat</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0073</td>
<td>1.93</td>
<td>14.49</td>
<td>4.43</td>
<td>0.31</td>
</tr>
<tr>
<td>0.0022</td>
<td>0.99</td>
<td>16.01</td>
<td>4.72</td>
<td>0.29</td>
</tr>
<tr>
<td>0.0368</td>
<td>4.68</td>
<td>12.68</td>
<td>4.10</td>
<td>0.32</td>
</tr>
<tr>
<td>0.0002</td>
<td>0.29</td>
<td>19.25</td>
<td>4.83</td>
<td>0.25</td>
</tr>
</tbody>
</table>

To estimate the distribution function of DON concentrations in the 13-ton lot of wheat, the following procedure was used. Each one of the bulk samples was numbered $I = (1, 2, \ldots N)$, and the test samples $j = (1, 2, \ldots r)$. A DON concentration matrix $c_{ij}$ was determined ($N \times r = 144$ test samples). The estimator of the mean concentration of the $i$ test sample is

$$\hat{c}_i = \frac{1}{r} \sum_{j=1}^{r} c_{ij}$$

(Eq. 1)

and the variance of this estimator is

$$\hat{s}_i^2 = \frac{1}{r(r-1)} \sum_{j=1}^{r} (c_{ij} - \hat{c}_i)^2$$

(Eq. 2)

Now if $c$ is the true concentration and the reduced variate is formed $x_i = (c - \hat{c}_i)/s_i$, it is well-known that $x_i$ has a Student’s $t = \text{distribution with } r-1$ degrees of freedom, assuming that $c$ is normally distributed in the small sample (but not in the biglot). Therefore, the probability density function (PDF) determined from the $i$ sample alone would be

$$\frac{dp}{dc} = \frac{1}{\hat{s}_i} t_{r-1}(x_i)$$

(Eq. 3)

where

$$t_{r-1}(x) = \frac{\Gamma\left(\frac{r}{2}\right)}{\sqrt{\pi(r-1)}} \left[1 + \frac{x^2}{r-1}\right]^{-\frac{r}{2}}$$
is the normalized Student’s distribution and \( \Gamma \) denotes Euler’s gamma function. The equation 1 gives a symmetric peak of probability around \( \hat{c} \). However, there is a collection of samples, with \( i = 1, \ldots, N \), whose corresponding ensemble of peaks is exactly the experimental histogram of the lot; each contribution is weighted with the inverse standard deviation \( 1/s_i \) of the individual samples. The normalized PDF for the whole lot is

\[
\frac{dp}{dc} = \frac{1}{N} \sum_{i=1}^{N} \frac{1}{s_i} \Gamma_{r-1}(x_i) \tag{Eq 4}
\]

It is more convenient to average out random fluctuations by working with the cumulative distribution function (CDF), which is the integral of Equation 4; then

\[
p(c) = \frac{1}{N} \sum_{i=1}^{N} T_{r-1}(x_i) \tag{Eq. 5}
\]

where

\[
T_{r-1}(x) = \frac{1}{2} + \frac{\Gamma(r)}{\Gamma(r-1)} \frac{x}{\sqrt{\pi(r-1)}} F\left(\frac{1}{2}, \frac{r}{2}, \frac{3}{2}; \frac{x^2}{r-1}\right)
\]

and \( F \) is the hypergeometric function.

This procedure yields a smooth function \( p(c) \), which can be further analyzed. To determine whether the distribution has an asymmetric tail for high values of the concentration, it is convenient to examine the new variable

\[
\eta(c) = -1\ln(-1np)
\]

which is linear with \( c \) in the so-called extreme-value type I distribution, used in hydrology for flood probability estimates. In fact, it has been observed in all the distributions determined in this work, that \( \eta \) is a linear function of concentration at high values of \( c \). However for low values of \( c \), near the limit of detection \( \eta(0) \) is negative and \( \eta'(0) \approx 0 \). In all the range, \( \eta \) can reasonably be fitted by a cubic polynomial in \( c \). Such polynomials have enough flexibility to accommodate even bimodal distributions (see bran, Figure 3d). With such expression for \( \eta (c) \) the CDF and the PDF can be computed from the equations:

\[
p = \exp\left[-e^{-\eta(c)} \right]
\]

\[
\frac{dp}{dc} = (p \ln p) \frac{d\eta}{dc}
\]

and the mean, standard deviation (SD), skewness, and kurtosis are then calculated by straightforward numerical integration. The observed DON distribution function in wheat is compared with a normal distribution with the same mean and SD in Figure 3a.
Figure 3: The experimental distribution function of DON contamination vs. DON concentration (ppm) is compared with a normal distribution function of the same mean and standard deviation.

(a) wheat grains, (b) wheat flour, (c) gluten, (d) the experimental distribution function of DON contamination in bran having two separated peaks is compared with the sum of two normal distribution functions

\[ p = 0.0732 \times e^{-0.4(2.7-c)^2} \quad \text{and} \quad p = 0.373 \times e^{-0.66(5.13-c)^2} \]

The sampling distribution function of DON concentrations in the other products was estimated in the same manner, and the distribution functions obtained are compared with normal distributions with the same mean and SD for wheat flour (Figure 3b), gluten (Figure 3c), and bran (Figure 3d).

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 is 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 those 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.
Future task WP3
Corn produced in Argentina represented c.a. 30% of the total cereal and oilseed production. Natural occurrence of Fumonisins in Argentinean corn is a factor not only in grains but also in derivate products. In the last Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2001) was established a group provisional maximum tolerable daily intake (PMTDI) for Fumonisins B\textsubscript{1}, B\textsubscript{2} and B\textsubscript{3} alone or in combination, of 2µg/kg of body weight per day; and, Fumonisins B\textsubscript{1} NOEL (no observed effect level) for animals ranged between 0.2 mg/kg of body weight per day (induced porcine pulmonary oedema, and liver cancer) and 1.9 mg/kg of body weight per day (liver cancer in female mice).

Fumonisins are stable under storage conditions and different physical and chemical processes (high temperature, etc.). These processes may change fumonisin distribution in all process fractions.

In this part of the research, it was looked after to determine fumonisin contamination levels in the several fractions obtained in corn wet milling. This process permits to obtain corn products such as starch, germ, gluten, fibre and wash water, and corn based products like: modified starches, oil, feed and sugar products. The production in Argentina is (in thousands Ton per year): starches (70), fructose (300), glucose (150), others sugar products (70), corn oil (30) and gluten feed and meal (180).

The industrial process of corn wet milling consists in two principal steps:

• Corn steeping under controlled conditions of temperature, time, SO\textsubscript{2} and lactic acid concentrations. This process permits greater water absorption and improves the separation between starch and protein matrix.
• Separation process of several fractions: germ (by different density), fibre (by filtration), and gluten and starch (by centrifugation). The water fractions obtained would also be collected.

Based on the above information it was proposed to work together with CIM-UnLu (partner 10), the following future tasks:

- A laboratory-scale procedure to reproduce corn wet milling at industrial-scale would be evaluated to identify CCPs.
  The method to be applied would reproduce corn steeping in batch conditions and the separation process through sieves.
- Industrial batch process in an Argentine plant would be evaluated through fumonisin determination at the CCPs which were identified at the laboratory scale.
- Mycotoxin levels in corn are reduced at the storage entrance by cleaning with sieves. We have begun with this part of the research using different sieve sizes in collaboration with a private company.

TASK WP4&5 - INTA, CIM-UnLu and UBA
Cultural practices, including crop rotation, tillage, planting date, and management of irrigation and fertilization, have some effects on the plant infection and subsequent mycotoxin accumulation.
Because most mycotoxin problems could develop in the field, strategies are needed to prevent infection of growing plants by toxigenic fungi. The general strategy is to alter the conditions under which the crop is grown so that infection by the offending fungus or fungi is avoided. Tactics employed in this struggle include those used to battle most plant disease: tillage practices, fertilization practices, crop rotation, and plant population.

The major source of inocula for the *Fusarium* spp. is colonized plant debris. *F. graminearum* produces large number of perithesia on infested residues. In Argentina there is an increase in conservation tillage practices and to cropping systems in which corn is rotated with wheat or in which wheat is grown each year in the same field.

Scabs resistance breeding programs have identified wheat lines with moderately high resistance to the spread of *F. graminearum* over the world and also in Argentina. The purpose of this research is to identify wheat lines susceptible to *F. graminearum*, to evaluate actual conservation tillage practices applied in the region, as well as the influence of planting date, location, agrochemical application (Metsulfurom), on DON contamination levels (280 pl/m² with nutrient fertilization balance considering 3500 kg/ha yield).

The wheat samples collected in different locations by INTA group (partner 8) are sent to CIM-UNLu for analysis. Samples from S.A. de Areco, Junín, Arrecifes and Bragado just arrived. A sub sample 100gr is separated before milling. Milling is made through a Romer mill and extraction of trichothecenes and clean up of the extracts is performed in CIM-UnLu. Clean extracts for DON quantification and 100gr sub sample will be sent to UBA (partner 9) for fungal isolation and identification.

**References produced**


Submitted for publication

Supervision of PhD Thesis (Director Dr Silvia L. Resnik, Departamento de Industrias. Facultad de Ciencias Exactas y Naturales, U.B.A)

Partner 10 - University of Lujan

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
FIRST ANNUAL REPORT

Task WP1 Harmonization and standardisation of the analytical methods for mycotoxin analysis.
FAPAS reference samples were also used to evaluate clean up columns available in Argentina for deoxynivalenol, aflatoxins and zearalenone.
Control samples analysis for aflatoxins, zearalenone and fumonisins on corn and DON in wheat fell in the reference range provided by FAPAS.

Future task WP1
Samples provided by Brazil partners would be analysed at reception.

Task WP2 Risk assessment of human exposure to ochratoxin A

Ochratoxin A (OTA) is a mycotoxin produced by some species of the genera *Aspergillus* (e.g. *A. alutaceus*, *A. niger*, and *A. carbonarius*), and *Penicillium* (e.g. *P. verrucosum*), which can contaminate a great variety of foods. It has been found in starch-rich foodstuff such as cereals and derived products (e.g. beer), legumes and pulses, but it can also appear in other commodities such as coffee, cacao, nuts, spices, dried fruit, wine, etc. and in animals fed with contaminated foods (Krogh, 1987). OTA-producing cultures of *A. carbonarius* and *A. niger* have been isolated from dried vine fruit (Codex Alimentarius Commission, 1999, Abarca et al. 2001, Magnoli et al., 2001, Da Rocha Rosa et al., 2002, Olsen, 2002, 2003, Olsen et al., 2002 a and b).

Because OTA is known to have toxicological effects for human and animal, such as nephrotoxic, immunotoxic, genotoxic and carcinogenic effects (Krogh, 1987, IARC, 1993, Dirheimer, 1998), several countries have specific regulations for OTA in one or more commodities at levels ranging from 1 to 50 µg/kg for foods.

The Joint FAO/WHO Expert Committee on Food Additives met in Geneva February 6-15, (2001) retained the previously established provisional tolerable PTWI of 100 ng/kg body weight per week, pending the results of ongoing studies on the mechanisms of nephrotoxicity and carcinogenicity, and recommended a further review of ochratoxin A in 2004. The provisional tolerable daily intake of OTA in foods proposed by the Commission of the European Community (1998) is 1.2–14 ng/kg body weight; and recently in May 2003, the European Commission proposed, as an initial basis for discussion, limits of 3-4 µg/kg for roasted coffee, 6-10 µg/kg for instant coffee, and 2 µg/kg for wine and grape juice; moreover a new tolerable daily intake (TDI) of ochratoxin A ranged from approximately 0.13 to 3.14 ng/kg bw/day in total population and from 0.88 to 3.55 ng/kg bw/day for consumers was proposed, due to the incorporation of unreported sources such as wine and coffee (Mycotoxicology Newsletter, 2003). Significant contamination of wine has been reported all over the world (Zimmerli and Dick, 1996, Burdaspal and Legarda, 1999, Visconti et al., 1999, Otteneder and Majerus, 2000, Filali et al., 2001, JECFA, 2001, Pietri et al., 2001, Soleas et al., 2001, Lopez de Cerainy et al., 2002, Shepard et al., 2003), so wine was indicated as one of the possible sources of OTA exposure. Codex Committee on Food Additives and Contaminants (1998) reported that wine (especially red wine) is
the second major source of human exposure to OTA following cereals, giving a total dietary intake of 15%.

The work presented in this report focused on two aspects. The first one is the methodology standardization to be applied in Argentina on blood samples of human and pigs. The second one was related to the increased awareness of the potential risk for consumer health due to the possible exposure to OTA through wine consumption which implies, each country carry out systematic measurements of OTA levels in domestic and imported wines.

Materials and methods used for OTA analysis

The major problem encountered in the frame of WP2 was the difficulty of the University of Lujan (partner 10) in getting the immuno-affinity Ochraraprep columns (for use in OTA determination). Indeed, with the help of Dr Monica Olsen, we could get a special price offer for column purchase from Biopharm company. With the aim of optimising the financial resources and project running, the columns were ordered in Europe by the project general coordinator and sent to Argentina by the end of 2003. However, due to high complications for material clearance from the local customs, the materials are still not delivered to the partner lab. We are awaiting for the help of Biopharm company for solving this problem.

Ochratoxin A was purchased from Sigma-Aldrich (USA). The standard solutions were made in benzene: acetic acid (99:1) according to the concentration established using a UV spectrophotometer (ε333: 5500 M⁻¹ cm⁻¹). The required quantity was evaporated to dryness and dissolved in the mobile phase indicated under chromatographic conditions.

Extraction and clean-up of Ochratoxin A in serum and plasma

As it was expressed at Montevideo Meeting, Scott et al. (1998) method was followed with slightly modifications.

One ml of serum or plasma (containing anticoagulant) was mixed (vortex) for 15´´ with 0.25ml saturated sodium chloride solution in a centrifuge tube. Then, 5ml MeOH was added, vortexed again and centrifuge at 2000 rpm (830 x g) for 15-20 min.

The supernatant (serum-MeOH) was transferred to another tube and mixed with 5 ml 0.015M o-phosphoric before adding to a C-18 solid phase extraction, preconditioned with 10 ml MeOH followed by 6 ml MeOH : (0.015M) o-H₃PO₄ (1:1, v/v). Solvents passed through the column by gravity or under reduced pressure at a flow rate of 1-2 drops/sec. The column was washed with 5ml 0.015 M o-H₃PO₄ followed by 5ml MeOH: o-H₃PO₄ (1:1, v/v).

OTA was eluted with 2ml MeOH and the eluate evaporated to dryness under vacuum at 30°C.

The evaporated extract was dissolved in 3 x 2.5 ml PBS solution- MeOH (85:15) and added to an OchraPrep column. All solvents were passed through the column by gravity flow.
The column was washed with 5 ml PBS solution- MeOH (85:15) followed by 10 ml distilled water. OTA was eluted with 3ml MeOH (back-flushing) into a silanized vial and then more 1.5ml MeOH. The two aliquots were evaporated to dryness together under vacuum, at 30ºC. The evaporated extract was dissolved in 250 µl of mobile phase.

The PBS (Phosphate- buffered saline) solution consist of a mixture of 19 ml 0.2M monobasic sodium phosphate, 81ml 0.2M dibasic sodium phosphate, 14.04 g sodium chloride, 0.402 g potassium chloride and 1.0 g sodium azide was adjusted to pH: 7.4, diluted to 200 ml and then a 10ml aliquot further diluted 10-fold.

**HPLC determination of OTA**

The mobile phase was Acetonitrile: H2O: Acetic Acid (421.5: 570: 8.5). The flow rate was 1ml/min and injection volume 100 µl. Fluorescence excitation and emission wavelengths were 330 nm and 470 nm, respectively.

Retention time of OTA was 6 min. Figure 1 showed the blank.

![Figure 1](image)

**Figure 1**

**Collected samples:** Two pool of human plasma were provided by two different laboratories (General Rodríguez Hospital, and Roberto Carrara). Sixty pigs serous were taken from a slaughter manufacture near Luján (San Antonio de Areco). It was extracted at the slaughter moment from the neck vein.

**Results OTA in plasma and serum**

At the beginning it was to systematize the method (extraction, detection and confirmation) and to obtain better recovery values.

**Recoveries:** Human plasma as well as pig serum was contaminated at different levels.
Table 1: Examples of OTA recovery in spiked plasma samples

<table>
<thead>
<tr>
<th>CIM Number</th>
<th>Type of Sample</th>
<th>ml of Sample</th>
<th>St. Concentration µg/ml</th>
<th>Spiked µl</th>
<th>Percentage of recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>7145</td>
<td>Human</td>
<td>1</td>
<td>0,2426</td>
<td>100</td>
<td>116</td>
</tr>
<tr>
<td>7494</td>
<td>pig</td>
<td>1</td>
<td>0,2426</td>
<td>100</td>
<td>49,3</td>
</tr>
<tr>
<td>7552</td>
<td>pig</td>
<td>1</td>
<td>0,2426</td>
<td>100</td>
<td>49,2</td>
</tr>
<tr>
<td>7557</td>
<td>pig</td>
<td>1</td>
<td>0,2426</td>
<td>100</td>
<td>17</td>
</tr>
<tr>
<td>7607</td>
<td>Human</td>
<td>1</td>
<td>0,2426</td>
<td>100</td>
<td>20</td>
</tr>
<tr>
<td>7612</td>
<td>pig</td>
<td>1</td>
<td>0,2426</td>
<td>100</td>
<td>65,6</td>
</tr>
<tr>
<td>7676</td>
<td>pig</td>
<td>1</td>
<td>0,2426</td>
<td>100</td>
<td>50</td>
</tr>
</tbody>
</table>

Because most of the pig serum sample showed haemolysis, it has been centrifuged and filtrated to clearer as possible. In spite of that, some interference was detected. On the other hand, it can be see that higher contamination has worst recover, it could be to a “saturated phenomena” of the column due to so high contamination. Any way, it is necessary to improve the clean up step, to obtain repetitibility and reproducibility results, as well as greater recoveries in smaller levels of OTA contamination.

Clean up of Ochratoxin A for determination on wine

The extraction and quantification was based on Castellari et al. (2000) with minor modification. Column (Ochrarep, Rhône Diagnostics Technologies) was placed on an SPE vacuum manifold (Baker) and was at first washed with 5 ml of PBS (Scott et al., 1998) before used (PBS: dissolve 7.02 g sodium chloride, 0.201 g potassium chloride, 1.14 g disodium hydrogen orthophosphate, 0.26 g sodium dehydrogenate phosphate and 0.5 g sodium azide in 1 l HPLC grade water; adjust pH to 7.4). Then 10 ml of wine adjusted to pH 7.8 using 1 M NaOH were diluted with 10 ml of PBS and applied to the column, at a flow-rate of about 1-2 drops per second. The eluted was applied once more onto the column. 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 three times back flushing in each fractions (4.5 ml and three 1.5 ml fractions), from the column with HPLC-grade methanol at a flow-rate of about 1 drop per second. Eluate was evaporated into a silanized glass vial under vacuum at 40°C and the residue re-dissolved in 250 µl of mobile phase.

Detection of OTA. Apparatus and Chromatographic condition

Detection of OTA was achieved at 333 nm excitation, 460 nm emission wavelength. Injection volume of the samples extract was 100 µl. Calibration curve was established by injecting six standard solutions with OTA concentration ranging from: 0.026 to 2.65 µg/l (R²: 0.9997). Recovery experiments were performed on OA-free wines samples spiked with different OA levels. A mean recovery of OA was greater than 90 %. Results were not corrected for recovery. Detection limit of the employed method was 0.008 µg/l and quantification limits was 0.015 µg/l .HPLC equipment was Hewlett-Packard 1100 model, equipped with fluorescence detector and
chromatographic column Hypersil (125 x 4 mm) with 5 µm BDS C-18 and guard column Lichrocart, packed with 5 µm RP-18 were used. Computer program used was Chemstation A.08.03 for chromatographic analysis. The system was employed at 40°C temperature, with a mobile phase of acetonitrile: water: acetic acid (85:114:1 v/v/v) at 1ml/min. Retention time of OTA peak was approximately 5.8 min.

Results OTA in wine

Eighty samples of red wines, as well as one dessert wine, and three white wines were bought in 2003, from manufacturers stock and retail markets at Luján and Buenos Aires cities of Argentina (70). The samples represent a considerable variety of Argentina wines, screening the most important argentine wineries. The study also included wine samples from other countries (8 from Italy, 5 from Spain, 2 from France and one from South Africa) available in Argentina markets. Information about the origin of the commercial samples was taken from the bottle labels. None of the red wine produced in Argentina were contaminated. Two reports expressed a low toxicogenic capacity for those fungi isolated from grapes in the region corroborate these results. Among the few other wines analysed, 6 from Italy (included dessert wine), 3 from Spain, 1 from France and the only one from South Africa, have had OTA contaminations, but at different levels, that means 11 from 16 imported wine analysed (68.8%). An example of chromatograms obtained from wine sample and its methyl ester derivative are shown in figure 2.

Figure 2: Chromatogram of: a) dessert wine sample with 1.32 µg/l OTA  
b) OTA methyl ester derivate of the dessert wine
Although the limit number of imported wine samples analysed (Table 2), the results showed that OTA contaminations from European wines were in coincidence with authors who said dessert wine was more contaminated than others and those authors who said wines produced in Mediterranean climate could have higher contaminations levels.

**Table 2:** Ochratoxin A contamination of imported wines in Argentina.

<table>
<thead>
<tr>
<th>Year Crop</th>
<th>Vintage</th>
<th>Origin Country</th>
<th>Region</th>
<th>OTA µg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>1993</td>
<td>Vin de Pays D’OC (white)</td>
<td>France</td>
<td>Narbonne</td>
<td>0.02</td>
</tr>
<tr>
<td>1997</td>
<td>Cabernet Sauvignon - Cabernet franc - Merlot</td>
<td>France</td>
<td>Medoc</td>
<td>nd</td>
</tr>
<tr>
<td>1994</td>
<td>Superiore (white)</td>
<td>Italy</td>
<td>Frascati</td>
<td>nd</td>
</tr>
<tr>
<td>1997</td>
<td>Chianti</td>
<td>Italy</td>
<td>Sensi</td>
<td>0.31</td>
</tr>
<tr>
<td>1997</td>
<td>Chianti Classico Riserva</td>
<td>Italy</td>
<td>Firenze</td>
<td>0.34</td>
</tr>
<tr>
<td>1997</td>
<td>Chianti</td>
<td>Italy</td>
<td>Unknown</td>
<td>0.11</td>
</tr>
<tr>
<td>1999</td>
<td>Merlot</td>
<td>Italy</td>
<td>Sensi</td>
<td>0.18</td>
</tr>
<tr>
<td>1999</td>
<td>Merlot Veneto</td>
<td>Italy</td>
<td>Firenze</td>
<td>0.02</td>
</tr>
<tr>
<td>2000</td>
<td>Merlot</td>
<td>Italy</td>
<td>Verona</td>
<td>1.32</td>
</tr>
<tr>
<td>Unknown</td>
<td>Marsala Fine</td>
<td>Italy</td>
<td>Unknown</td>
<td>0.03</td>
</tr>
<tr>
<td>1999</td>
<td>Chardonnay (white)</td>
<td>South Africa</td>
<td>Unknown</td>
<td>0.03</td>
</tr>
<tr>
<td>1995</td>
<td>Reserva Tinto</td>
<td>Spain</td>
<td>Rioja</td>
<td>nd</td>
</tr>
<tr>
<td>1997</td>
<td>Merlot</td>
<td>Spain</td>
<td>Barcelona</td>
<td>nd</td>
</tr>
<tr>
<td>1997</td>
<td>Cabernet Sauvignon - Syrah - Petit Verdot</td>
<td>Spain</td>
<td>Rioja</td>
<td>nd</td>
</tr>
<tr>
<td>1997</td>
<td>Tempranillo -Garnacha</td>
<td>Spain</td>
<td>Cenicero</td>
<td>0.12</td>
</tr>
<tr>
<td>1999</td>
<td>Garnacha y Carineña</td>
<td>Spain</td>
<td>Barcelona</td>
<td>0.48</td>
</tr>
</tbody>
</table>

*nd: No detected*
Moreover, the only South Africa wine analysed, was also contaminated, in agree with Shepard et al. (2003).

Exposure: The 56th meeting of JECFA (2001) set a PTWI for OTA at 100 ng/kg body weight/week, which equivalent to a suggested by Commission of the European Community (1998) maximum tolerable daily intakes (MTDI) for humans of 1.2-14 ng/kg body weight day. On the other hand figure 3 showed the tendency in wine consumption (included red, white, dessert wines) of both countries, and the mean intake of the last five years was 95.27 ml for Argentine people (FAO, Food Balance Sheet).

Figure 3: Wine consumption from 1980 to 2001 year from Argentina

[Graph showing wine consumption from 1980 to 2001 for Argentina]

Argentina will be exposure through imported wine consumption, and because the wine intake (figure 3). Taking into account the half detection limit (0.004 µg/l) of the none contaminated samples; OTA exposure through national wine intake for Argentine people could be 0.0054 ng/kg b.w./day. Moreover, taking into account mean level of the imported wines analysed (0.186 µg/l) OTA exposure of Argentine people would be 0.256 ng/kg b.w./day, which should implied a risk according to Commission of the European Community (1998) MTDI suggested.

Up to now, there has been a lack of quantitative studies in the literature concerning wine OTA contamination in Argentina. It is a preliminary report about wine contamination in these countries.

The presence of OTA in wines would appear a widespread problem of concern, because its contribution to OTA exposure in populations, but also because the international trade

Future task WP2
Implementation of the selected and standardized method for OTA determination in 200 blood obtained in an hospital (Centro Regional de Hemoterapia, Mar del Plata, Buenos Aires) and exploitation of dietary questionnaires to blood donors.
TASK WP3 Evaluation of milling procedures as potential CCPs

The work develop in this period, together with UBA, focused on statistically analyzing the contamination occurring with DON in wheat and derived milling fractions and with fumonisins in maize.

The methodology for DON analysis was divided in two steps to optimise resources. Extraction and cleanup of analytical samples, first step, were performed in CIM-UnLu and derivatization and Gas Chromatography quantification in UBA (second step).

Extraction and cleanup was realized, as described by Truckssess et al. (J. AOAC Int., 1996, 79, 883-887) with slight modifications: The mixture acetonitrile–water (84 + 16) was adjusted taking into account the water content of each sample; ca 8 mL extract was placed in an 8 x 15 mm culture tube, and four 0.5 mL portions were successively passed through the cleanup column. Each was transferred to a different tube, evaporated to dryness in a 60°C water bath under vacuum, and stored at –18°C. Only the first portion was analysed.

Sub samples were analysed to determine the presence of FB1, FB2 and FB3 according to the method of AOAC International (2000). Briefly, aqueous methanol extracts were prepared for each of the samples, the pH was adjusted before they were applied to strong anion exchange (SAX) solid-phase extraction cartridges. Available SAX columns were assayed before application. The columns tested were Merck Adsorbex cat. no 19845 and J. & W. Scientists Fisons Accubond #1601. The cartridges were washed to remove other compounds, the fumonisins selectively eluted with an acetic acid methanol solution, and the eluates were collected and dried. o-Pthalialdehyde (OPA) derivatives of each purified sample extract were prepared and analysed by reversed-phase HPLC using fluorescence detection with a stainless steel LC column, 25 cm x 4mm i.d., packed with Lichrosorb 5m C18 reversed-phase material (Merck Co., Darmstadt, Germany). Identification and quantification of the fumonisins were by comparison of the retention times and peak areas in the samples with those observed for fumonisins standards. Detection limits were: FB1, 10; FB2, 10; and FB3 10 ng g -1 (signal noise ratio 3:1). Results were published in JAOAC.

An assay was also conducted for determining the pattern of contamination distribution and the variability associated with the fumonisins on 100 maize sub samples from Argentina. As shown in figure 4, the distribution of fumonisins in maize presents a great right asymmetry.
Future task WP3
Corn produced in Argentina represented c.a. 30% of the total cereal and oilseed production. Natural occurrence of Fumonisins in Argentinean corn is a factor not only in grains but also in derivate products. In the last Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2001) was established a group provisional maximum tolerable daily intake (PMTDI) for Fumonisins B₁, B₂ and B₃ alone or in combination, of 2µg/kg of body weight per day; and, Fumonisins B₁ NOEL (no observed effect level) for animals ranged between 0.2 mg/kg of body weight per day (induced porcine pulmonary oedema, and liver cancer) and 1.9 mg/kg of body weight per day (liver cancer in female mice).

Fumonisins are stable under storage conditions and different physics and chemistry processes (high temperature, etc.). These processes may change fumonisins distribution in all process fractions.

In this part of the research, it was looked after to determine fumonisin contamination levels in the several fractions that it was obtained in corn wet milling. This process permits to obtain corn products such as starch, germ, gluten, fibre and wash water, and corn based products like: modified starches, oil, feed and sugar products. The production in Argentina is (in thousands Ton per year): starches (70), fructose (300), glucose (150), others sugar products (70), corn oil (30) and gluten feed and meal (180).

The industrial process of corn wet milling consists in two principal steps:

- Corn steeping under controlled conditions of temperature, time, SO₂ and lactic acid concentrations. This process permits greater water absorption and improves the separation between starch and protein matrix.
- Separation process of several fractions: germ (by different density), fibre (by filtration), and gluten and starch (by centrifugation). The water fractions obtained would also be collected.
Based on the above information it was proposed to work together with UBA (partner 9), the following future tasks:

- A laboratory-scale procedure to reproduce corn wet milling at industrial-scale would be evaluated to identify CCPs.
- The method to be applied would reproduce corn steeping in batch conditions and the separation process through sieves.
- Industrial batch process in an Argentine plant would be evaluated through fumonisin determination at the CCPs which were identified at laboratory scale.
- Mycotoxin levels in corn are reduced at the storage entrance by cleaning with sieves. We have begun with this part of the research using different sieve sizes in collaboration with a private company collaboration.

**TASK WP 4&5 (INTA, CIM-UnLu and UBA)**

Cultural practices, including crop rotation, tillage, planting date, and management of irrigation and fertilization, have some effects on the plant infection and subsequent mycotoxin accumulation. Because most mycotoxin problems could develop in the field, strategies are needed to prevent infection of growing plants by toxigenic fungi. The general strategy is to alter the conditions under which the crop is grown so that infection by the offending fungus or fungi is avoided. Tactics employed in this struggle include those used to battle most plant disease: tillage practices, fertilization practices, crop rotation, and plant population. The major source of inocula for the *Fusarium* spp. is colonized plant debris. Large number of perithesia is produced by *F. graminearum* on infested residues. In Argentina there is an increase in conservation tillage practices and to cropping systems in which corn is rotated with wheat or in which wheat is grown each year in the same field. Scabs resistance breeding programs have identified wheat lines with moderately high resistance to the spread of *F. graminearum* over the world and also in Argentina. The purpose of this research is to identify wheat lines susceptible to *F. graminearum*, to evaluate actual conservation tillage practices applied in the region, as well as the influence of planting date, location, agrochemical application (Metsulfurom), on DON contamination levels (280 pl/m2 with nutrient fertilization balance considering 3500 kg/ha yield).

The wheat samples collected in different locations by INTA group (partner 8) are sent to CIM-UNLu for analysis. Samples from S.A. de Areco, Junín, Arrecifes and Bragado just arrived. A sub sample 100gr is separated before milling. Milling is made through a Romer mill and extraction of trichothecenes and clean up of the extracts is performed in CIM-UnLu. Clean extracts for DON quantification and 100gr sub sample will be sent to UBA (partner 9) for fungal isolation and identification.
References produced


Micotoxinas en nuestros paises. Conferencia Magistral.


Submitted for publication
Partner 11 – UCON DBND

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
FIRST ANNUAL REPORT

SUMMARY
During development of MYCOTOX project, the University of Concepción, UDEC, has been participating in Work packages 1, 2, 3 and 4.
The first activity in the frame of the project was the attendance to initial meeting performed in Montevideo, Uruguay in February 2003. Once the tasks were defined for the different WP where the University of Concepcion has participation, just coming back to Chile, the purchasing of consumables and implementation of different analytical methodologies started. At this moment two coordination meetings were performed with INIA in relation with WP4, one in Santiago at INIA Presidency with the aim to inform at this level about MYCOTOX project. The other one, was in Concepcion with Guy Henry (partner 1) and Ricardo Madariaga (partner 7) and the administrative and technical staff of El Globo Mill with the aim to involve them in the project, specifically in WP3 and WP4. Later on, UdeC was visited by Martin Nagler (partner 2), again with purpose of coordination between partners 7 and 11 for WP 4&5.

During 2003, at least two coordination trips to Collico Mill in Valdivia were done, with the participation of Professor Ricardo Villegas and Mario Vega (partner 11) and Ricardo Madariaga (partner 7).

Participation of UDEC in WP1
The first task in relation to this WP, was purchasing of all the material to perform the analytical work involved, this mean purchase of standard for the analysis of aflatoxins $B_1$, $B_2$, $G_1$, $G_2$, zearalenone DON, Fumonisina $B_1$ and $B_2$, all these standard where Sigma. Besides standards, Romer columns 224 and 225 for extract purification were also purchased.
The second task was to analyze FAPAS samples. Analysis were performed for maize samples with AFT $B_1$ and $B_2$, maize samples for Zearalenone, maize samples for Fumonisins and wheat samples for DON. Between September and December analysis for Aflatoxins, Zearalenone and DON was done. The results are presented in Table 1.
Wheat flour sample with DON arrived in January and was analyzed at the end of February. The results sent to the leader of WP1 this month. By this time the method for Fumonisins by HPLC is in process of development.
In the frame of the MYCOTOX project a Pharmacy postgraduate student, Paulina Riquelme, will develop the thesis entitled “Determinación de los puntos críticos de control para el análisis de peligro de contaminación con micotoxinas del genero Fusarium y Alternaria en la cadena del trigo del sur de Chile” (Determination of the control critical point for the hazard of Fusarium and Alternaria mycotoxins in a wheat chain in the south of Chile” This work is involved in aspects of WP 1, WP 3 and WP 4. The thesis will start in 2004.
# FAPAS interlaboratory works: UdeC results

## Report Resulting Sheets: Aflatoxin

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>UdeC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxin analysed</td>
<td>AFL, FUM, ZEA, DON, OTA</td>
</tr>
<tr>
<td>Sample</td>
<td>wheat, Corn, wheat products, corn products, others</td>
</tr>
<tr>
<td>Sample weight</td>
<td>5Kg, 3Kg, 1Kg</td>
</tr>
<tr>
<td>Subsample</td>
<td>25g, 50g</td>
</tr>
<tr>
<td>Extration procedure</td>
<td>shaker with H2O and CHCl3, Blend with MeOH and KCl sol, shaker with NaCl, H2O and MeOH, shaker with NaCl, H2O and MeOH, shaker with ACN and H2O, shaker with ACN and MeOH, Blender with ACN and H2O, shaker with H2O and MeOH</td>
</tr>
<tr>
<td>Clean-up</td>
<td>SPE column (florisil and C-18), LC-Si SPE tubes 1000mg, immunoafinity column, ROMER column, ROMER or Trilogy column, SAX or Baker Amino, Ochrapred or Vicam columns</td>
</tr>
<tr>
<td>Determination</td>
<td>HPLC, HPTLC, CGL and ECD</td>
</tr>
<tr>
<td>HPTLC datas: Plate:</td>
<td>HPTLC Silicagel 60 F254, Mobile fase: Chloroform+Xylene+Acetone; (60+30+10, v/v/v), Spoted vol: 50 µL, Cromaovisor, Densitometer</td>
</tr>
<tr>
<td>HPLC datas: Column:</td>
<td>Mobile fase, Injection vol.</td>
</tr>
<tr>
<td>CGL and ECD datas: Column:</td>
<td>Gas, Injection vol.</td>
</tr>
<tr>
<td>Analyst(s)</td>
<td>One, Two</td>
</tr>
</tbody>
</table>
Contract number: ICA4-CT-2002-10043

Reference

Percentage recovery

Date

Standard deviation (σ)

Corrected Date

Report Resulting Sheets: Zearalenone

Laboratory: UdeC

Toxin analysed

- AFL
- FUM
- ZEA
- DON
- OTA

Sample

- wheat
- corn
- wheat products
- corn products
- others

Sample weight

- 5Kg
- 3Kg
- 1Kg

Subsample

- 25g
- 50g

Extraction procedure

- shaker with H₂O and CHCl₃
- Blend with MeOH and KCl sol
- shaker with NaCl,H₂O and MeOH
- shaker with NaCl,H₂O and MeOH
- shaker with ACN and H₂O
- shaker with ACN and MeOH
- Blender with ACN and H₂O
- shaker with H₂O and MeOH

Clean-up

- Liq-liq extraction
- SPE column (florisil and C-18)
- LC-Si SPE tubes 1000mg
- Immunoaffinity column
- ROMER column
- ROMER or Trilogy column
- SAX or Baker Amino
- Ochrapred or Vicam columns

Determination

- HPLC
- HPTLC
- CGL and ECD

HPTLC datas:

Plate: HPTLC Silicagel 60 F254
Mobile fase: Toluene+Ethyl Acetate+Formic Ac; (60+30+10, v/v/v)
Spoted vol. 30 µL
- Cormatovisor
- Densitometer

HPLC datas:

Column:
Mobile fase: Flow: gradient:
Injection vol. 

CGL and ECD datas: Column:

<table>
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<tr>
<th>Gas</th>
<th>Flow</th>
<th>Gradient</th>
<th>Temp</th>
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</thead>
</table>

Analyst (s)  

| X | One | Two |

Reference

Percentage recovery 

Date

Standard deviation (σ)

Corrected Date

<table>
<thead>
<tr>
<th>AFT</th>
<th>B₁</th>
<th>B₂</th>
<th>ZEA</th>
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<td>1,08</td>
<td>190,69</td>
<td></td>
</tr>
<tr>
<td>2,64</td>
<td>0,97</td>
<td>209,80</td>
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<td>2,55</td>
<td>0,74</td>
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<td></td>
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<td>2,07</td>
<td>0,75</td>
<td></td>
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</tr>
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<td>2,52</td>
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<td></td>
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</tr>
<tr>
<td>2,41</td>
<td>0,75</td>
<td></td>
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<tr>
<td>2,15</td>
<td>0,55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Prom (ppb) | 2,73 | 0,80 | 200,25 |
SD | 0,5375345 | 0,1510298 | 13,5128106 |
CV% | 19,6971233 | 18,9023527 | 6,74813882 |
n | 10 | 10 | 2 |

AFT Series 22 Round 09  
T2209  

ZEA Series 04 Round 46  
T0446
Table 1: Results of the interlaboratory works performed by the partner 11 using Fapas material.

<table>
<thead>
<tr>
<th>Mycotoxin</th>
<th>Assigned value (μg/kg)</th>
<th>Satisfactory range (μg/kg)</th>
<th>Determined value (μg/kg)*</th>
<th>SD</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFL B1</td>
<td>6.78</td>
<td>3.8-9.76</td>
<td>2.73</td>
<td>0.54</td>
<td>-----</td>
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<tr>
<td>AFL B2</td>
<td>1.66</td>
<td>0.93-2.39</td>
<td>0.80</td>
<td>0.15</td>
<td>-----</td>
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<tr>
<td>Total AFL</td>
<td>8.66</td>
<td>4.85-12.46</td>
<td>3.53</td>
<td>-----</td>
<td>-----</td>
</tr>
<tr>
<td>ZEA</td>
<td>228</td>
<td>137-319</td>
<td>200.25</td>
<td>13.5</td>
<td>-----</td>
</tr>
</tbody>
</table>

* Value not corrected by the recovery

Problems in WP1
The main problems have been the lack of accuracy of the result obtained, specially for the analysis of Aflatoxins B\textsubscript{1} and B\textsubscript{2}, this is a big concern for the staff of UDEC and it why all the efforts will be done to determinate the critical point that are the cause of this lack of accuracy expressed as a very low recovery (about 60-70%).

Outline plans in WP1 for 2004:
During 2004 UDEC, to work in the implementation of corrected method for the analysis of the main mycotoxins concerned to the project (Aflatoxins, DON), the implementation of the method for Fumonisin B1 and B2. Once the methods are ready we will participate in the inter-laboratory test that will be done in the frame of WP1 as defined in the initial meeting at Montevideo.

Participation of UDEC in WP2
The frame of WP2, analysis of OTA in blood, UDEC has been working in the implementation of the method to analyze OTA at the level expected could be find in blood accordingly described in literature. At the moment calibration curves, limit of detection and limit of quantification are already estimated; LOD = 0.2 ng/ml , LOQ = 0.7 ng/ml. The first assay have been performed using Vicam column, but it is clear that for the project OCHRAPREP column have been purchased and will be the same columns used by the Argentinean partners in this WP. In the last part of this month the validation of the method will be ready to start the analysis of the blood. Because during the Project, cereal samples will be also analyzed, the validation for the analysis of OTA in wheat it is also in progress. In this case, HPTLC will be used, considering that upper detection limits are enough for OTA estimation in cereals. The validation of this procedure is summarized in the annex 1.

At the moment, three places in different parts of the country have been chosen for blood samples collection. The reason is because Chile is a very long country with a big variety of climatic conditions. At the south part of Santiago is where we think the condition for production of OTA is possible. And our places have been chosen, the first at 100 Km from Santiago, the second at 250 Km, the third at 500 Km and the fourth at 700 km from Santiago. In this WP, also a Postgraduate student, Katherine Muñoz, will participate with her thesis. The thesis has the intended title “Determinación de la concentracion de Ocratoxina A en Sangre Humana en la Zona Agrícola Centro Sur de Chile” (Determination of Ochratoxin A in human blood from the Central-South Agricultural zone from Chile”) The blood samples will start to be
collected at the end of this month, by now the implementation of the method using Ochraprep columns it is in progress.

**Outline plans in WP2 for 2004**

During 2004 blood samples collection and analysis will be performed. At the same time, cereal samples will be collected near the places where the human blood samples will be taken, in this way we will be able to have a first correlation between OTA in blood and food products.

**Validation of OTA analysis in wheat by HPTLC**

**Sensitivity**

a) Calibration curve

![Calibration Curve](image)

**Table 2: Raw data for calibration curve.**

<table>
<thead>
<tr>
<th>ng</th>
<th>Area 1</th>
<th>Area 2</th>
<th>Area 3</th>
<th>A. Prom.</th>
<th>s</th>
<th>RSD</th>
</tr>
</thead>
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<tr>
<td>4,50</td>
<td>2286,78</td>
<td>2228,61</td>
<td>2194,95</td>
<td>2236,78</td>
<td>46,46</td>
<td>2,08</td>
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<td>7,49</td>
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<tr>
<td>8,99</td>
<td>4176,70</td>
<td>4121,38</td>
<td>4102,30</td>
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<td>4718,49</td>
<td>4812,32</td>
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<td>59,50</td>
<td>1,24</td>
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<tr>
<td>13,48</td>
<td>6027,23</td>
<td>5888,90</td>
<td>5715,05</td>
<td>5877,06</td>
<td>156,43</td>
<td>2,66</td>
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</table>

b) Limit of Detection and Limit of Quantification

**Table 3: Data for estimation of LOD and LOQ.**

<table>
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<tr>
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<th>Area 2</th>
<th>Area 3</th>
<th>A Prom</th>
<th>s</th>
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<td>450,78</td>
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<td>875,04</td>
<td>847,38</td>
<td>854,74</td>
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<tr>
<td>3,00</td>
<td>1605,64</td>
<td>1577,66</td>
<td>1518,68</td>
<td>1567,33</td>
<td>44,39</td>
</tr>
</tbody>
</table>

LOD = 273 pg equivalent to 2 ppb
LOQ = 331 pg equivalent to 4 ppb
Recovery

\( n = 2 \)
Addition : 149.8 ng
Results:
\( n_1 = 150.32 \text{ ng} \)
\( n_2 = 140.92 \text{ ng} \)
Mean = 145.62 ng
Recovery = 97.2 %

Repeatability (Day 1)

<table>
<thead>
<tr>
<th>Sample g</th>
<th>Result ng</th>
<th>Conc ppt</th>
</tr>
</thead>
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<tr>
<td>22,50</td>
<td>3,81</td>
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<td>20,20</td>
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<td>22,10</td>
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<td>20,90</td>
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<td>29,80</td>
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<tr>
<td>22,90</td>
<td>3,98</td>
<td>41,80</td>
</tr>
</tbody>
</table>

Mean 34.58
s 5.60
RSD 16.19

Repeatability (Day 2.)

<table>
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<tr>
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</tr>
</thead>
<tbody>
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<td>4,946</td>
<td>41,03</td>
</tr>
<tr>
<td>23,8</td>
<td>4,311</td>
<td>47,03</td>
</tr>
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<td>21,3</td>
<td>3,76</td>
<td>36,71</td>
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<td>19,9</td>
<td>3,875</td>
<td>35,34</td>
</tr>
<tr>
<td>21,2</td>
<td>5,043</td>
<td>49,00</td>
</tr>
</tbody>
</table>

Promedio 41.82
s 6.07
RSD 14.51

Participation of UDEC in WP3

Identification of the Mill to perform the study of distribution of mycotoxins during dry milling (in Chile there are not plant in where wet milling is done, not either maize or wheat). Knowing the structure of the Mill, a sampling plan was designed, this plan is shown in the Figure 1.
Problems in WP3

During 2003, changes in administration in El Globo mill from Concepción were produced, these changes produced a lot of difficulties that made impossible to do the sampling accordingly the designed plan. For the second year (2004) the sampling will be performed at least in two occasions, at the beginning of the year where silos are filled just with national wheat and later when imported wheat come to fulfill the country necessities.

It is also important to say that is not easy to find contaminated samples with DON in national wheat. In the meantime 65 samples obtained from Collico Mill from Valdivia, all of them obtained during the harvest 2003 were analyzed with the aim that some diagnosis could be done in relation to Fusarium mycotoxins. All samples resulted negatives to DON, T-2 toxin, DAS, HT-2 toxin, Zearalenone. Accordingly the prevalence of fungal contamination that resulted to be Alternaria, also Alternaria toxins where looked for, again all these samples resulted negative. In this moment these samples are in analysis to check OTA.

The University of Concepción counts with HPTLC system implemented for the analysis of mycotoxins, the same mycotoxins that will be analyzed in the project. The mycotoxin measured through Planar Chromatography includes, DON, ZEARALENONE, AFLATOXINS. The methodology implies the use of MYCOSEP 224 and 225 Romer columns for purification. The extraction is don with
CH3CN/WATER ; 84+16, v/v., purification with the multifunctional Mycosep columns, and chromatography using HPTLC plates. Detection and quantification is performed by densitometry and/or VideoStore/Videoscan.

**Figure 2: Wheat sampling plan for analysis**

**Outline plans in WP3 for 2004**

During 2004, GC –ECD system will be available, this will improve the analytical capacity of UDEC Lab for mycotoxin specially Thriothecenes (DON, T-2, DAS, HT-2). The plans for 2004 in this point is to implement the methods similarly to those used in UBA with the aim to harmonize the methodology for Fusarium mycotoxins analysis, both for extraction and derivatization.

In the frame of this work package, during 2004, Gisela Rios, member of the UDEC team, will start her Doctorate, in a collaborative thesis involving CIRAD, INRA UDEC.
Outline plans in WP4 and WP5, 2004

Through close relationships with the partner 7, UDEC will participate in WP4 and WP5 for 2004 supporting these worpackages with analytical tools doing the mycotoxins analysis for the samples collected by partner 7 in those Wps. Apart of this, UDEC is participating in the HACCP team with Ricardo Villegas.

Participation in meetings and conferences

- Project kick off meeting, Montevideo (Uruguay), 17-19 February 2003
- Progress meeting of WP 4 &5 in Buenos Aires (Argentina), 20-22 August 2003
- IV Congreso Latinoamericano de Micotoxicología, la Habana, Cuba, 24-26 de Septiembre 2003.

Papers and publications


Partner 12 - LATU

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT
Covering period from 1 January to 31 December 2003

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.
FIRST ANNUAL REPORT

The Mycotoxin Department of Technological Laboratory of Uruguay participated in the MYCOTOX Project within two workpackages: WP1 and WP4 as collaborator. 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 this reported period.

Participation in WP1

1- NIR Methodology. Denis Bastianelli visit
LATU has available for use an Infratec 1241 Grain Analyzer Foss Tecator (NIT). It would be extremely useful to use it for determining mycotoxin. When Dr. D. Bastianelli visited Mycotoxin Department, explained NIR/NIT theory and a joint work on evaluation of NIT methodology for Deoxynivalenol was planned and carried out in this period.
Mycotoxin Department of LATU determined DON by visual TLC and 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, something never done in this area.

The report done by Bastianelli concluded that more samples must be processed in order to demonstrate that the prediction model detects really a trace contamination. Following its implementation and positive evaluation, LATU has the possibility to send more samples to Dr. Bastianelli. This is planned for 2004. DON levels and quantity must be arranged.

2- Protocols with the detail of infrastructure and analytical procedures were sent to the WP1 leader as requested in the forms.

3- FAPAS reference materials were purchased for conducting the interlaboratory studies for Aflatoxin B1, B2, G1, G2 in maize T0446, Deoxynivalenol in wheat flour T2210, Zearalenone in maize T2209 and Fumonisin B1 and B2 in maize T2208. Results of the studies were reported to coordinator. (Table 1)

4- During the period 2003-2004 participation in all planned FAPAS rounds, analyzing 6 sample test materials for: zearalenone (maize Round 09), ochratoxin A (cereal Round 21,24) deoxynivalenol (wheat flour Round 10), aflatoxin M1 (milk Round 61); and fumonisin B1, B2 (maize Round 11) 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 \( \leq 2 \) Z score values (Table 2).

Aflatoxins B1, B2, G1 and G2 (maize Round 59), DON (wheat flour Round 12) and fumonisin B1, B2 (maize Round 13) have not still been analysed.
Cost of this rounds was paid by LATU.
5- A WP1 meeting was carried out at the IV Congreso Latinoamericano de Micotoxicología in La Habana Cuba (September 24-27). All assigned tasks were completed.
LATU sent to the WP1 leader the assigned value of each FAPAS reference material received. As LATU is a routine participant of FAPAS Programme, it was asked to send to the WP1 leader the statistical studies of each round in which we participated in the period 2002-2003 related to Aflatoxin B₁, B₂, G₁, G₂, Deoxynivalenol, Zearalenone and Fumonisin B₁ and B₂.

6- Experimental results

<table>
<thead>
<tr>
<th>FAPAS interlaboratory works</th>
</tr>
</thead>
</table>

### Report Resulting Sheets: Aflatoxin

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>LATU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toxin analysed</td>
<td>AFL</td>
</tr>
<tr>
<td>Sample</td>
<td>wheat</td>
</tr>
<tr>
<td>Sample weight</td>
<td>5Kg</td>
</tr>
<tr>
<td>Subsample</td>
<td>25g</td>
</tr>
<tr>
<td>Extration procedure</td>
<td>shaker with H₂O and CHCl₃</td>
</tr>
<tr>
<td>Clean-up</td>
<td>Liq-liq extraction</td>
</tr>
<tr>
<td>Determination</td>
<td>HPLC</td>
</tr>
<tr>
<td>TLC data:</td>
<td>Plate:</td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
</tr>
<tr>
<td>Mobile fase</td>
<td>CHCL₃: acetone (9:1)</td>
</tr>
<tr>
<td>Spotted vol.</td>
<td>10 y 20 microlitros</td>
</tr>
<tr>
<td>X</td>
<td>Cromatovisor</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>HPLC data:</th>
<th>Column:</th>
<th>Temp.:</th>
<th>Mobile fase</th>
<th>flow</th>
<th>gradient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection vol.</td>
<td>Detector:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CGL and ECD data:</th>
<th>Column:</th>
<th>Gradient Temp</th>
<th>Gas</th>
<th>flow</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection vol.</td>
<td>Detector:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference</th>
<th>AOAC 2000 993.17</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Percentage recovery</th>
<th>B₁ 94%</th>
<th>B₂ 89%</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Data</th>
<th>5.5 µg/kg</th>
<th>1.6 µg/kg</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Standard deviation (σ)</th>
<th>0.9</th>
<th>0.3</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Corrected Data</th>
<th>5.9</th>
<th>1.8</th>
</tr>
</thead>
</table>

**Report Resulting Sheets: Fumonisins**

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>LATU</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Toxin analysed</th>
<th>AFL</th>
<th>X FUM</th>
<th>ZEA</th>
<th>DON</th>
<th>OTA</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Sample</th>
<th>wheat</th>
<th>X corn</th>
<th>wheat products</th>
<th>corn products</th>
<th>others</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Sample weigt</th>
<th>5Kg</th>
<th>3Kg</th>
<th>1Kg</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Subsample</th>
<th>25g</th>
<th>50g</th>
<th>X 10g</th>
</tr>
</thead>
</table>

| Extraction procedure | shaker with H₂O and CHCl₃ | Blend with MeOH and KCl sol |
shaker with NaCl, H2O and MeOH
shaker with ACN and H2O
shaker with ACN and MeOH
Blender with ACN and H2O
X shaker with H2O and MeOH

Clean-up

□ Liq-liq extraction
□ SPE column (florisil and C-18)
X LC-Si SPE tubes 100mg
□ Immunoaffinity column
□ ROMER column
□ ROMER or Trilogy column
□ SAX or Baker Amino
□ Ochraprep or Vicam columns

Determination

X HPLC □ TLC □ CGL and ECD

TLC datas:
Plate:
Mobile phase:
Spoted vol.
□ Cromatovisor □ Densitometer

HPLC datas:
Column: C18
Temp.: 30°C
Mobile phase: Methanol: bufferflow 1ml/min gradient phosphate
Injection vol.: 20 μl
Detector: Fluorescence

CGL and ECD datas:
Column:
Gradient Temp
Gas:
Injection vol.
Detector:

Reference
AOAC
2000
995.15

Percentage recovery
FB1 94% FB2 89%

Data
420.3 μg/kg 239.3 μg/kg

Standard deviation
38.0 15.6

Corrected Data
447.1 μg/kg 268.9 μg/kg
Report Resulting Sheets: Zearalenone

Laboratory
LATU

Toxin analysed
AF
FUM
ZEA
DON
OTA

Sample
wheat
X corn
wheat products
corn products
others

Sample weight
5Kg
3Kg
1Kg

Subsample
X 25g
50g

Extraction procedure
shaker with H2O and CHCl3
Blend with MeOH and KCl sol
shaker with NaCl, H2O and MeOH
X shaker with NaCl, H2O and MeOH
shaker with ACN and H2O
shaker with ACN and MeOH
Blender with ACN and H2O
shaker with H2O and MeOH

Clean-up
X Liq-liq extraction
SPE column (florisil and C-18)
LC-Si SPE tubes 1000mg
immunoaffinity column
ROMER column
ROMER or Trilogy column
SAX or Baker Amino
Ochrhapred or Vicam columns

Determination
HPLC
X TLC
CGL and ECD

TLC data:
Plate:
Merck, Sílica gel 60
Mobile fase
CHCl3: acetone (9:1)
Spotted vol.
10 y 20 microlitros
X Cromatovisor
Densitometer

HPLC data:
Column:
Temp.
Mobile fase
flow
gradient
Injection vol.
Detector:

CGL and ECD Column:
Gradient Temp
Datas:

<table>
<thead>
<tr>
<th>Gas flow</th>
<th>Injection vol.</th>
<th>Detector</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Reference: AOAC 2000
970.45

Percentage recovery: 85%

Data: 246.5 μg/kg

Standard deviation (σ): 16.5

Corrected Data: 290

---

**Table 1: FAPAS reference materials results**

<table>
<thead>
<tr>
<th>Toxin/substrate/Identification</th>
<th>Assigned value μg/kg</th>
<th>Satisfactory range μg/kg</th>
<th>Our assigned value to FAPAS Round μg/kg</th>
<th>Z score*</th>
<th>Our value reported to coordinator μg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B₁, B₂, G₁, G₂ in maize T0446</td>
<td>B₁ 6.78, B₂ 1.66</td>
<td>B₁ 3.8-9.76, B₂ 0.93-2.39</td>
<td>7.3, 2.1</td>
<td>0.3, 1.2</td>
<td>5.5, 1.6</td>
</tr>
<tr>
<td>Zearalenone in maize T2209</td>
<td>ZON 228</td>
<td>ZON 137-319</td>
<td>308.8</td>
<td>1.8</td>
<td>246.5</td>
</tr>
<tr>
<td>Fumonisin B₁ and B₂ in maize T 2208</td>
<td>FB₁ 879.1, FB₂ 305.9</td>
<td>FB₁ 432.1-1326.1, FB₂ 150.4-461.3</td>
<td>958.6</td>
<td>-</td>
<td>420.3, 447.1</td>
</tr>
<tr>
<td>Deoxynivalenol in wheat flour T2210**</td>
<td>DON 463</td>
<td>DON 297-630</td>
<td>340</td>
<td>-1.4</td>
<td>-----</td>
</tr>
</tbody>
</table>

* satisfactory Z<2

** reference material was purchased and arrived after the report of results was informed to coordinator. We await for instructions.
Table 2: FAPAS proficiency rounds results period 2003-2004

<table>
<thead>
<tr>
<th>Toxin/substrate/Identification</th>
<th>Assigned value µg/kg</th>
<th>Satisfactory range µg/kg</th>
<th>Our assigned value to FAPAS Round µg/kg</th>
<th>Z score*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zearalenone in maize T2209</td>
<td>ZON</td>
<td>ZON 137-319</td>
<td>308.8</td>
<td>18</td>
</tr>
<tr>
<td>Ochratoxin A in cereal T1721</td>
<td>OTA</td>
<td>OTA 3.70-9.52</td>
<td>6.7</td>
<td>0.1</td>
</tr>
<tr>
<td>Ochratoxin A in cereal T1724</td>
<td>OTA</td>
<td>OTA 13.10-33.69</td>
<td>16.5</td>
<td>-1.3</td>
</tr>
<tr>
<td>Deoxynivalenol in wheat flour T2210</td>
<td>DON</td>
<td>DON 297-630</td>
<td>340</td>
<td>-1.4</td>
</tr>
<tr>
<td>Aflatoxin M1 in milk T0461</td>
<td>M1</td>
<td>M1 0.05-0.13</td>
<td>&lt;0.5</td>
<td>---</td>
</tr>
<tr>
<td>Fumonisin B₁, B₂ in maize T2211</td>
<td>FB₁, FB₂</td>
<td>FB1 319-980</td>
<td>FB₁ 512</td>
<td>-0.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>FB2 113-347</td>
<td>FB₂ 278</td>
<td>0.8</td>
</tr>
</tbody>
</table>

* satisfactory Z<2

Participation in WP4

1- Participation in the constitution of HACCP team in Uruguay, within the activities of WP 4&5. LATU will analyse the samples which will be collected from field.

2- 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.

3- Mycotoxin Department (LATU) surveillance data year 2002 related to Aflatoxin B₁, B₂, G₁, G₂, Deoxynivalenol, Zearalenone and Fumonisins B₁ and B₂ was sent to WP4 coordinator.

4- 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 payied by FAO.

5- 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.

6- A third informal meeting (December 2003) was undertaken with the attendance of S. Stewart and J. Cea to evaluate the design survey, sample collection, number of samples and costs. We agree a special cost for the analysis to be done. In first instance 50 samples will be analysed.
Participation in meetings and conferences

- Project kick off meeting, Montevideo (Uruguay), 17-19 February 2003
- Progress meeting of WP 4 &5 in Buenos Aires (Argentina), 20-22 August 2003
- IV Congreso Latinoamericano de Micotoxicología, la Habana, Cuba, 24-26 de Septiembre 2003. An oral presentation of the MYCOTOX Project including description of goals, activities, participating countries and institutions was presented at this congress.

Papers and publications


**Contract number:** ICA4-CT-2002-10043

**FIRST ANNUAL REPORT:**
Covering period from 1 January to 31 December 2003

---

**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**
PARTNERSHIP

Shared-cost Rtd

Contract number: ICA4-CT-2002-10043

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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ANNEXES
Meeting reports
"KICK-OFF" Meeting
17 to 19 February 2003
Montevideo (Uruguay)
FINAL PROGRAM

SUNDAY FEBRUARY 16  
Arrival of participants to Montevideo

MONDAY FEBRUARY 17 MORNING  
INSTITUTIONAL ASPECTS

9h00 – 9h15  
Welcome of participants and presentation of PROCISUR  
Ing. Manuel Otero, representative of IICA in Uruguay

9h15 – 9h30  
(Auto)-presentation of participants

9h30 – 10h30  
Presentation of the MYCOTOX Project (activities and planning)  
Dr. Nadine Zakhia-Rozis, CIRAD (Montpellier, France)

10h30 – 11h00  
Coffee Break

11h00 – 12h00  
Presentation of objectives and expected outputs of the meeting  
Dr. Gérard Chuzel, CIRAD (Montpellier, France)

12h00 – 13h30  
Lunch

MONDAY FEBRUARY 17 AFTERNOON  
SCIENTIFIC ASPECTS

13h30 – 15h30  
Working groups for separate discussions  
Nadine Zakhia (Moderator)

Discussion and consensus on a “harmonised” and “standardised” dialogue between the project’s partners, mainly on the following topics:

1. Glossary on Food Quality and Safety Management Systems  
e.g. Risk assessment, food quality, control measures, supply chain, “filière”, agribusiness, food sector, etc

2. Sampling  
2.1. Samples for lab analysis (WP1, WP2 and WP3)  
2.2. Field sampling through the commodity flow (WP4, WP5 and WP6)

Main issues to be discussed:  
  a) sampling management (sample history & environment, identification number, etc)  
  b) data analysis (statistics, adapted softwares and methods, etc)
3. Methodological framework for the field work (mainly WP4, 5 and 6 and their connexion with WP 1, 2 and 3)

Discussion of the pluri-disciplinary approach that will be adopted in the project, i.e. the integration of Laboratory X Technical X Socio Economic Aspects in the maize and wheat supply chains.

15h30 – 16h00    **Coffee Break**

16h00 – 17h30    Plenary session: presentation of the conclusions of each working group (10’ each WG, followed by plenary discussion).
                  **Guy Henry (Moderator)**
                  **Martin Nagler & Bete Salay (rapporteurs)**

**TUESDAY FEBRUARY 18   PLANNING OF ACTIVITIES FOR 2003**

In this session, each WP leader will present a tentative planning which will be discussed by all partners

9h – 10h15    **Work Package 2**: Risk assessment of human exposure to ochratoxin A
               **Leader Dr. Ana Pacin, Universidad de Lujan, Argentina**
               **Rapporteurs: Mario Vega & Catherine Brabet**

10h15 – 10h30    **Coffee Break**

10h30 – 12h00    **Work Package 3**: Evaluation of milling procedures as potential CCPS
                  **Leader Dr. Silvia Resnik, Universidad de Buenos Aires, Argentina**
                  **Rapporteurs: Denis Bastianelli & Otniel da Silva**

12h00 – 13h30    **Lunch**

13h30 – 15h15    **Work Package 4**: Hazard analysis of mycotoxins and
                  **Work Package 5**: Identification and validation of mycotoxin control measures
                  **Leader Prof Ray Coker, NRI, UK. (Prof Coker will not attend the meeting. Dr. Martin Nagler will manage discussion on WP 4 & 5)**
                  **Rapporteurs: Guy Henry & Silvina Stewart**

15h15 – 15h30    **Coffee Break**

15h30 – 17h30    **Work Package 6**: Development of a Food Quality Management System
                  **Leader Dr. Ricardo Rodriguez, INTA, Argentina**
                  **Rapporteurs: Ricardo Madariaga & Nadine Zakhia**
WEDNESDAY FEBRUARY 19   

PLANNING OF ACTIVITIES FOR 2003

8h – 09h15       Work Package 1: Development and standardisation of effective analytical tools for mycotoxin determination in cereals and by-products
                 Leader Dr. Tania Barreto, EMBRAPA, Brazil
                 Rapporteurs: Eugenia Vargas & Jacqueline Cea

09h15 – 09h30     Coffee Break

09h30 – 10h15     Work Package 1 (continuation)

10h15 – 12h30     Separate Meetings (WP1 + WP2 + WP3) and (WP4 + WP5 + WP6) for joint activity planning

12h30 – 13h30     Lunch

13h30 – 15h30     GENERAL ASPECTS

1. **Project Management**: Steering Committee + Administrative Aspects + Guidelines and Deadlines for Scientific Reports and Cost Statements

2. **Quick Place Site**: a virtual space for sharing information and reports

3. **Presentation of the main meeting conclusions**

15h30 -          Meeting closure
First Meeting – 17 to 19 February 2003 – Montevideo (Uruguay)
WORK PACKAGE 1 CONCLUSIONS

- Essential to start with an inventory of the mycotoxins analysed in each WP1 laboratory and the available methods and techniques. Tania (EMBRAPA-Brazil) is committed to collect these data from WP1 team members and diffuse them for further exchanges.

- MAA (Brazil) has the capacity of preparing high amounts of homogeneous samples for WP1 team, along with carrying out global interlaboratory studies.

- Reminder: for interlaboratory studies, at least 12 laboratories are needed whereas for collaborative studies, at least 8 laboratories are needed.

- Standardisation of the chromatographic analyses should be performed both on FAPAS materials (used as international reference) and standard well defined materials (prepared by MAA-Brazil for instance and sent to all WP1 team). (Tania is committed to manage the information exchanges between WP1 members on these analytical aspects and help for joint problem solving).

- A global purchase of FAPAS materials seems interesting to get a discount price. (Nadine is committed to contact FAPAS office and if OK, order the test materials for all WP1 partners. The cost of those materials will be deduced from each institution budget and the materials delivered if possible directly to each partner in his own country).

- A special attention should be paid to the transport and “circulation” of contaminated materials before analysis. There is a need for knowledge of official rules and requested sanitary certificates for sample circulation between partners (All WP1 members are committed to get these information in their own countries).

- The Toximet procedure was presented by M. Nagler (NRI –UK). It is an approved method suitable for monitoring and control (Martin will sent to Nadine more information and papers on Toximet for downloading on Quick Place site).

- Need for the elaboration of a protocol for comparison of traditional chromatographic techniques and alternative ones (such as BCL, NIRS, Toximet). A joint reflection is to be launched among WP1 members on this (once chromatographic techniques are standardised).

- As often discussed and pointed out during the meeting, sampling and sample preparation before analysis are key points both for analytical aspects (WP1 + WP2 + WP3) and for “field” aspects (WP 4&5&6). Concerning the analytical aspects, Tania and Eugenia are committed to write a draft for sampling and sample preparation and make them circulate between WP 1&2&3 members.

- Various issues were also raised concerning I) the minimum amounts required as significant sub-samples for analysis, ii) the need or not for a Romer mill and iii) the preparation of a sample slurry before analysis. (Nadine is committed to get information from Ray Coker-NRI on those issues and diffuse it to the concerned partners).
The WP2 work is crucial as a few data is available on OTA occurrence in Latin America. The most concerned commodities are presently wheat and coffee. Food susceptible to be contaminated with OTA are mainly cereals and derivatives, milk and cheese, pig meat.

OTA determination is delicate in terms of quantification thresholds. Current methods have high limits of quantification, over 50 ppb. New methods are emerging that allow OTA quantification in wheat with low thresholds (about 5 ppb). There is currently no universal analytical method. The method used by Dr. Monica Olsen (scientific advisor of the MYCOTOX project) can not be applied by the WP2 partners because of unavailable infrastructure. The method they then chose for OTA determination is Scott method (Scott et al., 1998. Food Additives and Contaminants, 15, 555-562). This method is based on LC determination and confirmation through OTA methyl ester derivatives. It is easily implemented and WP2 partners do own the necessary infrastructure and equipment. However, it would be interesting to compare Scott method to that used by Monica by analysing the same samples (to be discussed with Monica).

WP2 activities will start with the OTA determination in blood samples coupled with a survey on the donor diet through questionnaires. A questionnaire form is already elaborated. In case of high OTA content in blood, a correlation will be found with the most consumed food identified from the survey. The incriminated foods will then be analysed for OTA content determination.

WP2 activities in the first project year will be dedicated to:

i) selection and standardization of OTA determination methodology in “informally collected” blood samples. More blood samples (around 200) will be collected in the second year. Later, samples will be collected from healthy volunteers. M. Nagler recommended to also collect, if possible, blood samples from people with kidney disease, to try correlating this disease with OTA contamination. OTA determination is planned on cereals once blood sample analysis is performed and results discussed.

ii) standardization of the methodology for dietary enquiries. Later, the survey will be broadened to include healthy volunteers and the questionnaire will be widened to take into account information on the global environment and way of life of the surveyed people (Consultation will be done with M. Olsen for comparison and validation of the methodology with the European one).

Meetings are planned between the concerned partners in Argentina (A. Pacin and S. Resnik) and Chile (M. Vega) for standardisation and implementation of the methodologies, according to the food habits and the context in each country.

According to the planned work, it is more realistic to expect the delivery at month 18 of a report on the exposure assessment to OTA in Argentina and Chile, which is a part of the foreseen deliverable D7. The rest of D7 or “Risk Characterization for OTA in Latin America South Cone” is expected at month 24.
First Meeting – 17 to 19 February 2003 – Montevideo (Uruguay)
WORK PACKAGE 3 CONCLUSIONS

- In Argentina, the derived products from wet wheat milling go for human consumption, whereas maize is exported. Strong linkages exist between the concerned WP3 partners and the grain industry.

- The first activities are focusing on the determination of DON in the fractions obtained through wet wheat milling. Samples are withdrawn from the industry and analysed to evaluate the repartition of DON in the different fractions: flour, starch, gluten and bran. The same work will be repeated in relation with different industries in Argentina and Chile.

- The same methodology will be repeated for determination of DON in the fractions obtained through dry wheat milling.

- DON content is measured by gas chromatography after extraction and clean up. The analytical methodology is put in place and standardised in collaboration between Argentina (Silvia and Ana) and Chile (Mario).

- The same work is to be done on fumonisin in maize (sampling of fractions outcoming from wet and dry milling processes and fumonisin determination).

- Previous works of A. Pacin showed the occurrence of contamination peaks with DON and fumonisin throughout the year, according to changes in climatic conditions. It would be interesting to publish these data which might serve as in input to WP 4&5&6.

- Nadine is committed to gather information and literature on what has been done in France upon the distribution of mycotoxins in the fractions outcoming from different milling processes.

- Nadine will make contact with INRA Montpellier where a pilot plant for grain dry milling is available. A joint collaboration MYCOTOX – INRA is to be discussed for a possible use of the pilot plant for dry milling trials. This would allow a complementary work at a pilot scale, with the possibility of changing process parameters such as extraction grade, particle size, etc. The outcoming fractions can be then analysed either by gas chromatography and NIRS (D. Bastianelli). Another interesting collaboration to be discussed with INRA is to test some grain decontamination processes.

- Another input to WP3: Denis proposes to study the impact of mycotoxin content in the flour and bran upon the animal feeding (weight, productivity, etc). The NIRS technique can also be used for this purpose.

- Nadine and Denis continue exchanging information with Silvia about the last points in order to elaborate a joint work plan to be done in France, in coherence with the WP3 framework.

- According to the planned work, it is more realistic to expect the delivery at month 6 of a report on the standardised procedures for wheat sampling during milling processes, which is a part of the foreseen deliverable D1. The rest of D1 i.e. the report on standardised procedures for maize sampling during milling processes is expected at month 10.
First Meeting – 17 to 19 February 2003 – Montevideo (Uruguay)
WORK PACKAGES 4&5&6 CONCLUSIONS

- Discussions were held for the global workplan of WP4&5&6 as these work packages are closely related.

- A presentation was made by M. Nagler on the HACCP (systematic approach to identify, analyse and control a hazard) and the experience NRI has on its application (in the Philippines). During discussions, it appeared that there are some differences between the Codex Alimentarius version of 1997 and the most recent one. (S. Resnik and M. Nagler check those differences and transmit information to all partners) (see separate files sent by Silvia on Codex in Quick Place, “General Information” Room, Codex Alimentarius Section).

- It was agreed by all participants that the HACCP-based approach to be used in WP 4&5 should be integrative and take into account the socio economic and organizational issues in the local contexts of the 4 concerned countries.

- There is a need for an inventory of the economic tools that could be integrated in the different steps of the HACCP method in order to elaborate a “global” tool for mycotoxin contamination control (Guy, Bete and Martin are committed to collaborate on this).

- The need for constitution of a global team in each country was pointed out. This team should include at least a HACCP specialist and a socio economist (to be identified) besides the personnel from the project partners involved in WP 4&5.

- A training on HACCP method should be planned among the project partners (including the specialists to be identified in each country). This training could be organized in Uruguay with the support of PROCISUR. J. Cea (LATU) is identified as a potential member of the WP 4&5 team in Uruguay as she followed a training on the HACCP application for myxotoxin control in UK (with M. Nagler).

- As preliminary steps for WP 4&5, are the need for identifying priorities in commodity / mycotoxin combinations and selecting some benchmark (pilot) geographical sites in each country.

- PROCISUR can bring support by supplying the partners with a report on a global project dealing with various food supply chains. Cecilia Gianoni is committed to help with this.

- Two guidelines will be elaborated on i) the description of wheat and maize agroindustrial supply chains and ii) literature review on the mycotoxin occurrence in these chains (Guy, Bete and Martin are committed to prepare these guidelines and send them to the participants).

the drafts elaborated in Montevideo during the specific meeting of WP 4&5&6 team are located in Quick Place, WP 4&5&6 rooms. These files are entitled i) Chain Selection Matrix WP4&5 Feb03 Montevideo ; ii) Framework WP 4&5&6 Year1 Feb03 Montevideo). The frame elaborated by Martin for bibliography review on mycotoxin occurrence in cereal chains and sent to partners is entitled Bibliography Review Nagler April03).
First Meeting – 17 to 19 February 2003 – Montevideo (Uruguay)
MAIN CONCLUSIONS

1. It is essential to mention the European funds of the project in all scientific papers or oral presentations dealing with MYCOTOX results. A standard acknowledgment will be prepared and put on Quick Place site (Nadine is committed to prepare an acknowledgment model and diffuse it to all partners).

2. The discussions between participants in WP 4&5&6 pointed out the need for:
   i) Identification of motivated socio economists in each country to carry out the studies on the organisation of whole grain channels, the relationships between chain actors and the priority given to mycotoxin contamination in each context
   ii) Rapid constitution of HACCP teams in the 4 countries and training on HACCP principles

R. Madariaga (INIA-Chile), S. Stewart (INIA-Uruguay) and R. Rodriguez (INTA-Argentina) are committed to identify these competencies in their countries.

G. Henry and E. Salay (Brazil) are committed to prepare guidelines for getting information on the grain channels and the occurrence of mycotoxins in the 4 countries and diffuse them to WP 4&5&6 partners.

R. Coker and M. Nagler are committed to prepare the operational work plan for WP 4&5 and share it with the concerned partners. A reflexion is to be rapidly launched to define the best way for organizing a training on HACCP method to the concerned partners in the 4 countries.

3. All partners are aware of the importance of having a common dialogue and vocabulary for an efficient collaboration through MYCOTOX project. The work into small groups during the meeting was very fruitful and led to a finalized glossary in 4 languages (French, English, Spanish and Portuguese). All suggestions and efforts for improving this glossary are welcome.

4. The participants pointed out the need for sharing information about the topic, either info on seminars and scientific events, publications, informal reports, etc. The Quick Place site will be used for this purpose. S. Resnik proposed to share with WP 1&2&3 partners the scientific references and journals available in her laboratory, downloading them on Quick Place at regular periods.

5. Once the analytical methods standardised using reference materials, the WP1 laboratories will be able to offer a joint capacity for analysing the samples which will be collected in the field by WP 4&5&6 teams. This offer will clearly notify which type of mycotoxin and number of samples each laboratory is able to analyse, at a “reasonable” non-profit cost.

6. The WP 1&2&3 participants agreed to participate in the forthcoming IV Latin American Congress on Mycotoxicology to be held in Cuba in September 2003 and to present a joint poster on the project. T. Correa is committed to prepare a summary draft and make it circulate among WP1 team before sending to the organizing committee by May 31.
Progress meeting for WP 4&5
20-22 August 2003
Buenos Aires (Argentina)
WORK PROGRAMMES 4 & 5  
FIRST CO-ORDINATION MEETING  
INTA – Argentina; 20 – 22 August 2003

PROGRAMME

TUESDAY 19 AUGUST  
Arrival of participants

WEDNESDAY 20 AUGUST MORNING

0900 – 0930  Welcome of participants and presentation of INTA  
*Ricardo Rodriguez, INTA-Argentina*

0930 - 1000  Introduction to meeting  
*Dr Nadine Zakhia-Rozis, CIRAD, Montpellier, France*

1000 - 1030  Aims of meeting  
*Prof Raymond Coker, NRI/UoG, Chatham, UK*

1030 - 1100  Coffee Break

1100 - 1730  Summary of Progress:  
- HACCP teams & training  
- Collection & collation of mycotoxin occurrence data  
  - from literature  
  - from other sources  
- Need for further occurrence data  
- *Deliverable D2*, WP4 (Report documenting occurrence data; 31 July 03)  
- Hazard Analysis  
- Discussion

1100 - 1200  Brazil

1200 - 1300  Uruguay

1300 - 1430  Lunch

WEDNESDAY 20 AUGUST - AFTERNOON

Summary of Progress (continued)

1430 - 1530  Chile

1530 - 1545  *Coffee Break*

1545 - 1645  Argentina

1645 - 1730  Conclusions  
*Martin Nagler, NRI/UoG, Chatham, UK*
THURSDAY 21 AUGUST - MORNING

0900 - 1300  Next Steps (Issues, problems, opportunities, exchange of ideas): Views of individual HACCP Teams and general discussions

0900 - 0915  Introduction
Prof Raymond Coker, NRI/UoG, Chatham, UK

Work Package 4

0915 – 1115  Group Discussions : HACCP Team
The following issues will be discussed by the individual HACCP teams

0915 - 1000  Hazard Analysis
- Deliverable D3, WP4 (Description of hazard analysis & justification of selected commodity-mycotoxin combination; 31 August 03)

1000 - 1115  Verification of Commodity Flow Diagram (CFD)
- Deliverable D6, WP4 (Document verifying CFDs; 31 December 03)

1115 - 1130  Coffee Break

1130 - 1200  Determination of steps where mycotoxin hazard requires control (surveillance performed where necessary)
- Surveillance & sampling plans
- Sample preparation methods
- Performance of analyses
- Assignment of responsibilities
- Deliverable D14, WP4 (Description of steps where mycotoxin hazard(s) originate; 1 April 04)
  Including presentations of S. Resnik (University of Buenos Aires, Argentina) and A. Pacin (University of Lujan, Argentina)

Work Package 5

1200 - 1230  Understanding of Stakeholders
Lead by Guy Henry, CIRAD, Campinas, Brazil
- Deliverable D9, WP5 (Description of socio-economic constraints & opportunities affecting implementation of control measures; 30 June 04)
  Presentation of A. Engler (INIA Chile) on the methodological tools in socioeconomics to be adopted and adapted

1230 - 1300  Development & validation of control measures
- Deliverable D13, WP5 (Reports describing control measures & CCPs; 31 December 04)

1300 – 1400  Lunch

THURSDAY 21 AUGUST - AFTERNOON

1400 - 1430  Ochratoxin A & preharvest control of Fusarium toxins – the European experience

1430 - 1615  Plenary Session: Reports of HACCP Team Rapporteurs & General Discussion
1615 – 1630 Coffee Break

1630 - 1645 Introduction to field visit
   Ricardo Rodriguez, INTA-Argentina

1645 - 1730 Summary of key issues & agreed actions (including Progress Review Tables)
   Dr Nadine Zakhia, CIRAD, Montpellier, France

FRIDAY 22 AUGUST

FIELD VISIT
INCO-DEV MYCOTOX PROJECT
Work Packages 4 & 5 – First Progress Meeting
INTA, Buenos Aires, Argentina; 20-22 August 2003

<table>
<thead>
<tr>
<th>Those present:</th>
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<tr>
<td><strong>Argentina</strong></td>
<td>Ricardo Rodriguez (Team Leader)</td>
<td>INTA</td>
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<td></td>
<td>Fernando Carduza</td>
<td>INTA</td>
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<td></td>
<td>Marcelo Masana</td>
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<td>Norma Pensel</td>
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<td></td>
<td>Alejandra Ricca</td>
<td>INTA</td>
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<td></td>
<td>Guillermo Sanchez</td>
<td>INTA</td>
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<td></td>
<td>Ana Pacin</td>
<td>UNLU</td>
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<td></td>
<td>Silvia Resnik</td>
<td>UBA</td>
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<td><strong>Brazil</strong></td>
<td>Elisabete Salay (Team Leader)</td>
<td>UniCamp</td>
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<td></td>
<td>Guy Henry</td>
<td>CIRAD (UniCamp)</td>
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<td></td>
<td>Cathy Brabet</td>
<td>CIRAD</td>
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<td></td>
<td>Otniel Freitas</td>
<td>EMBRAPA</td>
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<td><strong>Chile</strong></td>
<td>Ricardo Madariaga (Team Leader)</td>
<td>INIA</td>
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<td></td>
<td>Alejandra Engler</td>
<td>INIA</td>
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<td>Mario Vega</td>
<td>UDEC</td>
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<td>Ricardo Villegas</td>
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<td><strong>Uruguay</strong></td>
<td>Silvina Stewart</td>
<td>INIA</td>
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<td>Gonzalo Gutierrez</td>
<td>INIA</td>
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<td></td>
<td>Cecilia Gianoni</td>
<td>PROCISUR</td>
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<td><strong>France</strong></td>
<td>Nadine Zakhia</td>
<td>CIRAD</td>
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<td><strong>UK</strong></td>
<td>Raymond Coker</td>
<td>NRI/UoG</td>
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<td>Martin Nagler</td>
<td>NRI/UoG</td>
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<tr>
<td><strong>Sweden</strong></td>
<td>Monica Olsen</td>
<td>NFA</td>
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<tr>
<td>Country</td>
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<td>Comments</td>
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| **Argentina** | **Progress at time of meeting** HACCP Team | • Team assembled primarily from within INTA  
• Inputs from Silvia Resnik (UBA) & Ana Pacin (UNLU) will add value to the team  
• Socio-economist still required  
• Industrial team members required  |
| | **Deliverable D2** (Report documenting occurrence data, 31/7/03) | Not yet completed  |
| | **Deliverable D3** (Description of hazard analysis & justification of selected mycotoxin-commodity combination, 31/8/03) | • Wheat selected as commodity  
• Mycotoxins to be confirmed, but will include DON  |
| **Next Steps** | **Deliverable D2** (Report documenting occurrence data, 31/7/03) | Complete review of literature & collate data  |
| | **Deliverable D3** (Description of hazard analysis & justification of selected mycotoxin-commodity combination, 31/8/03) | • Deoxynivalenol (DON) considered to be a high risk (health impact)  
• Zearalenone considered to be a medium risk  
• Confirmed that wheat selected as commodity:  
  - Pre-harvest contamination very important  
  - Thus, pre-harvest control measures required  
• High levels of fumonisins B₁ & B₂ in corn (economic impact)  |
| | **Deliverable D6** (Document verifying CFDs, 31/12/03) | To be actioned (Ana Pacin & Silvia Resnik to assist with identification of mills)  |
| **Martin Nagler’s Visit Report** | HACCP Team | • Inputs from socio-economist & industrial partners required  
• Marcelo Masana will be attending HACCP course in Cuba, and will play a key role in the HACCP team  |
| | **Deliverable D2** (Report documenting occurrence data, 31/7/03) | • Importance of completing review of literature and collating data emphasised  
(Collated data subsequently sent to Prof. Coker)  |
| | **Deliverable D6** (Document verifying CFDs, 31/12/03) | • Preliminary CFD drafted for production of wheat flour in Argentina  
• Confirmation & validation of CFD to be completed  |
## Brazil

### Progress at time of meeting

<table>
<thead>
<tr>
<th>Deliverable D2 (Report documenting occurrence data, 31/7/03)</th>
<th>Very comprehensive bibliography of mycotoxin occurrence data assembled</th>
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</table>
| Deliverable D3 (Description of hazard analysis & justification of selected mycotoxin-commodity combination, 31/8/03) | Hazard analysis in progress:  
- *Corn* selected as commodity (used as poultry feed)  
- Wheat largely imported (ca 70%) from 'good' regions in Argentina  
- *Aflatoxins, fumonisins, deoxynivalenol & zearalenone* identified as most important toxins  
- Parana State identified as area to be studied  
- See Powerpoint presentation on MYCOTOX website (Mycotoxins in Brazilian grain) |
| Deliverable D6 (Document verifying CFDs, 31/12/03) | To be actioned |
| Deliverable D9 (Description of socio-economic constraints & opportunities affecting implementation of control measures, 30/6/04) | Studies underway |

### Next Steps

<table>
<thead>
<tr>
<th>Deliverable D2 (Report documenting occurrence data, 31/7/03)</th>
<th>Report to be completed (subsequently completed)</th>
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<td>Deliverable D3 (Description of hazard analysis &amp; justification of selected mycotoxin-commodity combination, 31/8/03)</td>
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- *Ochratoxin A* added to selected mycotoxins  
- Sampling/sample preparation implications of involvement of aflatoxins & ochratoxin A to be considered – both these toxins require large samples (e.g. 10kg corn) |
| Deliverable D6 (Document verifying CFDs, 31/12/03) | To be actioned |
| Deliverable D9 (Description of socio-economic constraints & opportunities affecting implementation of control measures, 30/6/04) |  
- Workshop to be organised at Campinas, in order to define the appropriate methodology for the successful integration of technological & socio-economic issues  
  *(Workshop will be held 1-2 December 2003)*  
- See Powerpoint presentation on MYCOTOX website (Technical & Socio-economic Integration) |

### Martin Nagler's Visit Report

| HACCP Team |  
- Strong HACCP Team assembled  
- Agronomist required  
- Dr Valedicir Dalpasquale, post-harvest corn specialist (Univ. Maringa)  
- Alexandria Alves, agronomist (Univ. Maringa) |
**Chile**

**Progress at time of meeting**

**HACCP Team**

- Balanced HACCP team assembled, but veterinary specialist may be added
- Private sector collaborators have been identified:
  - Baer Breeder & Seed Producer
  - Kunsmann Group – Collico Mill

**Deliverable D2** (Report documenting occurrence data, 31/7/03)

- Completed

**Deliverable D3** (Description of hazard analysis & justification of selected mycotoxin-commodity combination, 31/8/03)

- Wheat-based salmon feed selected as commodity
- Mycotoxin(s) still to be selected (no clear mycotoxin problem)
- Agroclimatic conditions differ to those in Brazil, Argentina & Uruguay
- F. graminearum group II has not been observed in Chile (but trichothecene mycotoxins produced under lab. conditions)
- See Powerpoint presentation on MYCOTOX website (Summary of Progress in Chile)

**Deliverable D6** (Document verifying CFDs, 31/12/03)

- Preliminary studies undertaken

**Next Steps**

**Deliverable D3** (Description of hazard analysis & justification of selected mycotoxin-commodity combination, 31/8/03)

- Confirm nature of mycotoxin-commodity combination

**Deliverable D6** (Document verifying CFDs, 31/12/03)

- Activities to be continued

**Martin Nagler’s Visit Report**

**HACCP Team**

- No additional comments

- Mycotoxin-commodity combination still to be confirmed
<table>
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<th>Deliverable</th>
<th>Description</th>
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| D6          | **Preliminary CFD produced for production of wheat flour in Chile**  
**Confirmation & validation of CFD to be completed** |
| D13         | Potential control measures discussed, including:  
- Beneficial characteristics of some wheat varieties  
  - Kumpa & Dollinco with low density spikes dried more easily  
  - Drooping ears dried more easily  
  - Darker grains less susceptible to fungal attack (presence of fungicidal polyphenols/tannins?)  
- Clean crops with air density system after harvest (also reduces moisture content by up to 2%)  
- Biological control with atoxigenic strains of *Fusarium*  
- Minimum tillage (involving removal of surface debris but no turning of soil)  
- Stubble burning in Chile is a major issue – most of wheat & corn stubble is burnt. The absence of FHB could be related to this environmentally unfriendly practice. |

**Uruguay**

**Progress at time of meeting**

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<th>Deliverable</th>
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| **D2**      | **Wheat** selected as commodity  
**Deoxynivalenol** (DON) selected as highly problematic mycotoxin  
Increased *Fusarium* contamination linked to zero tillage, high rainfall during growth period & above average temps.  
Post-harvest drying is also a problem because of continued use of wood-fired driers  
See Powerpoint presentation on MYCOTOX website (Summary of Progress in Uruguay) |

**Next Steps**

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<th>Description</th>
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| **D6**      | Preliminary studies performed  
Activities to be continued, possibly involving a workshop that includes carefully targeted, key players |
| Deliverable D14 (Description of steps where mycotoxin hazard originates) | Analytical infrastructure in place:  
- INIA has purchased a Romer subsampling mill  
- Quantitative analyses will be performed at LATU |
|---|---|
| Martin Nagler’s Visit Report | Deliverable D6 (Document verifying CFDs, 31/12/03) | Preliminary CFD produced for production of wheat flour in Chile  
Confirmed & validation of CFD to be completed |
| Deliverable D13 (Reports describing control measures & CCPs, 31/12/04) | Potential control measures discussed, including:  
- Use of most effective fungicides (Carambar or Folicur)  
- New fungicide – Prothioconazole – being evaluated  
- Most cost effective to spray once with fungicide when flowers first appear  
- Spray fungicide from ground level  
- Practice appropriate crop rotation  
- Resistant variety of wheat (Chinese/Mexican crossed cultivar) is close to release |
| Monica Olsen’s presentation | Control of ochratoxin A: Monica Olsen presented the findings of an EU-funded project that studied the occurrence & control of ochratoxin A in Europe. This particular project was one of several studies, including the control of Fusarium toxins in wheat, that were completed under the Mycotoxin Prevention Cluster. | See www.mycotoxin-prevention.com  
See www.ifa-tulln.ac.at/fucomyr/  
See Powerpoint presentation on MYCOTOX website (Control of ochratoxin A in Europe)  
Key points:  
- *Penicillium verrucosum* is the only ochratoxin A (OA)-producing mould on European cereals  
- *P. verrucosum* is not present in the field  
Potential pre-requisites & control measures:  
- GAP:  
  - Keep drying & storage facilities clean  
- CCPs:  
  - Dry rapidly after harvest  
  - Appropriate storage conditions  
  - *P. verrucosum* very competitive at 15°C & aw 0.90 to 0.95 |
| Field Visit | Experimental plots at San Antonio de Areco | Discussions with agronomists reinforced the impact of agroclimatic conditions on the occurrence of *Fusarium* toxins on wheat; e.g. deficit of rain before flowering but excessive rain during flowering is problematic |

*Outstanding actions are in **bold** font*
INTERNAL REGIONAL WORKSHOP
“Formulation of socio economic approach, methods and instruments”
1-2 December 2003
Campinas (Brasil)
### Workshop Program

<table>
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<tr>
<th>Time</th>
<th>Theme</th>
<th>Presenter / Moderator</th>
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<tr>
<td><strong>MONDAY - 1 DEC</strong></td>
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<tr>
<td>08:30-09:00</td>
<td>Welcome + objectives</td>
<td>Salay + Henry</td>
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<tr>
<td>09:00-09:20</td>
<td>Mycotox project’s socio-econ research questions + discussion</td>
<td>G. Henry</td>
</tr>
<tr>
<td>09:20-09:35</td>
<td>HACCP method and implications when applied to a chain + questions</td>
<td>E. Salay</td>
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<tr>
<td>09:35-10:30</td>
<td>Presentation of innovative approaches &amp; methods</td>
<td>E. Farina</td>
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<tr>
<td>10:30-11:00</td>
<td>Café</td>
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<tr>
<td>11:00-12:30</td>
<td>Discussion + First draft of hypotheses</td>
<td>B. Daviron</td>
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<tr>
<td>12:30-13:30</td>
<td>Lunch – sandwich at Hotel Premium</td>
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<tr>
<td>13:30-13:45</td>
<td>Organization/characterization of chain actors – Brasil</td>
<td>E. Salay</td>
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<tr>
<td>13:45-14:00</td>
<td>Organization/characterization of chain actors – Chile</td>
<td>A. Engler</td>
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<td>14:00-14:15</td>
<td>Organization/characterization of chain actors – Uruguay</td>
<td>G. Guttiérez</td>
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<td>14:15-14:30</td>
<td>Organization/characterization of chain actors – Argentina</td>
<td>D. Iglesias</td>
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<tr>
<td>14:30-15:00</td>
<td>Clarifications &amp; questions</td>
<td>G. Henry</td>
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<td>15:00-15:20</td>
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<tr>
<td>15:20-16:20</td>
<td>Building a consensus on selected economic theory and approach + Hypotheses validated</td>
<td>B. Daviron</td>
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<tr>
<td>16:20-17:30</td>
<td>Implications for appropriate methods and instruments</td>
<td>B. Daviron</td>
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<td>20:00</td>
<td>Caipirinhas + bifes at Gloria &amp; Guy’s</td>
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<td>(Ave Paulo Castro Puppo Nogueira, 600, Novo Campinas, Fone: 3251 1303)</td>
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<td><strong>TUESDAY – 2 DEC</strong></td>
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<tr>
<td>08:30-11:00</td>
<td>Adaptation of proposed methods + instruments to the country, chain &amp; actor conditions</td>
<td>Daviron + Henry</td>
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<tr>
<td>11:00-12:00</td>
<td><strong>Socio-economic elements for CFD construction</strong></td>
<td>G. Henry</td>
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<tr>
<td></td>
<td>• Example of the Brazilian corn sub-chain</td>
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<td></td>
<td>• Group work to construct wheat chains</td>
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<tr>
<td>12:00 – 13:00</td>
<td>Any other business</td>
<td>G. Henry</td>
</tr>
<tr>
<td>13:00</td>
<td>Closing of workshop</td>
<td>E. Salay</td>
</tr>
<tr>
<td>13:15</td>
<td>Lunch</td>
<td></td>
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</tbody>
</table>
Workshop participants

Ing. Alexandre Florindo, UEM, Maringa-PR, Brasil
Daniel Iglesias, PhD INTA-La Pampa, Argentina
Ing. Gonzalo Guittiérez, UdelAR, Montevideo, Uruguay
Alejandra Engler, PhD INIA- Quilamapu, Chillan, Chile
Dr. Cathy Brabet, Cirad-Amis/ProsPER, Campinas, Brasil
Guy Henry, PhD Cirad-Amis/ProsPER, Campinas, Brasil
Prof. Dr. Elisabete Salay, Unicamp-Nepa, Campinas, Brasil
Valeria Tolentino, MSc Unicamp-Fea (doctoral student E. Salay), Brasil
Katia Martini Rodrigues Unicamp-Fea (doctoral student, E. Salay), Brasil

Invited experts

Prof. Dr. Elizabeth Farina, USP-Fea/Pensa, Sao Paulo, Brasil
Dr. Benoit Daviron, CIRAD-Amis/SEA, Montpellier, France
SUMMARY OF PRINCIPAL RESULTS

This 2-day workshop had the following objectives. (i) Present the challenges and formulate socio-economic research questions in the Mycotox project, (ii) Present innovative socio-economic theory and approaches that may be appropriate to answer the research questions, (iii) Formulate appropriate methods and instruments.

The workshop was attended by 8 Mycotox project partners from the four Southern Cone countries, complemented by another two Brazilian researchers of the subject, and moderated by two invited experts, from Brazil and France.

The principal workshop results are summarized as follows :

Socio-economic research questions discussed & formulated
1. Which are the instruments (i) economic incentives, (ii) regulations, (iii) collective actions, to promote the adoption of QAS (incl. HACCP)?
2. Which are the consequences and/or necessary conditions regarding the SAG organization?
3. Does the full implementation of a HACCP system in the chain, require a high degree of vertical integration, or 3rd party certification?
4. What is the role of public institutions (legislation, enforcement mechanisms, …) to promote….?

Related hypotheses to the research questions
1. There is a problem recognised by all actors of the system (consensus).
2. This problem is a sufficient condition for adoption. An actor is key, when participating in a productive phase structured in an oligopoly or oligopsony.
3. Key to adoption is the condition that economic benefits are greater than the costs for the key actors.
4. Benefits are distributed in an equal way
5. An interprofessional organization is needed to manage potential problems (benefit distribution for example). Certification, favors concentration, due to economies of scale.
7. Adoption favors stronger coordination of actors in the chain.
8. Certification appears as a solution when there are many buyers (reduces transactions costs by reducing info costs)
9. If one cannot measure contamination in products, certification of the production process could be an appropriate solution.
10. Adoption of HACCP favors the concentration of actors (due to scale economies and the incapacity of small enterprises).

Conditions:
From the hypotheses the group derived a set of necessary conditions to facilitate the implementation of QAS (including HACCP) that are going to be tested or justified in the socio-economic studies. The majority of the conditions are directly related to benefits and costs of different key elements. The estimation / calculation of these costs and benefits are the subject of several proposed studies, either as desk studies, analysis with secondary or primary data.
Summary of approach and methods by identified conditions

1. Benefits greater than costs **ADDRESS IN PROPOSED METHODS**

2. There is no equality problem with Benedit distribution **ADDRESS IN PROPOSED METHODS**

3. Problem is recognized by all key actors. **ADDRESS IN PROPOSED METHODS**

4. The problem of recognition by all actor as a sufficient condition for adoptrion. **FURTHER REFLEXION NEEDED**

5. Greater coordination in the chain favors adoption of HACCP systems **DESK STUDY**

6. Certification as an appropriate solution when there are many buyers **DESK STUDY**

7. When measurement of product contamination is imposible, process certification appears as an appropriate solution. **DESK STUDY**

8. There exists a causalita between HACCP and chain concentration? **DESK STUDY**

Division of work

Given that the meeting identified a set of activities (methods and studies to be checked, or further reflections) to be developed in terms of additional key information needed, the workload of this was divided amongst the workshop participants. A deadline of end of 2003, was set for submission of first results and/or drafts.

Conclusion

While most time was spent on the specific workshop objectives, it was also seen as an opportunity to have a discussion on the socio-economic inputs in the traditional CFD (commodity flow diagram), as described in the formal HACCP approach. The discussion resulted in some first drafts. One of which is included/attached as Annex 3.

In conclusion, it can be said, that the workshop did not produce ready-to-go solutions. However, the workshop succeeded in generating in-depth reflections and discussions amongst the participants. In addition, the workshop generated a set of key methodological entry points and conditions that represent concrete starting points for the socio-economic research agenda in 2004.

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Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT:
Covering period from 1 January to 31 December 2003

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

Contract number: ICA4-CT-2002-10043

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Publications in peer-reviewed scientifics journals

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ANNEXES

Papers and publications

Oral presentations in conferences and congress
MICOTOXINAS EN NUESTROS PAÍSES

Autores: Dra. Silvia Resnik (1,2) – Dra. Ana Pacin (1,3)
(1) Comisión de Investigaciones Científicas de la Provincia de Buenos Aires CIC
(2) Facultad de Ciencias Exactas y Naturales de la Universidad de Buenos Aires
(3) Centro de Investigación en Micotoxinas Universidad Nacional de Luján

El punto de partida de un trabajo serio es un buen diagnóstico, por esta razón y recorriendo conceptos conocidos, volvemos a repetir que las micotoxinas son sustancias químicas, que contaminan materias primas y alimentos, a partir de la colonización de hongos toxicogénicos, que cuando las condiciones ambientales son propicias, son capaces de sintetizar metabolitos secundarios tóxicos, denominados micotoxinas.

¿Cuáles son las características de las micotoxinas?

Estos compuestos son tóxicos, y en cantidades pequeñas, y justamente por esta razón se hace necesario su control.

Son contaminantes naturales de los alimentos, y las medidas de prevención son hasta el momento insuficientes, con el agravante que contaminan materias primas destinadas a elaborar alimentos para la población humana, y animal, como maíz, soja, sorgo, arroz, trigo, etc.

La distribución de la contaminación no es homogénea, y además depende de la matriz alimenticia y de la micotoxina

Las matrices alimenticias sobre las que colonizan los hongos toxicogénicos son muy variadas y la contaminación puede iniciarse y/o incrementarse en diferentes etapas:

- Cultivo, donde se debería tener en cuenta, prácticas agronómicas, regiones geográficas, híbridos, climas,
- Manejo post-cosecha, donde deberían tenerse en cuenta inóculo inicial, tipo de almacenamiento, tiempo de almacenado, temperatura, humedad
- Acondicionamiento de la matriz, donde debería tenerse en cuenta, la separación para la molienda, las moliendas húmeda y seca, el perlado
- Procesos, donde es necesario considerar las múltiples formas de cocción de matrices: con o sin fermentación, horneado microonda, horneado a gas, eléctrico, frito y finalmente es necesario considerar las implicancias económicas derivadas de la comercialización y/o regulaciones, que perjudican a los compradores (mayor exposición a la intoxicación) y a los vendedores (menor ganancia).

Estas son algunas características de la contaminación por micotoxinas, a lo que tenemos que agregar, la peculiaridad en cuanto a la metodología analítica, que tantos encuentros, simposios, jornadas y estudios colaborativos ha merecido, y aquí hay dos puntos:

El muestreo, referido a la distribución, pero al decir del profesor Whitaker, es el 80% del resultado.
Y el método, fisicoquímico, cuando está destinado a la investigación, que es costoso (equipos, solventes, personal entrenado), la repetitibilidad, la dispersión de la información,

Las micotoxinas no son fáciles. Las características mencionadas, a las que se les podrían agregar otras, como la dificultad en los diagnósticos de intoxicaciones o la versatilidad de las demandas de compradores, hacen a la problemática de la micotoxinas de difícil manejo.

América Latina y el Caribe han trabajado en forma despareja con respecto a la contaminación, sin embargo existe un crecimiento en esta década, que posibilita expresar que actualmente existen dos grandes limitantes: la económica y la científica.
La económica, por todos conocida, entiendo que si todos tuviéramos personal bien pago, entrenado, equipos suficientes, sería factible en nuestros países llegar a un mejor diagnóstico de la contaminación por micotoxinas.

La científica, en cambio, está relacionada con:
La inexistencia de planes de muestreo, absolutamente necesario, ya que son diferentes para cada matriz alimenticia y para micotoxinas
Las metodologías analíticas de detección y cuantificación.
Los materiales de referencia
La necesidad de utilizar los estudios toxicológicos para defender los valores que se quieren imponer para las regulaciones y/o la legislación.
El desconocimiento de la exposición de las poblaciones de los países a estas sustancias
El mal conocimiento, por falta de rigor científico en el diseño de los estudios en animales, que son utilizados como punto de partida para establecer los límites de ingesta admisible en el hombre.
El desconocimiento, por falta de rigor científico en el diseño de los estudios en animales, del metabolismo de estas sustancias en animales de producción, cuando sus alimentos pueden tener un alto porcentaje de una materia prima con elevada contaminación.

Que se propone

Muestreo, llevar a cabo estudios de variabilidad de la distribución para obtener los parámetros necesarios para el diseño de muestreos representativos de la contaminación de un lote.

Metodología, estandarizar un grupo de metodologías que sean simples, que permitan analizar en forma económicas, reproducibles, con adecuada recuperación para ser utilizadas para trabajos de rutina y de “screening”.

Metodología, estandarizar un grupo de metodologías que permitan analizar en forma confiable, reproducibles, con adecuada recuperación para ser utilizadas para trabajos de investigación.

Mejorar, y organizar estudios colaborativos sobre metodologías de referencia accesibles de las micotoxinas, que se definan como más necesarias en aquellos matrices alimenticias que sean de interés para la Región.
Enfatizar que los métodos inmunológicos, salvo Cuba, se fabrican fuera de nuestros países o con materias primas provenientes de otros países lo que los hace caros, además, que algunos de ello no cumplen con las exigencias actuales de reproducibilidad, confiabilidad o de exactitud necesarias para preservar la salud de la población o disminuir las pérdidas económicas en la producción pecuaria.

Incrementar los estudios sobre las modificaciones que cualquier etapa del proceso de elaboración de alimentos, pueda hacer sobre el contenido final de micotoxinas.

Con respecto a las regulaciones y/o la legislación, destacar la importancia de intervenir, no solo ser depositarios de la legislación elaborada en otros países, que no está basada en datos toxicológicos.

Estimar la exposición de la población, para poder evaluar el riesgo y trabajar sobre las micotoxinas y los alimentos que pueden afectar en orden prioritario a humanos y animales.

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Proyecto MYCOTOX N° ICA4-CT-2002-10043 (2003-2005) financiado por la Comision Europea (INCO-DEV)

REDUCCIÓN DE LA CONTAMINACIÓN POR FUMONISINAS DURANTE LA LIMPIEZA DE MAÍZ

Autores: Pacin Ana M (*1,2) Taglieri Daniela (1,2), Cano Gabriela (1,2), Resnik Silvia L. (2,3),
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RESUMEN

El maíz es una matriz alimenticia susceptible a la contaminación por micotoxinas, en especial aflatoxinas y fumonisinas, ya que la contaminación es natural y depende de las condiciones ambientales (meteorológicas u almacenamiento) por lo que es necesario llevar a la práctica toda medida que permita disminuir dicha contaminación inicial.

El objeto del presente trabajo fue evaluar el efecto de la utilización de zaranda, paso previo a la molienda del maíz, con mallas de diferentes calibres, sobre la contaminación de fumonisinas en maíz.

Se procesaron a través de una malla de 6.5 mm, 13.452 toneladas de maíz, contenidas en 16 silos distintos, que se analizaron separadamente. El total de muestras analizadas fue 45 (maíz antes de pasar por la zaranda, maíz limpio y descarte). En el caso de malla 7 se procesaron 5553 toneladas (7 silos, 21 muestras).

Cuando se utilizó zaranda con malla calibre 6.5 mm, la mediana del maíz limpio fue 1144 μg/kg y la mediana del descarte fue de 10471 μg/kg.

Ambas mallas permiten una importante reducción de la contaminación, pero la malla 6.5 mm "limpia" casi el doble que la malla de 7 mm, ya que la relación entre descarte y maíz limpio es de 17.25 para el primer caso y 9.2 para el segundo.
CHILE, MICOTOXINAS, GLOBALIZACIÓN. ¿ LAS MICOTOXINAS SON UN PROBLEMA EMERGENTE ?

Autores: Mario Vega H., Roberto Saelzer F., Gísela Ríos G., Erika Herlitz B., Corina Bastias T.
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RESUMEN
En Chile, no existe data estadística que de cuenta de episodios de micotoxicosis, tampoco del grado de contaminación de alimentos y piensos con micotoxinas. No existe conciencia clara en los organismos de control, ni infraestructura analítica en laboratorios públicos, que permitan dimensionar si constituye o no un problema sanitario. La situación cambia en algunos sectores, como el avícola y la piscicultura, que cuentan con laboratorios de control o demandan a las universidades, control micotoxicológico de sus insumos y productos. Con la globalización de la economía y la suscripción de diversos tratados de libre comercio, que facilitan el libre acceso de granos y otros insumos utilizados por este sector, se hace indispensable el contar con el apoyo analítico de control con el fin de dimensionar el problema y establecer los procedimientos preventivos que sean necesarios.

La Universidad de Concepción, a través del Departamento de Bromatología, Nutrición y Dietética, presta una activa colaboración al sector productivo en el análisis de micotoxinas en piensos y granos básicos. El presente trabajo, informa de los resultados de 726 análisis realizados durante los años 2001, 2002 y 2003 en diversas matrices (maíz, trigo, centeno, soya, triticale, sorgo, avena, lupino, gluten, semilla de algodón, alimentos balanceados para ganado, aves y peces) dando una visión parcial de la situación país respecto a esta problemática.

La metodología aplicada se basa en la infraestructura analítica disponible, preferentemente cromatografía planar instrumental, para el screening y cuantificación de las siguientes micotoxinas: Aflatoxina B1, B2, G1, G2, Acido Penicílico, Esterigmatocistina, Ocratoxina A, Citrinina, Zearalenoana, DON, DAS, Toxina T-2 y HT-2.

Screening de micotoxinas: 234 muestras, 1 muestra positiva a Aflatoxinas (torta de maní)
Aflatoxinas: 260 muestras, una muestra positiva (torta de maní)
Fusariotoxinas 232 muestras: 9 muestras positivas a DON (maíz)
12 muestras positivas a ZEA (sorgo)
1 muestra positiva a Toxina T-2 (maíz)

Las Aflatoxinas presentaron contaminaciones entre 1,3 y 17 ppb (µg/Kg). La muestras con DON positivo variaron entre 0,12 y 16 ppm (mg/Kg) y las positivas con Zearalenona fluctuaron entre 4,7 y 17,8 ppm (mg/Kg). Los resultados obtenidos, si bien parciales, permiten concluir la necesidad de efectuar un mayor números de análisis prospectivos con la finalidad de establecer normativas y proyectar en mejor forma al país en un mundo con comercio globalizado.
Proyecto MYCOTOX N° ICA4-CT-2002-10043 (2003-2005) financiado por la Comisión Europea (INCO-DEV)

SISTEMA DE GERENCIAMIENTO DE LA CALIDAD PARA EL CONTROL DE MICOTOXINAS EN LAS CADENAS DE PRODUCCIÓN Y PROCESAMIENTO DE CEREALES DE LOS PAÍSES DE EL CONO SUR(*)

Autores: Corrêa, T.B.S.¹; Vargas, E. A.²; Cea, J.³; Vega, M.⁴; Resnik, S.⁵; Souza, M.L.M.¹; Freitas-Silva, O.¹; Zakhia, N.⁶

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RESUMEN

El Cono Sur, con apoyo de la Unión Europea, inicia la implementación de Proyecto de Cooperación técnica para el Desarrollo de Sistema de Gerenciamiento de Calidad de Alimentos para Control de Micotoxinas en las Cadenas de Producción y Procesamiento de Cereales (Maíz y Trigo) de los Países del Cono Sur, a través de la formación de una Red de Laboratorios. El sistema pretende desarrollar y patronizar herramientas analíticas (muestreo, preparación de muestras y métodos analíticos), para garantizar la seguridad del consumidor y asegurar el mercado, principalmente de cereales y subproductos producidos y consumidos por los países comprometidos.

Por lo tanto, es fundamental en los laboratorios, implementar un Sistema de Garantía de la calidad referenciado por normas internacionales. Esta es una propuesta inédita en América del Sur, con la participación de los Laboratorios de EMBRAPA y MAPA (Brasil), LATU (Uruguay), UDEC (Chile) y UBA (Argentina), además de alianzas CIRAD (Francia) e NRI (Inglaterra). Los planos de muestreo serán establecidos y/o armonizados buscando un compromiso entre representatividad y viabilidad en la evaluación de los puntos críticos de las cadenas de trigo y maíz para las toxinas de interés.

Los métodos analíticos oficiales validados deberán atender a los requisitos de criterios de performance establecidos por el Comité Européen de Normalisation (CEN, 1999) y serán utilizados por los laboratorios para los análisis de Aflatoxinas, Zearalenona y Fumonisinas en las matrices de maíz y Deoxynivalenol, Zearalenona y Fumonisinas en trigo. Este proyecto busca también la validación de un método rápido para cuantificación de micotoxinas. El desempeño de los métodos serán evaluados por el uso de los materiales de referencias adquiridos del Food Analysis Performance Assessment Scheme (FAPAS)/CSL/DEFRA/UK y de materiales homogeneos que serán producidos en el ámbito del proyecto. El control intralaboratorial será realizado en cada grupo de muestras por medio del análisis de muestras artificiales y naturalmente contaminadas, ciegas o no al analista. Los resultados de los análisis serán aceptados cuando los valores de recuperación se encuentren dentro de los criterios establecidos por el CEN. La implementación de un Sistema Gerencial de calidad en el control de micotoxinas, permitirá el establecimiento de políticas y procedimientos, tales como: control de documentos,
de registros adquisición de servicios y suministros, atención al cliente, acciones correctivas y preventivas, auditorías internas y análisis críticos para la mejora continua del sistema. Con esto se puede garantizar la confiabilidad de los datos micotoxicológicos generados y obtener reconocimiento y competitividad en el mercado interno y externo, así como permitir el uso de la red como herramienta en la evaluación de cadenas productivas buscando la implantación de el sistema HACCP.

(*) Proyecto Mycotox ICA 4-CT-2002-10043
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.

Autor: Nadine Zakhia, CIRAD, TA 40/16, 73 rue JF Breton, 234398 Montpellier Cedex 5, Francia. email: zakhia@cirad.fr

RESUMEN

La inocuidad de los alimentos es una demanda creciente por parte de los consumidores. Además, las reglamentaciones y estándares oficiales son más y más estrictos en los negocios internacionales. En este sentido, la contaminación de los productos agrícolas y alimentos por micotoxinas es un asunto de primera importancia, tanto para la salud humana como para la productividad animal. Entonces, reducir esta contaminación es esencial para desafiar retos de salud pública y comerciales. A través del proyecto MYCOTOX, colaboran 4 países de América Latina (Argentina, Brasil, Chile y Uruguay) y 3 países europeos (Francia, Reino Unido y Suecia) para desarrollar y implementar un sistema integrado y global de manejo de la contaminación por micotoxinas en las cadenas productivas de trigo y maíz, de acuerdo con el contexto socio económico de cada país. El proyecto trata de aspectos analíticos (estandarización y homogeneización de métodos para el muestreo y la determinación de micotoxinas, desarrollo de técnicas alternativas precisas y menos costosas), así como de aspectos de identificación de peligros y manejo de riesgos relacionados con la contaminación por las micotoxinas. Así, el proyecto se basa sobre una metodología sistemática e interactiva, con alto relacionamiento entre todos los actores y mediante controles frecuentes a lo largo de las cadenas en lugar de un control analítico puntual antes de la venta de los productos. Este sistema global se usará como estrategia de responsabilización de todos los actores y como herramienta para toma de decisiones en las cadenas de trigo y maíz en el Cono Sur de América Latina.

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Covering period from 1 January to 31 December 2003

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

Posters presented in congresses

Contract number: ICA4-CT-2002-10043

FIRST ANNUAL REPORT:
Covering period from 1 January to 31 December 2003

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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

ANNEXES

Papers and publications

Diffusion and dissemination documents
Other relevant information

- Links were made with the European Cluster on Mycotoxins, with the help of Dr Monica Olsen, scientific advisor of MYCOTOX project and member of the European Cluster. Participation in the Third European Mycotoxin Cluster Workshop, 2-4 June 2003, Uppsala, Sweden (see section Scientific Annual Report, paragraph Publications and Papers) and presentation of MYCOTOX project through a dissemination-type poster.

- Links were made with the National Consortium on Mycotoxins in France. Some members of this consortium are also members of the European Cluster on Mycotoxins. This opens the way for scientific collaborations with French institutions or laboratories and logistic support in some specific areas (e.g. the possible use of a milling pilot plant; the access to analytical techniques; the infrastructure for welcoming a Chilean PhD student in 2004; and information exchange on the application of HACCP method throughout various commodity agrichains in Northern countries).

- 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 INCO-DEV Programme (FP5) of the European Commission.

The CEREFER project is organising a conference on small-scale producing units of traditional fermented foods, to be held in Jaen (Spain), September 6-8, 2004 (http://www.ujaen.es/huesped/foodsafety). One session is dedicated to mycotoxins in fermented foods. The general coordinator of MYCOTOX project (ICA4-CT-2002-10043) is member of the Conference Scientific Committee and is committed to give an oral presentation on mycotoxins and to chair a session.
### 1. Dissemination activities

<table>
<thead>
<tr>
<th>Activity</th>
<th>Total (cumulative)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of communications in conferences (published)</td>
<td>5</td>
</tr>
<tr>
<td>Number of communications in other media (internet, video, ...)</td>
<td>1</td>
</tr>
<tr>
<td>(Within a French Network on Food)</td>
<td></td>
</tr>
<tr>
<td>Number of publications in refereed journals (published)</td>
<td>1</td>
</tr>
<tr>
<td>(2 submitted for publication)</td>
<td></td>
</tr>
<tr>
<td>Number of articles/books (published)</td>
<td>-</td>
</tr>
<tr>
<td>Number of other publications - Posters in conferences</td>
<td>8</td>
</tr>
<tr>
<td>Dissemination flyers or posters for project presentation</td>
<td>2</td>
</tr>
</tbody>
</table>

### 2. Training

<table>
<thead>
<tr>
<th>Activity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of PhDs last year of PhD supported by Mycotox project</td>
<td>2</td>
</tr>
<tr>
<td>Number of MScs</td>
<td>-</td>
</tr>
<tr>
<td>Number of visiting scientists</td>
<td>-</td>
</tr>
<tr>
<td>Number of exchanges of scientists (stays longer than 3 months)/Trainers</td>
<td>4</td>
</tr>
</tbody>
</table>

### 3. Achieved results

<table>
<thead>
<tr>
<th>Activity</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patent applications</td>
<td>-</td>
</tr>
<tr>
<td>Number of patent granted</td>
<td>-</td>
</tr>
<tr>
<td>Number of companies created</td>
<td>-</td>
</tr>
<tr>
<td>Number of new prototypes/products developed</td>
<td>-</td>
</tr>
<tr>
<td>Number of tests/methods developed</td>
<td>-</td>
</tr>
<tr>
<td>Number of norms/standards developed</td>
<td>-</td>
</tr>
<tr>
<td>Number of new softwares/codes de veloped</td>
<td>-</td>
</tr>
<tr>
<td>Number of production processes</td>
<td>-</td>
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</tbody>
</table>

### 4. Industrial aspects

<table>
<thead>
<tr>
<th>Activity</th>
<th>Yes/No</th>
</tr>
</thead>
<tbody>
<tr>
<td>Industrial contacts</td>
<td>yes X</td>
</tr>
<tr>
<td>Financial contribution by industry</td>
<td>yes X</td>
</tr>
<tr>
<td>Industrial partners : Large</td>
<td>yes X</td>
</tr>
<tr>
<td>SME(^1)</td>
<td>yes X</td>
</tr>
</tbody>
</table>

\(^1\) Less than 500 employees
5. Comments
Other achievements (use separate page if necessary)

- Relationships achieved with:
  i) The European Cluster on mycotoxins
  ii) The French Consortium on mycotoxins

- Participation of the most project partners 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) on the specific application of HACCP method to mycotoxin prevention and control.