Standards and Trade Development Facility

project SafeNut

http://www.stdf-safenutproject.com/

- STDF 114 -

"Validation and transfer to the key stakeholders of a sustainable and effective aflatoxin management system in the Brazil nut production chain for recovering and consolidating export markets, particularly in Europe."

FINAL REPORT

Covering Period from 1 June 2006 to 30 November 2008

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Last version: May 2009

CIRAD LIVSMEDELSVERKET SL r-biopharm Ministério da Agricultura, Pecuária e Abastecimento GOVERNO FEDERAL Embrapa

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1. GENERAL DATA ON THE PROJECT

STDF project number: 114

Date of Working Group approval: March 2006

Title: Validation and transfer to the key stakeholders of a sustainable and effective aflatoxin management system in the Brazil nut production chain for recovering and consolidating export markets, particularly in Europe.

Duration: 2 years (1 June 2006 – 31 May 2008)

In accordance with the Safenut progress and status reports produced and delivered to the STDF Working Group, the project partners had faced difficulties, such as equipping the laboratories, that impeded or delayed the implementation of some project activities and the delivery of the resulting outputs expected during the reporting periods.

☞ *For that reason, a project extension of 6 months (i.e. until 30 November 2008) was requested by the Safenut general coordinator and agreed by the STDF Working Group in November 2007.*

Region of execution: States of Acre and Pará, Brazil

Executing agency: CIRAD (Centre de coopération internationale en recherche agronomique pour le développement), France

Partners:
NFA (National Food Administration), Sweden
CSL (Central Science Laboratory), United Kingdom
R-Biopharm AG company, Germany – As a sub-contractor of CSL
MAPA (Ministério da Agricultura, Pecuária e Abastecimento), Brazil
EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária), Brazil

Specific objectives:
1. Characterization of the Brazil nut production chain, and formulation of organizational and incentive strategies for safety control.
2. Validation of recommended good practices in the Brazil nut production chain for aflatoxin control.
3. Validation and implementation of a rapid aflatoxin surveillance system for use along the Brazil nut production chain.
4. Knowledge and technology transfer to the key stakeholders.
5. To strengthen the public-private dialogue and partnership in the Brazil nut sector.

2. MANAGEMENT ACTIVITIES

The implementation of the Safenut project consisted in a series of management actions for ensuring the execution of the work programme.

2.1. Signature of contracts of technical and financial cooperation

Contracts of technical and financial cooperation were signed between CIRAD and the other Safenut partners in order to formalize the partnership and commit the Safenut partners to the execution of the collaborative project activities in a sound financial manner and with respect of the STDF and WTO rules. These contracts which were prepared by following the model of the contract signed by CIRAD and WTO are:

- Contract between CIRAD and NFA signed in July 2006,
- Contract between CIRAD and CSL signed in October 2006,
- Contract between CIRAD, EMBRAPA and FUNARBE signed in June 2007,
- Contract between CIRAD, MAPA and FUNARBE signed in September 2007.

☞ *A copy of the different signed contracts was sent to STDF.*

In Brazil, institutions usually make project commitments through contractual modalities with private foundations managing their financial resources, and especially when the budgets are coming from outside donors and international projects.
The FUNARBE foundation (Fundação Arthur Bernades - http://www.funarbe.org.br/), legal entity of private law, was selected to facilitate the transfer and management of the financial resources of the Brazilian partners (EMBRAPA and MAPA), and the preparation of their cost statements. CIRAD has already committed this foundation for work within the framework of European projects (INCO-DEV). In accordance with the Article 6 (Sub-contracting) of the CIRAD-WTO contract, the Safenut general coordinator has contacted the WTO for its prior written authorization to assign the contracts with FUNARBE.

2.2. Fund transfer to the Safenut partners

CIRAD has transferred to all the Safenut partners 50 % and 35 % of their total budget once the contracts of technical and financial cooperation were signed and 50 % and 35 % of the total Safenut budget received from WTO.

Delay in the fund transfer to EMBRAPA and MAPA occurred due to delay in the signature of the contracts with these partners (only in 2007. See 2.1). In the meantime, CIRAD assumed directly the expenses of the Brazilian partners as necessary in order not to compromise the execution of the project activities. The advanced amounts were invoiced to the Brazilian partners as supporting documents for their accounts, and deduced from the fund transfer to these partners.

The transfer to CSL of 35 % of their total budget was also delayed until an agreement with this partner and R-Biopharm on the activities they must support scientifically and financially by the end of the project based on the Safenut proposal.

CIRAD will transfer to all the Safenut partners the 15 % of their total budget once the 15 % of the total Safenut budget will be received from WTO, i.e. after approval by the STDF Working Group of the final project scientific and financial reports.

2.3. Safenut rules to be respected by all the partners

The rules of project running and management, including the STDF and WTO ones, were discussed and clarified for the whole consortium members during the Safenut kick off meeting. These rules were also included in the Safenut action plan (see 2.4.) and reminded to the whole consortium members during the Safenut annual progress meeting organized in September 2007.

The Figure 1 presents the project management and communication between the different Safenut partners.

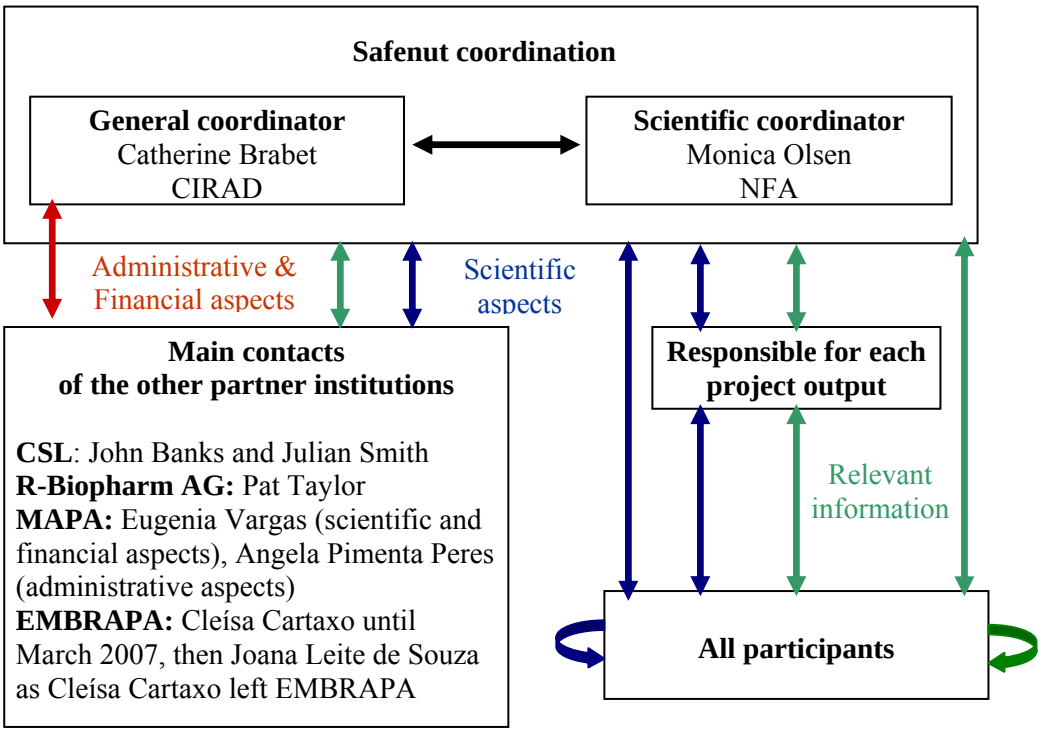

Figure 1: Project management and communication between the different Safenut partners

On the request of the general coordinator, main contacts were assigned in each partner institution in order to facilitate the follow up of administrative, financial and scientific issues.
A responsible for each project output was also assigned during the Safenut kick off meeting (see Safenut action plan). He (she) is in charge of the coordination of the activities to be implemented for output delivering by mobilizing the appropriate competence and relationships, and of the progress reporting to the general and scientific coordinators.

2.4. Use of the tool Lots® for developing a Safenut action plan

The tool Lots® (www.lots.mindo.com) was brought to the project and presented by Monica Olsen, NFA and used to develop a Safenut action plan during the project kick off meeting in July 2006. This plan, after validation by the Safenut partners, includes:

* A description of the present situation for the Brazilian nut sector in the main Amazon producing countries (Brazil, Bolivia, Peru), and of the research works and actions implemented, in progress or needs in order to control the Brazil nut contamination by aflatoxins. This description was based on the results of discussions at the international workshop on "Prevention and

Control of Brazil Nut Contamination by Aflatoxins" organized by the Safenut project at its beginning (July 2006).

* The rules for project running and management to be respected by all the partners:
  - Formalization of the partnership through the signature of technical and financial contracts,
  - Project management and communication between the different partners,
  - STDF and WTO rules based on the signed WTO-CIRAD contract: performance, sub-contracting, intellectual and industrial property rights, use and publication of information, termination, budget transfer,
  - Scientific and financial reporting (frequency, deadlines and templates).

* A detailed work plan for each project specific objective: actions to be carried out (what), methodology (how/where), expected outputs, human resources (who: responsible for each output, competence and relationships), chronogram (when), follow-up (list of check points to ensure the good progress of the activities) and identified risks.

  ☞ *A copy of the Safenut action plan was sent to STDF.*

At the request of the general coordinator, a synthesis of the main project results, conclusions and plans discussed during the Safenut progress meeting in September 2007, was prepared for each project specific objective (Specific objective 1: MG Piketty ; Specific objective 2: C. Brabet & M. Olsen ; Specific objective 3: John Banks ; Specific objective 4 & 5: C. Brabet). These syntheses were used to update the Safenut action plan and propose a new timetable as part of the request for project extension from June to November 2008.

2.5. Collaboration with the private sector as focus group of the Safenut project

Collaboration with the Brazil nut private sector has been developed and strengthened through the participation and involvement of key stakeholders (cooperatives and associations of Brazil nut producers/extractivists and processors/exporters) in:

- The execution of the activities of the project related to the project specific objectives 1 & 2. In particular, field participative research was conducted for evaluating and updating recommended good practices at a pilot scale, i.e. in two Brazil nut production chains selected in the states of Acre and Pará, where agents demonstrated a high interest and motivation for Brazil nut quality improvement. The general coordinator supported the Brazilian partners for making the contacts with key stakeholders at the beginning of the Safenut project and formalizing the collaboration. The pilot group of agents could be used now as a leader and serve as an example for the other agents of the region for the dissemination of the project results.

- The Safenut workshops and open meeting organized in the states of Acre and Pará in July 2006 (first workshop, Rio Branco, Acre), September 2007 (open meeting, Belém, Pará) and November 2008 (final workshop, Belém, Pará) (see 3.5.).

- The training courses in good practices and rapid methods (LFD kits) for aflatoxin surveillance along the Brazil nut production chain in both states of Acre and Pará, November 2008 (see 3.4.2. and 3.4.3.).

Key stakeholders, not only from Brazil but also from Peru and Bolivia, participated at the Safenut workshops and training courses.

The collaboration with the Brazil nut sector ensured that the safety management tools evaluated and recommended by the Safenut project (good practices and rapid methods for aflatoxin analyses) are acceptable and compatible with the local socio-economic and environmental context. It also facilitated the knowledge and technology transfer, in particular the dissemination of the Safenut results, to the stakeholders throughout the project, and contributed to strengthen the private/public sector dialogue in the Brazil nut area.

2.6. Organization and participation in Safenut project meetings

Safenut project meetings in Brazil

- *Kick off meeting, Rio Branco, state of Acre, 12-14 July 2006:*
  First meeting between all partners for project launching (see 3.5.1.).

- *First annual progress meeting, Belém, state of Pará, 26-27 September 2007:*
  Annual meeting between all partners for discussing the results and conclusions of the first year project, as well as future plans until the end of the project (see 3.5.2.).

- *Open meeting, Belém, state of Pará, 28 September 2007:*
  Meeting between all Safenut partners, open to the Brazil nut private sector and other Brazilian institution supporting this production chain, for presenting and discussing the Safenut project and first results (see 3.5.2.). This meeting counted with the participation of Dr Cameon Ivarsson, Director of Napasol AG (http://www.napasol.com/) as a representative of INC (http://www.nutfruit.org/).

- *Second annual progress meeting, Belém, state of Pará, 3-4 November 2008:*
  Annual meeting between all partners for presenting and discussing Safenut results, conclusions and presentations for the final workshop on 6-7 November 2008 (see 3.5.3).

- *Meetings of the general coordinator with the Brazilian partners for specific administrative & financial issues:*
  - Validation and signature of the contracts of technical and financial cooperation, Brasilia-DF, May 2007,
  - Instructions to Funarbe for the second annual financial reports of MAPA and EMBRAPA (June 2007-May 2008), Belo Horizonte-MG, May 2008

- *Meetings of the Safenut partners for organizing and follow up project activities (Annex 1):*
  - Various meetings in the states of Pará and Acre as well as in Belo Horizonte-MG for the organization and follow up of the field and laboratory activities, including methodological support for sample collection, preparation and analysis (project specific objective 2),
  - Meetings in São Paulo-SP, Belém-PA and Rio Branco-AC for the organization and follow up of the socio-economic activities (project specific objective 1).

Safenut project meetings in Europe

Local	Date	Objectives of the meeting	Participants
Darmstadt, Germany	25-27 June 2007	Finalisation of the statistical analysis, interpretation and discussion of the results of ELISA & LFD validation tests	John Banks, CSL R-Biopharm local team
Geneva, Switzerland At the STDF Secretary	4 September 2007	- Presentation of the project current status, problems encountered and actions taken to resolve them, and outline plans until the end of the project. - The extension of the Safenut project until the end of 2008 was discussed. The submission of a written official request by CIRAD to the STDF working group before their next meeting in November 2007 was required. - The realization of a training video has also been considered.	C. Brabet, CIRAD M. Olsen, NFA M. Roberts and M. Spreij, STDF
Uppsala, Sweden NFA	16-23 August 2008	- Compilation and statistical analysis of the results obtained from the Brazil nut samples collected in the states of Acre and Para / Identification of the Critical Control Points / Draft recommendations to update the existing manual of Brazil nut good practices (project specific objective 2) ; - Recommendations for the additional tests for kit validation (project specific objective 3) ; - Organization of the training courses in good practices and LFD kits for the private sector and trainers (project specific objective 4), - Organization of the final progress meeting and workshop (project specific objective 5).	C. Brabet, CIRAD M. Olsen and Mats Lindblad, NFA
Uppsala, Sweden NFA	27 September – 3 October 2008	Finalization of the statistical treatments of Safenut results (project specific objective 2) and of the synthesis of project conclusions/recommendations	C. Brabet, CIRAD M. Olsen and Mats Lindblad, NFA

2.7. Supervised students and contracted consultants for Safenut activity support

Tables 1 and 2 present the supervised students and contracted consultants, respectively, for Safenut activity support.

The Safenut project also contracted day-workers for Brazil nut dehusking and laboratory cleaning at EMBRAPA Acre and LANAGRO-PA/MAPA.

2.8. Reporting to the STDF Working Group

The following reports were delivered to the STDF Working Group:

Progress reports:
- First progress report for the reporting period June-September 06
- Second progress report for the reporting period October 06 – January 07
- Third progress report for the reporting period June-September 07
- Fourth progress report for the reporting period October 07 – January 08

Status reports:
- Status report for the reporting period June 06 - May 07
- Status report for the reporting period June-September 2007
- Status report for the reporting period October 07 – March 08
- Status report for the reporting period April-June 08
- Status report for the reporting period July-September 08

Annual reports:
- Scientific annual report for the reporting period June 06-May 07
- Financial annual reports for the reporting period June 06-May 07

☞ *Delay occurred in the delivery of the scientific and financial reports by the Brazilian partners, despite the various contacts made by the general coordinator by email and telephone.*

The general coordinator would like to highlight:

- the good cooperation among the Safenut partners for executing the project activities. Exchanges of technical and scientific information and experiences, in particular on methodological issues, were effective and essential for the implementation of the activities of the different project specific objectives;

- the good cooperation of the private sector which was involved in the Safenut activities, and its good participation and contribution at the Safenut workshops and training courses;

- the extensive work done for the implementation of the field activities in a difficult environment that represents the Amazon region (strong local constraints, in particular of logistics) and for the development of predictive models based on controlled laboratory experiments.

Table 1: Students under the supervision of Safenut partners for project activity support

Name	Institution of origin	Host institution	Supervisors	Safenut activities supported	Period
Olivier Devillers French engineering student	ENSAR (Ecole Nationale Supérieure Agronomique de Rennes)	EMBRAPA Acre	C. Brabet, CIRAD C. Cartaxo and J. de Souza, EMBRAPA Acre	Activities A2.1 and A.2.2 in the state of Acre (project specific objective 2)	December 06-May 07
Felicia Maria Nogueira Leite Brazilian student, MSc in agronomy	UFAC (Universidade Federal do Acre) / SEAPROF (Secretaria de Extensão Agroflorestal e Produção Familiar)	EMBRAPA Acre	C. Brabet, CIRAD C. Cartaxo and J. de Souza, EMBRAPA Acre M. Luzenira de Souza, UFAC	Activities A2.1, A.2.2 and A.2.4 in the state of Acre (project specific objective 2)	January 07-November 08
Sarita Maria de Azevedo Brazilian student, Graduated in agronomy	UFAC	EMBRAPA Acre	J. de Souza, EMBRAPA Acre	Brazil nut sample preparation and analyses (project specific objective 2).	November 07-May 08
Idaiane Lira Costa Undergraduated student in Biological Sciences	UNINORTE-FAC (União Educacional do Norte - Faculdade do Acre)	EMBRAPA Acre	J. de Souza, EMBRAPA Acre	Brazil nut sample preparation and analyses (project specific objective 2).	June-November 08
Sarah Biruel Brazilian student	Colégio Politécnico Bento Quirino	CIRAD / UNICAMP	C. Brabet, CIRAD	Safenut website design and hosting (project specific objective 4)	September 06-May 07
César Augusto Piedrahíta Aguirre Colombian student	UNICAMP-FEA (Universidade Estadual de Campinas-Faculdade de Engenharia de Alimentos)	CIRAD / UNICAMP	C. Brabet, CIRAD	Spanish translation of the Safenut website (project specific objective 4)	February 2007
Janaína Deane de Abreu Sá Diniz Brazilian student, PhD	UnB (Universidade de Brasilia)	EMBRAPA Belém	MG Piketty and C. Brabet, CIRAD J. Carvalho dos Santos, EMBRAPA Belém	Socio-economic activities (project specific objective 1): Field surveys in the state of Pará, and data analysis and synthesis. Updating of the legislation for Brazil nut commercialization	June-November 2008

Table 2: Consultants contracted for Safenut activity support

Name	Host institution	Safenut activities supported	Period
Douglas Storto Brazilian engineer	LANAGRO-PA / MAPA	Brazil nut sample preparation and analysis in the state of Pará (project specific objective 2)	3 months (March-June 07)
Maria Fatima Correa Brazilian technician	SFA-PA / MAPA	Brazil nut sample collection in the state of Pará (project specific objective 2)	3 months (March-June 07)
Edna Lúcia da Silva Lima Brazilian technician	LANAGRO-PA / MAPA	Brazil nut sample preparation in the state of Pará (project specific objective 2) Organization of the ELISA & LFD training course / Progress & Open meetings in September 07 (project specific objectives 4 & 5)	3 months (July-September 07)
Ricardo Marcio Souza de Carvalho Brazilian technician	LANAGRO-PA / MAPA	Brazil nut sample preparation and analysis in the state of Pará (project specific objective 2)	3 months (February-May 08)
Allan Santos de Souza Brazilian technician	LANAGRO-PA / MAPA	Brazil nut sample preparation and analysis in the state of Pará (project specific objective 2)	3 months (May-August 08)
Hugo Gimenes de Lima Website designer	CIRAD / UNICAMP	Updating of the Safenut website and implementation of the Safenut intranet with a restricted access to the Safenut partners (project specific objective 4)	June 2007-November 2008

3. SCIENTIFIC AND DISSEMINATION ACTIVITIES

The deliverables and milestones expected during the Safenut project are presented within the corresponding project specific objectives.

The Safenut project started with the organization of the kick off meeting and a first international workshop at mid-July (see 3.5.1.). The scientific and dissemination project activities were effectively launched after the kick off meeting, i.e. once the partners have discussed and defined in detail how they were going to organize themselves and interact with each other for the project implementation.

3.1. Specific objective 1: Characterization of the Brazil nut production chain, and formulation of organizational and incentive strategies for safety control.

The conditions of Brazil nut production and commercialization which vary significantly between the producing regions of Brazil were poorly defined and documented at the start of the Safenut project. A better knowledge of these conditions is necessary to identify the major constraints and opportunities in the Brazil nut production chain as well as formulate organizational and incentive strategies for the adoption of sustainable and effective aflatoxin control measures.

Within the framework of the Safenut specific objective 1, such a work was done in the two regions of execution of the project, i.e. the states of Acre and Pará.

3.1.1. Inventory of the regulations applied to Brazil nuts for commercialization

A report on the Brazilian and international regulations applied for Brazil nut commercialization, for both the local and export markets, was produced.

☞ *This report will be sent to STDF along with this final report.*

Main conclusions:

The Brazilian and international regulations for Brazil nut commercialization impose maximum limits for aflatoxins, and lay down sampling and analytical methods for the official control of aflatoxin levels.

The maximum limits for aflatoxins are quite variable between countries and concern total aflatoxins and/or B1 according to the country's laws. Harmonization of these limits is taking place in some free trade zones (European Union, Mercosur, Australia/New Zealand) and harmonization efforts are being undertaken by the Codex Alimentarius for goods moving in the international market. However, this harmonization is a slow process because of the different views and interests of those involved in the process. The European maximum limits for aflatoxins in nuts, including Brazil nuts, are the strictest (2 ppb for B1 and 4 ppb for total aflatoxins for direct human consumption).

In that context, as a result of the loss of the European market for unshelled Brazil nuts from 2003, Brazil switched to new export markets, in particular Bolivia but also Asian countries such as China, and increased Brazil nut commercialization in the domestic market with the aim to absorb the volume that could not be exported to Europe. The existence of these less strict alternative markets may make difficult the implementation of good practices in the Brazil nut production chain to reduce aflatoxin levels to the European standards, since the stakeholders are less forced to improve product quality.

In light of the conclusions of the JECFA risk assessment associated with aflatoxins in tree nuts (almonds, hazelnuts, pistachios, Brazil nuts), the Codex Alimentarius Commission (CAC) adopted in 2008 maximum total aflatoxin levels of 10 ppb and 15 ppb in almonds, pistachios and hazelnuts, respectively ready-to-eat and for further processing, as well as sampling plans.
On the request of the Delegation of Brazil, the Codex Committee on Contaminants in Foods (CCCF) agreed to start new work on draft Maximum Levels for total aflatoxins in Brazil nuts destined for further processing and ready-to-eat, both in-shell and shelled nuts, based on scientific evidence in order to protect human health with a minimum economical impact on international trade.
The CAC also adopted a Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Tree Nuts (CAC/RCP 59-2005, REV. 1-2006), which includes a specific Appendix, addressing Good Extractivistic Practice for Brazil Nuts.

3.1.2. Description of the current conditions of Brazil nut production and commercialization in the Brazilian states of Acre and Pará

A first broad view of the state of art on the Brazil nut production chain in the states of Acre and Pará was carried out by compiling information already available (literature review).

Field surveys were then undertaken by the project socio-economic team to complement the secondary data for a more comprehensive characterization of the Brazil nut production chain in both states of Acre and Pará. Questionnaires were applied to the different actors of the Brazil nut production chain:
- producers/extractivists by focusing on the associations of producers that were involved in the activities of project specific objective 2,
- intermediaries,
- all the agro-industries in Acre (4) and Pará (7),
- CONAB in the state of Acre as a key institution supporting Brazil nut commercialization.

Note that some actors did not answer all the questions since the information has been considered confidential, particularly for reasons of market strategy.

A report on the Brazil nut production chain in the states of Acre and Pará was produced, by documenting and analyzing in both states:
- the evolution and location of Brazil nut production,
- the domestic and export markets,
- the Brazil nut flow diagram,
- the different segments (input suppliers, producers/extractivists, associations and cooperatives, intermediaries, agro-industries and institutional environment) and their interactions.

This report also includes:
- an analysis of the costs/benefits related to the implementation of good practices in the Brazil nut production chain for aflatoxin control in the state of Acre,
- the recommended actions to be taken for the development of a safe and sustainable Brazil nut production chain based on an analysis of the major constraints and opportunities in the production chain (see 3.1.3.).

☞ *This report will be sent to STDF along with this final report.*

3.1.3. Formulation of organizational and incentive strategies

Based on the integration of the project technical and socio-economical data, i.e. the description of the current conditions of Brazil nut production and commercialisation in the states of Acre and Pará (see 3.1.2.) and the recommended good practices for aflatoxin control (see 3.2.4), the major constraints and opportunities affecting the implementation of aflatoxin control measures in the production chain were identified and recommended action strategies formulated.

Main conclusions and recommended action strategies:

* Update the existing technical recommendations for Brazil nut quality management and disseminate them along the production chain (under the responsibility of research and development institutes and extension services)

  - The Safenut technical results demonstrated that the good practices currently implemented in the Brazil nut production chain and recommended by the existing guidelines are not effective for reducing aflatoxins in unshelled nuts below the European tolerated limits, without adequate Brazil nut drying to a safe moisture content within 10-30 days after collection and storage conditions (see 3.2.4.);
  - Simple dryers must be tested in the extractivist communities to assess the technical and economical feasibility of these equipments to dry unshelled nuts to a safe moisture content that prevents fungal growth and aflatoxin contamination (applied research);
  - Strengthen large-scale dissemination of effective and sustainable good practices to the Brazil nut producers/extractivists (most of the communities are still not aware of good practices allowing to reduce aflatoxin contamination).

* Implement mechanisms for price differentiation of Brazil nuts according to product quality in order to incentive the adoption of good practices for aflatoxin control (under the responsibility of public institutions such as CONAB and the private sector: intermediaries, industries, exporters, importers)

  - The increase of about 20 % of the Brazil nut production costs due to the implementation of good practices is not reflected in prices, in general, even when there is a demand for better quality;
  - The procedure currently used by the private sector for Brazil nut quality control and payment does not differentiate the price when the percentage of nonconforming nuts is below the tolerated limits (between 0 and 10 %);
  - The CONAB which operates in the state of Acre for pre-payment of Brazil nuts, makes no differentiation in the guaranteed minimum price according to product quality. Furthermore, this price is below the market price and does not cover the production costs even for conventional practices. The CONAB may increase the guaranteed minimum price in the near future ;
  - There are export markets with less strict aflatoxin limits than the European Union that will continue to represent alternatives for substituting the European market if there is no price differentiation for Brazil nuts of better quality.

* Implement policies to improve transport logistics in Brazil nut production areas

  - Poor transport logistics prevents to sell Brazil nut extractive production in a few days, especially in remote areas depending on land transport;
  - Brazil nut harvest occurs in the rainy season when land transport is the most critical, making difficult rapid transport to a suitable place for drying.

* Support the expansion of accredited laboratories for mycotoxin analysis in the Amazon Brazil nut producing states

  Currently, it is costly and time-consuming to perform mycotoxin analysis in laboratories located outside the Amazon region (such as LACQSA/MAPA in the state of Minas Gerais). Moreover, the local governance of quality control is limited.

* Strengthen policies to support local Brazil nut processing

  - For Brazil nuts produced in remote regions, the implementation of effective good practices to reduce aflatoxin contamination in unshelled nuts below the current European tolerated limits would be very difficult. Moreover, the exportation of unshelled nuts (even of good quality) may aggregate low value compared to the exportation of shelled nuts or derived products.
  - Brazil nut processing involving nut shelling allows both adding value and reducing aflatoxin contamination risk through an effective selection process of shelled nuts.

3.2. Specific objective 2: Validation of recommended good practices in the Brazil nut production chain for aflatoxin control

Extensive field work was carried out in both states of Acre and Pará to assess the effectiveness of the good practices currently used in the Brazil nut production chain for aflatoxin control in unshelled nuts, based on the existing guidelines, in particular the Codex Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Tree Nuts which includes a specific Appendix addressing Good Extractivistic Practice for Brazil Nuts (CAC/RCP 59 -2005, REV.1-2006).

Extensive controlled laboratory experiments were also conducted for the development of predictive models for fungal growth and aflatoxin production in unshelled nuts during storage.

A particular attention was given to these activities since the deliverables of most of the other project activities (A.1.2, A.1.3, A.2.4, and A.4.3) and consequently the success of the Safenut project were based on these results.

3.2.1: Identify existing or set up Brazil nut production systems following recommended code of practices (A.2.1)

☞ Constitution of a multi-disciplinary team with relevant competences

A multi-disciplinary team with relevant competences was constituted in both regions of execution of the Safenut project, i.e. in the states of Acre and Pará, Brazil. In Acre, the team involves staffs from EMBRAPA and students (Table 3), whereas in Pará it involves staffs from MAPA and consultants (Tables 4).
In both states, the teams benefited of a scientific and organizational support from the other Safenut partners (Table 5).

Table 3**:** The multi-disciplinary team in the state of Acre

Team members	Institution	Fields of expertise	Responsibilities / Tasks
Embrapa staff*			
Cleísa Brasil da Cunha Cartaxo	EMBRAPA Acre	Food technology	Until March 2007: Contact with the Brazil nut stakeholders for the selection of the production chain in Acre. Support for the construction & verification of the Brazil nut flow diagram.
Joana Maria Leite de Souza	EMBRAPA Acre	Food technology	From March 2007: Organization and follow up of the field activities. Support for sample collection
Virgínia de Souza Álvares	EMBRAPA Acre	Food technology	Support for sample collection & analyses
Students			
Olivier Devillers	ENSAR	Engineering student in agronomy	Execution of the activities A2.1 and A2.2.
Felicia Maria Nogueira Leite	UFAC / SEAPROF	MSc in agronomy	Execution of the activities A2.1, A2.2 and A2.4
Sarita Maria de Azevedo	UFAC	Graduated in agronomy	Support for Brazil nut sample preparation and analyses
Idaiane Lira Costa	UNINORTE -FAC	Undergraduated student in Biological Sciences	Support for Brazil nut sample preparation and analyses

* Including various technicians/assistants for supporting Brazil nut sample collection, preparation and analyses.

Table 4**:** The multi-disciplinary team in the state of Pará

Team members	Institution	Fields of expertise	Responsibilities / Tasks
MAPA staff			
Mauricio Quaresma de Araújo	LANAGRO-PA	Chemistry	Brazil nut sample preparation Water activity analyses
Nilce Limeira Medeiros	LANAGRO-PA	Microbiology	Fungal analysis
Poliana Carla Góes de Souza	LANAGRO-PA	Microbiology	Fungal analysis
José Carlos Barroso Junior	SFA-PA	Brazil nut sampling and sanitary certification Good practices in Brazil nut production chain	Contact with the Brazil nut stakeholders for the selection of the production chain in Acre. Support for the construction & verification of the Brazil nut flow diagram. Coordination of the Brazil nut sample collection.
Gilson Pedrosa dos Santos	SFA-PA	Brazil nut sampling	Brazil nut sample collection
José Delfin de Figueiredo Filho	SEMAGRI (Secretária Municipal de Agricultura)	Brazil nut sampling	Brazil nut sample collection
Consultants For supporting Brazil nut sample collection (Maria de Fátima Pinheiro Corrêa), preparation and water activity analysis (Douglas Storto, Edna Lúcia da Silva Lima, Allan Santos de Souza, Ricardo Marcio Souza de Carvalho)			

Table 5**:** Scientific and organizational support from the other Safenut partners

Team members	Institution	Fields of expertise	Responsibilities / Tasks
Catherine Brabet	CIRAD	Food science ; Quality and safety management in food production chain	Scientific & organizational support for the activities in Acre and Pará
Monica Olsen	NFA	Mycology	Scientific support for sampling design, sample preparation & fungal analysis
Pernilla Johnson	NFA	Mycology	Scientific support for sampling design, sample preparation & fungal analysis
Mats Lindblad	NFA	Statistical analyses and modelling	Scientific support during data handling
Eugenia Azevedo Vargas	LANAGRO-MG / MAPA	Chemistry, Food Science	Scientific support for sampling design, sample preparation & aflatoxin analyses
Julio César Garcia	LACQSA / MAPA	Mycotoxins	Scientific support for sample preparation
Eliene Alves dos Santos	LACQSA / MAPA	Mycotoxins	Scientific support for sampling design, sample preparation & aflatoxin analyses Aflatoxin analyses by HPLC*

* With the support of LACQSA temporary and permanent technicians

☞ Selection of a Brazil nut production chain in each of the states of Acre and Pará

The selection of the Brazil nut production chains was based on the involvement of the stakeholders in good practices of production, including the availability of storage facilities in the forest, their high interest and motivation for Brazil nut quality improvement and the access to their location.

The production chains concern the steps from the rainforest (tree) to the storage of the dried unshelled Brazil nuts in the processing plant.

In Acre, two associations of producers and a processing plant have been selected:

- The association of producers 1 gathers 21 producers whose 12 are nut collectors and fully involved in good practices. The association is accessible by road.

- The association of producers 2 gathers 125 families of rubber-tappers whose 80 are also nut collectors. Many of them are already involved in good practices of production even if these practices are relatively disparate among the producers. The association is accessible only by boat during the rainy Brazil nut harvest season (January-June), then by road.

- The processing plant produces shelled and unshelled nuts sold to the domestic market and Bolivia. It is concerned by quality issues and thus would like to improve its practices.

In Pará, the communities of an association of producers and a processing plant have been selected.

- The association of producers gathers 32 communities. In 2004, this association participated in training courses in good practices of Brazil nut production which were organized by the MAPA

within the framework of a project funded by the European commission. This project also allowed the construction of common warehouses. The communities are accessible by boat.

- The processing industry is integrally certified in ISO 9001 and HACCP. It has two factories for processing shelled and unshelled nuts, and its own laboratory for analysing and controlling the product quality. The company sells its products to the foreign and domestic markets.

☞ Construction and verification of the Brazil nut Flow Diagrams (BnFD)

The BnFD of the selected production chains in Acre and Pará were constructed and verified through field visits and meetings with the stakeholders. These BnFD are presented in Annex 2. They differ in part between the selected production chains. In Acre, there is no washing step after collecting Brazil nuts in the forest and before drying them in the community, and the flow diagram in the industry is much simpler than in Pará (fewer steps of nut cleaning, classification and selection).

3.2.2: Collection of data (A. flavus/A. parasiticus, aflatoxins, water activity, moisture content, relative humidity, temperature, insect infestation) (A.2.2)

Collection of data was conducted in both states of Acre and Pará in different Brazil nut production areas ("castanhais") and along the selected production chains during two harvest seasons (2007 and 2008) in order to have a more comprehensive study for identifying the critical control points and good practices.

☞ Definition of sampling plans for the selected Brazil nut production chains

A strategy was built not only for the Brazil nut sample collection but also for the laboratory work in both states of Acre and Pará.

Sampling plans were elaborated for each of the selected Brazil nut production chains. These plans detailed:
- The sampling points, populations, lots and replicates along the production chain, as well as the quantity of samples and incremental samples to be collected;
- The procedures to be followed for Brazil nut collection at each of the sampling points, the sample handling and transport to the laboratory;
- The registration of the environment factors (temperature and relative humidity).

Adjustments of the sampling plans and instructions for the laboratory work have been made between the two harvest periods as necessary, in particular on the basis of the production context and the availability of Brazil nut samples at different stages of the production chain.

The sampling points along the selected Brazil nut production chains in Acre and Pará with illustrating photos, and the flow diagram of sample transportation from sampling areas to laboratories are presented in Annex 3 and 4, respectively.

Detailed instructions were also elaborated for sample reception, preparation and storage at the laboratory, water activity analysis, cleaning and decontamination of the working area, equipment and materials, and discard of the remaining prepared Brazil nut samples.
A flow diagram of sample reception, preparation, storage and analysis is given and illustrated by photos in Annex 5.

All these protocols were discussed and agreed among the Safenut partners involved in the project specific objective 2, and based on the Commission regulation (EC) No. 401/2006 on sampling

procedures for official mycotoxin analysis in foods. They also include the documents to be filled and joined to the collected samples (term of sending, list of the sample identification numbers), the data and information to be recorded, and the equipment and consumables needed.

Instructions for fungal analysis in the collected Brazil nut samples were produced as a result of the training course organized in October 2006 in the AFPA agar plate methodology.

☞ Building of the laboratory capacity in the states of Acre and Pará to attend the Safenut activities and requirements

This included the following activities:
- Assessment of the laboratory infra-structure and organization at EMBRAPA Acre and LANAGRO-PA to implement the Safenut activities such as Brazil nut sample reception, preparation and storage, water activity analysis and fungi isolation and quantification;
- Elaboration of a plan for the adequacy of the laboratory infra-structure to attend the minimum needs of the Safenut requirements;
- Elaboration of list of complementary equipment and consumables for the improvement of laboratory facilities, followed by cost quotation into Brazilian market;
- Changes into laboratory lay-out and final installation of laboratory equipment and administrative facilities;
- Testing the equipment capacity for Brazil nut sample grinding and homogenization, and buying of complementary equipment as necessary;
- Ring-tests for fungi analysis in conjunction with project specific objective 4.

☞ Collection and analysis of Brazil nut samples, and environmental factor registration through the selected production chains

Brazil nut sample collection with environmental factor registration (temperature and relative humidity) at sampling points was performed in the selected Brazil nut production chains in both states of Acre and Pará during two harvest periods (2007 and 2008). The collected samples were then transported to the laboratories for sample preparation and analysis (water activity, aflatoxin-producing fungal isolation and quantification, aflatoxins).

In 2007, the collection of Brazil nut samples was initiated mid April in both states of Acre and Pará (i.e. at the end of the harvest period) whereas in 2008, in January (i.e. at the beginning of the harvest season). In 2008, the Brazil nut samples were collected in the same selected production chains than in 2007, at the exception of the state of Acre where sampling in the rainforest was carried out only in the association of producers 1 (and not in the association of producers 2) because of the improved production practices applied by the extractivists and better access to the locality. Samples that could not be collected at some sampling points during the 2007 harvest period (for example, pods recently fallen from the tree in the state of Acre) were collected during the 2008 harvest period.

The number and origin of Brazil nut samples collected in the states of Pará and Acre are presented in Tables 6 and 7, respectively. In 2007, 20 samples of leaves and soil were also collected in five production areas of the state of Acre (4 samples in each production area) and fungi analyzed in order to evaluate the risk of contamination of Brazil nut pods once they have fallen from the trees.

Water activity and fungal analyses were performed by the laboratories of EMBRAPA, state of Acre and LANAGRO-PA/MAPA, state of Pará, by using a Pawkit meter and the AFPA agar plate methodology (Method No 177, Nordic Committee on Food Analysis), respectively.

The quantification of aflatoxins B1, B2, G1 and G2 was carried out at LACQSA/LANAGRO-MG/MAPA, Belo Horizonte-MG by using a standard HPLC method rather than the ELISA method (originally scheduled in the Safenut proposal). Because of the delay of the completion of the kit validation (deliverable report available only by the end of August 2007) and consequently their implementation at the laboratory level, the general and scientific coordinators agreed that the HPLC method would be used for analysing aflatoxins in the Brazil nut samples collected within the project specific objective 2. The aflatoxin results were only accepted when the analytical quality control check complied with the Regulation EC No 401/2006, i.e. when the recoveries obtained for the Brazil nut spiked samples were within the range of 70 to 110%. If the quality control check presented a result out of this range, the analytical batch was supposed to be re-analysed.
In total, 315 Brazil nut samples were analyzed from the state of Acre and 215 samples from the state of Pará, resulting in a total of 530 samples and 2120 determinations.

Field data were extensively studied by NFA together with CIRAD (general coordinator) at the end of the Safenut project (the general coordinator visited NFA, Sweden during two 1-week visits). Modelling/predicting and statistical work was performed by NFA and illustrations provided for the presentation of the results of project specific objective 2 at the Safenut final workshop in November 2008. The main results and conclusions are summarised in 3.2.4.

NFA has also developed a protocol with photo illustrations (Annex 6) to enable the laboratories in Pará and Acre to isolate fungal strains for further examinations to species level. Laboratory and packing material was sent to the laboratory in Pará. A limited number of strains, about 20, were sent to Sweden in August 2008 and all the strains were identified to *A. flavus*. These strains were used for training of a staff member from one of the Brazilian partners (MAPA) who visited NFA during 2 weeks in September 2008 (see 4. Measurable project impacts).

Table 6: Collected Brazil nut samples in the state of Pará

Sampling points	**Collected global Brazil nut samples**	
	2007	**2008**
In the rainforest and communities of an association of producers		
Point 1: Effect of contact time of pods with ground 1.1 New pods (0-5 days) 1.2 Old pods (15-20 days) 1.3 Old pods (about 2 months)	**10** 10 samples of new pods from **5 production areas**	**24** 12 samples of new pods, 9 samples of old pods (15-20 days), 3 sample of old pods (about 2 months) from **3 production areas**
Point 2: Impact of Brazil nut selection after pod cracking 2.1. Non selected nuts 2.2. Selected nuts 2.3. Discarded nuts	**14** 5 non selected nut samples 5 selected nut samples 4 discarded nut samples from **5 production areas**	**12** 2 non selected nut samples x 2 lots 2 selected nut samples x 2 lots 2 discarded samples x 2 lots from **2 production areas**
Point 3: Impact of washing and drying	**3** 1 sample x **3 production areas**	**4** 1 sample x 2 lots x **2 production areas**
Point 4: Impact of storage in common warehouse (lots stored in bulk)	**5** 1 sample x 1 lot x **5 common warehouses** with different storage times (3 days, 2 and 4 months)	**23** 2 samples x 2 small lots x 2 different storage times (0 and 30 days) 3 samples x 1 large lot x 5 different storage times (0, 17, 29, 39, 54 days) in a **common warehouse**
TOTAL	**32**	**63**
In a processing plant (1 large lot processed per year, coming from a common warehouse of a selected community, Point 4 – see above)		
Point 1: Quality control (QC) at reception (2007+2008) Delivered nuts "Good" nuts from QC "Bad" nuts from QC	**3** 1 sample of delivered nuts 1 sample of "good" nuts 1 sample of "bad" nuts	**5** 3 samples of delivered nuts 1 sample of good nuts 1 sample of bad nuts
Point 2: Impact of storage at the end of storage (2007+2008)	**1** (30 days of storage)	**3** (1 month of storage)
Point 3: Impact of first selection (2007) Selected nuts & Discarded nuts	**2** 1 sample of selected nuts 1 sample of discarded nuts	**-**
Point 3: Impact of first selection & drying (2008) Selected nuts & Discarded nuts	**-**	**5** 3 samples of selected nuts 2 samples of discarded nuts
Point 4: Impact of second selection & drying (2007) Discarded nuts	**1**	**-**
Point 4: Impact of second and third selection / Quality control (QC) (2008) Selected nuts & Discarded nuts "Good" and "Bad" nuts from QC	**-**	**27** 3 samples of selected nuts 2 samples of discarded nuts x 2 selection processes 10 samples of "good" nuts 10 samples of "bad" nuts
Point 5: Impact of third selection & cooling (2007) Selected nuts & Discarded nuts	**2** 1 sample of selected nuts 1 sample of discarded nuts	**-**
Point 5 (in 2008) and 6 (in 2007): Impact of final storage	**1** (26 days of storage)	**3** (20 days of storage)
TOTAL	**10**	**43**

Table 7: Collected Brazil nut samples in the state of Acre

Sampling points	**Collected global Brazil nut samples**	
	2007	**2008**
In the rainforest and two associations of producers (in the association of producers 2, only in 2007)		
Point 1: Effect of contact time of pods with ground 1.2 New pods (0-5 days) 1.2 Old pods (15-20 days) 1.3 Old pods (about 2 months)	**-**	**31** 10 samples of new pods, 9 samples of old pods (15-20 days), 12 sample of old pods (about 2 months) from **3 production areas**
Point 2: Impact of Brazil nut selection after pod cracking 2.1. Non selected nuts 2.2. Selected nuts 2.3. Discarded nuts	**18** 6 non selected nut samples 6 selected nut samples 6 discarded nut samples from **6 production areas**	**33** 11 non selected nut samples 11 selected nut samples 11 discarded samples from 3-4 lots in **3 production areas**
Point 3: Impact of Brazil nut drying and selection 3.1. Selected nuts 3.2. Discarded nuts	**8** 5 selected nut samples 3 discarded nut sample from **3 production areas**	**22** 11 selected nut samples 11 discarded samples from 3-4 lots in **3 production areas**
Point 4: Impact of storage in common warehouse	**27** 1 sample x 4 large lots in bags x 6 storage times (0, 14, 28, 42 days, 5 months) in **the association of producers 1** 3 samples x 1 large lot in bulk x 1 storage time in **the association of producers 2** (4-6 months)	**78** 54 samples = 2 samples x 4 small lots in bags x storage times (0, 15, 30, 60, 90, 120 days) 3 samples x 2 large lots (one in bags and one in bulk) x 4 storage times (0, 15, 40, 60 days) in **the association of producers 1**
TOTAL	**53**	**164**
In a processing plant (large lots coming from the common warehouse of the association of producers 1, Point 4 stored in bags – see above)		
Point 1 (in 2008): Quality control (QC) at reception Delivered nuts "Good" nuts from QC "Bad" nuts from QC	**-**	**20** 10 samples of "good" nuts 10 samples of "bad" nuts
Point 2 (in 2008): Impact of storage	**-**	**6** 3 samples x 2 storage times (53 and 71 days)
Point 1 (in 2007): Impact of storage, drying and selection Selected nuts & Discarded nuts	**8** 1 selected nut sample x 4 large lots 1 discarded nut sample x 4 large lots	**-**
Point 3 (in 2008) **Impact of drying and selection** Selected nuts & Discarded nut	**-**	**5** 3 selected nut samples 2 discarded nut samples
Point 2 (in 2007) and 4 (in 2008): Impact of final storage	**8** 1 sample x 4 lots x 2 storage times (30 and 60 days)	**3** 3 samples x 1 storage time (9 days)
TOTAL	**16**	**34**

3.2.3: Development of a simple predictive model for aflatoxin and fungi production in the Brazil nut production chain (A.2.3)

This activity was carried out by NFA through the development of two experimental studies.

First study carried out from March to June 2007

Seventeen kilos of Brazil nuts in shell were collected in a community of the association of producers selected in the state of Pará and brought into Sweden in the beginning of March 2007.
An experiment started with storage of Brazil nuts in a respirometer. This experimental study aimed at gaining more knowledge of the growth of aflatoxigenic moulds and aflatoxin production in Brazil nuts in relation to humidity conditions and storage time. For this purpose, the fresh unprocessed Brazil nuts in shell were inoculated with an aflatoxin producing fungal strain of *Aspergillus nomius* previously isolated from Brazil nuts. The nuts were stored at 27 °C under three humidity conditions (97, 90 and 80 % relative humidity) in the respirometer for up to 3 months. Samples were taken on a regular basis for the analyses of the growth of aflatoxigenic moulds and aflatoxins by an improved and validated HPLC method for Brazil nuts.
The results of the experimental study were statistically analyzed and evaluated against the results from the field study conducted in the state of Pará, Brazil.
A full project report was delivered in November 2007 and from this material, a scientific article has been written and published in The World Mycotoxin Journal (see 3.4.5).

☞ *The full project report will be sent to STDF along with this final report.*

Second study carried out from March to April 2008

Further storage experiments were performed with the aim to:
- Test differences in growth rate and toxin formation between aflatoxigenic strains and species isolated from Brazil nuts, essentially according to the same temperatures and humidity as for the previous model experiment carried out in 2007;
- Test the impact of inoculation size on growth rate and toxin formation;
- See if different temperatures and humidities have any impact on growth rate and toxin formation;
- Discuss, on the basis of the results from this work, the results and the robustness of the previous model work.

A report was delivered to the Safenut general coordinator in June 2008.

☞ *This report will be sent to STDF along with this final report.*

The main conclusion from this study is that the results produced in the previous report are robust and can be used to predict and set the maximum storage time of Brazil nuts, to avoid levels above the maximum limit, under conditions allowing fungal growth such as in the rain forest or in the storage facility of the community or processing plant.

The models were used together with data from the field to the updated and validated good practices in the Brazil nut production chain at the prospect of the training course in good practises in the Brazil nut production chain (see 3.4.3).

The aflatoxin-strain used in the storage experiment was previously isolated from Brazil nuts imported to Europe in another project and tested for aflatoxin-production. The aflatoxin analyses showed that this strain was a good producer of both B and G aflatoxins and hence it was assumed that this isolate was an *Aspergillus parasiticus*. However, closer examination and with confirmation

by CBS in the Netherlands we have now showed that this is an *Aspergillus nomius.* This species may earlier been misidentified and has not been regarded as an important aflatoxin-producer in food commodities. Two more strains from this earlier project have been identified as *Aspergillus nomius.* This work, which is not a part of Safenut, has also been published in the World Mycotoxin Journal (see 3.4.5). It was indicated that *A. nomius* may be an important aflatoxin producer in Brazil nuts. During the Safenut final workshop in November 2008 this finding was also confirmed by unpublished data of Dr M Tanawaki from Instituto de Tecnologia de Alimentos (ITAL), Campinas, Brazil.

Main conclusions

Aflatoxin levels could be predicted from mould levels. There were high accuracy using laboratory data, and regional differences using field data.

The increase in probability of exceeding different aflatoxin maximum limits with time was described, based on field data.

The models can be used in practice to:
- evaluate the effect of storage humidity on the risk of high aflatoxin levels,
- predict aflatoxin levels based on mould levels,
- estimate how fast the risk of high aflatoxin levels increases under field conditions.

3.2.4: Formulate recommendations to update the existing manual of safety and quality in Brazil nuts (A.2.4)

The results of A2.2 and A2.3 were used for identifying the Critical Control Points along the Brazil nut production chain for aflatoxin contamination, and formulating recommendations to update the current Codex Code of Practice for Aflatoxin in Tree nuts (CAC/RCP 59 -2005, REV.1-2006).

Main conclusions:

The Safenut results (see Figures in Annex 7) showed that the practices currently used in the Brazil nut production chain, based on the existing manual of good practices, are not effective for aflatoxin control in unshelled nuts (levels above the European limits). The Critical Control Point is the drying step in the community which is not effective to dry nuts at a safe moisture level (corresponding to water activity less than 0.70). On the contrary, the moisture contents reached are often around the optimal range for aflatoxin production. This results in an increase of aflatoxin content during storage over several months. The Brazil nut lots delivered at the processing industries are already contaminated with high levels of aflatoxins, and the selection processes used in these industries are not effective to remove the contaminated unshelled nuts (nuts must first be broken).

Most of the collected Brazil nut samples contained equal amounts of aflatoxins B1 and G1 and a much lesser number of samples contained mostly/only B1, confirming earlier findings.

The Safenut results also showed that the aflatoxin producing fungi infect the nuts early in the forest.

Based on the above conclusions, the following recommendations were formulated to update the existing manual of good practices:

- The area under the Brazil nut trees should be cleared from residual pods and nuts from the former crop (Pre-collection). Broken pods, discarded nuts and old pods should not be left close to the collection area.

- The collection of the pods should proceed continuously as soon as they have fallen from the trees, by removing broken and damage ones. At this step, it is recommended that collectors are protected from accidents due to falling pods by using helmets.

- The nuts should be dried to a safe moisture level (water activity less than 0.70) within 10 days from the collection in the forest, after removing damaged, rotten, empty and rancid ones.

  The current recommendations of the Codex Code of Practice concerning the drying and storage in the extrativist communities are built on the assumptions that the nuts can be dried to a safe level by "spreading them out in thin layers, in open air, on clean surfaces, above ground level, and exposing them to sun drying and/or to a natural air circulation, with a regular turn" in the rain forest area, but the Safenut project has showed that this is not the case. The existing drying in the communities is not sufficient.

  Therefore, drying in the communities must be made more efficient and simple drying equipments are necessary.
  Or, the nuts should be transported within 10 days after collection in the forest to a processing plant for an immediate effective drying.

- An intermediate storage of the nuts, before reaching the processing facility, is only possible if the moisture content corresponds to a water activity below 0.70.

- It is also recommended that the current quality control used by most processing plants for evaluating the percentage of "bad" and "good" nuts through visual inspection after broken the unshelled nuts is further developed and validated. By doing so, this method may be used as a tool for decision if a lot can be used for production of in-shell nuts or if it should be shelled and sorted to eliminate bad nuts. Bad nuts are likely to contain very high levels of aflatoxins according to the results of the Safenut project and ConforCast project (www.agricultura.gov.br under the ConforCast logo).

The ideal flow chart recommended by the Safenut project for in-shell Brazil nuts is given in Annex 8.

3.3. Specific objective 3: Validation and implementation of a rapid aflatoxin surveillance system for use along the Brazil nut production chain

3.3.1: Adapt and validate existing rapid ELISA and on-site Lateral Flow Device (LFD) brought in to the project for aflatoxins in Brazil nuts (A3.1 & A3.3)

These activities have been carried out by CSL in conjunction with R-Biopharm AG.

Currently, Brazil nut inspection regarding aflatoxin levels is performed prior to export by using conventional High Performance Liquid Chromatography (HPLC). In Brazil, the certification of the consignments is performed by the Ministry of Agriculture ensuring that they were sampled and analysed by their Laboratory of Food Quality and Safety Control (LACQSA) in accordance with the Commission Regulation (EC) No 401/2006. The LACQSA is the only Brazilian laboratory recognized by the Commission Decision 493/2003/EC to perform aflatoxin analysis in Brazil nuts destined to exports. The HPLC method is validated for aflatoxin quantification in Brazil nuts and meets the legislative requirements. However, it is time consuming, expensive, requires highly qualified technicians and very specialized equipment, and uses large volumes of organic solvents.

There is a need for the Brazil nut industry to access rapid, robust and low-cost on site aflatoxin analytical methods that can be used throughout the Brazil nut production chain to monitor aflatoxin contamination and aid the implementation of a HACCP (Hazard Analysis and Critical Control Point) type control schemes based on preventive and correctives measures in order to meet the legislative limits for aflatoxins.

Immunoassays and in particular Enzyme-Linked ImmunoSorbent Assay (ELISA) have won wide acceptance over the past 25 years in clinical and non-clinical fields. R-Biopharm AG has developed an ELISA system for aflatoxins, and more recently a one step, on-site and robust Lateral Flow Device (LFD) for total aflatoxins. The LFD is an immunochromatographic test which results are read visually by observing the development of coloured bands (test and control lines) whereas the ELISA test requires a microtiter plate spectrophotometer for aflatoxin quantification.

On-the-market R-Biopharm AG rapid ELISA and LFD assays for the detection of aflatoxins were brought in to the project:
- RIDASCREEN® FAST Aflatoxin multi-standards (R5202) and RIDASCREEN® FAST Aflatoxin SC single control (R9002) for the measurement of total aflatoxins,
- RIDASCREEN® Aflatoxin B1 30/15 (R1211) for the measurement of aflatoxin B1,
- RIDA®QUICK Aflatoxin LFD (R5204) for the measurement of total aflatoxins.

Two fit-for-purpose studies were carried out to validate these R-Biopharm assays for the detection of aflatoxins in Brazil nut samples by comparing the ELISA and LFD formats against the standard HPLC method (for second study only). Assessments were based on samples that were spiked with aflatoxin B1 in the first study and naturally contaminated with aflatoxins in the second study (with varying concentrations of aflatoxins B1 and G1). The research on both occasions was undertaken at the R-Biopharm laboratories in Germany.

Note that HPLC and ELISA provide quantitative data; whereas LFD is semi-quantitative, determining minimum detection levels at set incubation time intervals (Table 8).

Table 8: Specifications of the ELISA and LFD assays

Name of the assay	**Principle / Format**	**Standard range (ppb)**	**Test implementation (incubation time)[1] (min)**	**Detection limit / Technical equipment / Result interpretation**
RIDASCREEN® FAST Aflatoxin	ELISA / 48 well MTP[2]	0 – 45	15	< 1.7 ppb Photometer Quantitative measurement
RIDASCREEN® FAST Aflatoxin SC	ELISA / 48 well MTP	0	15	Approx. 2 ppb Photometer Quantitative measurement
RIDASCREEN® Aflatoxin B_1 30/15	ELISA / 96 well MTP	0 - 50	45	1 ppb Photometer Quantitative measurement
RIDA®QUICK Aflatoxin	LFD / 20 reaction strips	-	reading at 4, 8, 16 min	Approx. 4 ppb Visual result interpretation[3] Semi-quantitative measurement

[1] Without including time for sample preparation ; [2] MTP = microtiter plate

[3] ≥ 4 µg/kg (ppb), 10 µg/kg (ppb) and 20 µg/kg (ppb) depending on incubation time and colour intensity of the test line.

☞ First fit-for-purpose study on Brazil nuts spiked with aflatoxin B1

Brazil nut samples with "0" and 20 ppb of aflatoxin B1 were prepared in duplicate, and analyzed by using the ELISA (RIDASCREEN® FAST Aflatoxin, RIDASCREEN® FAST Aflatoxin SC, RIDASCREEN® Aflatoxin B1 30/15) and LFD (RIDA®QUICK Aflatoxin) assays. The methods and results are presented in the deliverable report produced in December 2006.

☞ *This report will be sent to STDF along with this final report.*

For the ELISA RIDASCREEN® formats, all the "zero" samples gave result of < the limit of detection (LOD) and for the 20 ppb samples, results were within 1 ppb of the spike value for the RIDASCREEN® Fast Aflatoxin and Aflatoxin B1 and between 19.3 and 22.8 ppb for the RIDASCREEN® Fast Aflatoxin SC.

For the LFD, all the "zero" samples gave a negative result which was below the limit of detection of 4 ppb. For the 20 ppb samples, a band was present from 5 minutes which was just after the usual reading time of 4 minutes indicating that the analysed level was close to 20 ppb.

Based on this first study, it was concluded that any of these assays seemed to be suitable for the detection of aflatoxin levels in Brazil nuts.

☞ Second fit-for-purpose study on Brazil nuts naturally contaminated with aflatoxins

This second study has been carried out to add data to the preliminary study and further substantiate the previous findings and conclusions.

Brazil nut samples naturally contaminated with aflatoxins in a slurry form (1 part nut : 0.8 water) were obtained from a previous project and prepared at CSL over a range of about 2 to 300 ppb total aflatoxins (24 in total: 4 individually prepared sub-samples of six levels of aflatoxin contamination). Paired sub-samples were analysed by both the ELISA (RIDASCREEN® FAST Aflatoxin, RIDASCREEN® FAST Aflatoxin SC, RIDASCREEN® Aflatoxin B1 30/15) and LFD (RIDA®QUICK Aflatoxin) assays and HPLC.
In addition, a short ad hoc study was carried out to determine if the presence of peptone water in Brazil nut samples in a slurry form affected the performance of the ELISA assays (RIDASCREEN® FAST Aflatoxin, RIDASCREEN® FAST Aflatoxin SC). This was because it was envisaged to use the same samples for the mycological analysis (therefore containing peptone water) and the aflatoxin determinations by using the kits.

A deliverable report on the second fit-for-purpose study was produced in August 2007.

☞ *This report will be sent to STDF along with this final report.*

The following observations were made:

ELISA based methods were shown to detect aflatoxins at the lowest European threshold levels for total (4 ppb) and B1 (2 ppb) aflatoxins. The LFD assay was sensitive to 4 ppb total aflatoxins.

The ELISA RIDASCREEN® Fast aflatoxin tended to exceed the HPLC measurement at lower total aflatoxin concentrations and to be slightly lower at the higher concentrations but was still within the 95 % confidence limits throughout the range of samples tested.
The ELISA RIDASCREEN® FAST Aflatoxin SC tended to exceed the HPLC measurement and was not within the 95 % confidence limits throughout the range of samples tested.

The RIDASCREEN® Aflatoxin B1 30/15 tended to exceed the HPLC measurement and was not within the 95 % confidence limits throughout the range of samples tested.
Moreover, greater data variability was observed within replicate samples for the ELISA RIDASCREEN® Fast aflatoxin compared to the HPLC method.
For the RIDA®QUICK Aflatoxin (LFD), every measurement interpretation matched the concentration in the samples as measured by HPLC.
Peptone had only a negligible or no effect on the performance of the ELISA kits tested, but more measurements would be required to confirm this statistically.

At the Safenut progress meeting in September 2007, there were discussions on the possible effect of the lower cross reactivity of the antibodies to aflatoxin G1 compared to B1 used in the RIDASCREEN® FAST Aflatoxin kit for total aflatoxin measurement. This expressed concern was because of the high G1 to B1 ratio in most of the Brazil nut samples due to fungal species producing both toxins, such as *A. nomius.* Samples analyzed in the second fit-for-purpose study contained high ratios of G1 to B1 (5 groups of 4 sub-samples had an average ratio of 0.8, 1.2, 1.3, 1.9 and 2.8 respectively) but despite this, the line of equality in the statistical analysis was within the 95% Prediction Limits. Questions were also raised on the effect of high dilutions.

As consequence, it was agreed among the Safenut partners that additional tests should be performed as a double check for completing the evaluation of the ELISA and LFD kits. As for the previous fit for purpose studies, these tests will consist in a comparison of results obtained from the analysis of Brazil nut samples by both the kits and HPLC.
The general coordinator prepared a work plan proposal that was validated by CSL and R-Biopharm. This work plan included the following additional tests:

- Evaluation of the kits in samples with high amount of G1 to test the effect of lower cross reactivity of the antibodies to aflatoxin G1 compared to B1;
- Evaluation of the kits in samples with high amount of aflatoxins to test the effect of high dilutions;
- Further comparisons between the ELISA and LFD kits and HPLC as necessary, by analysing Brazil nut samples collected in the states of Acre and Pará, in order to provide a more complete series of data.

By considering the prepared work plan, a fully comprehensive protocol/Standard Operating Procedure was produced by the Brazilian team of LACQSA/LANAGRO-MG/MAPA with an estimation of the associated costs. This protocol was approved by the Safenut coordination but could not be implemented within the project's time frame and remaining CSL allocated budget, even trying to be restricted to the most relevant tests and reducing the associated costs.

☞ *The complete protocol will be sent to STDF along with this final report.*

No systematic analysis for ring testing the ELISA and LFD methods amongst laboratories for aflatoxin detection in Brazil nuts was undertaken. This would be a useful activity to consider in establishing inter-lab variability and the development of appropriate Standard Operating Procedures.

☞ Conclusion

The LFD assay was considered the most appropriate for use within the Brazil nut production chain for monitoring aflatoxin contamination. This assay is rapid, easy-to-use and reliable in non-technical hands. Moreover, it requires low investment and running costs. In looking at these aspects, it has therefore a clear advantage over the ELISA methods that require relatively sophisticated analytical equipment and skilled staffs. During the Safenut training courses, it was observed that

participants had difficulties to use the ELISA methods but not the LFD assay. The most laborious and technical part of the LFD process related to sample preparation. For all methods the extraction process for aflatoxins is ostensibly similar, and all are requiring of grinding/homogenising samples and use of solvents and some investment of time.

Consequently, the LFD is well adapted for a screening purpose at both field and laboratory levels and could be used by Brazil nut processing industries or middlemen. For quantitative aflatoxin measurement, especially at low concentrations (less than 4 ppb of total aflatoxins), ELISA or HPLC would be necessary to check if aflatoxin B1 levels are below 2 ppb.

3.3.2: Set up in Brazil a rapid ELISA and on-site LFD for aflatoxins in the laboratory, the Brazil nut production area and one processing plant (A3.2 & A3.4)

The following three training courses were organized in conjunction with Project specific objective 4 (see 3.4.2):

- Two training courses for both the ELISA and LFD methods in the states of Acre (25-26 October 2006) and Pará (24-25 September 2007) mainly for the Safenut partners,

- A training course in LFD method in the state of Pará (5 November 2008) mainly for key stakeholders of the Brazil nut sector and for some Safenut partners.

3.4. Specific objective 4: Knowledge & technology transfer to the key stakeholders

Appropriate dissemination of knowledge delivered by the Safenut project represented one of the major issues for project complete success. This was achieved through the use of different information systems within the project specific objectives 4 and 5 to reach various target audiences.

A particular attention was given to the dissemination of the project outputs to the Brazil nut private sector by involving them in the project activities and events (workshops, open meeting and training courses). Efforts have been made for the project also benefits Peruvian and Bolivian key stakeholders for ensuring a significant regional impact. However, because of the difficulties in identifying in these two countries the right partners involved in the Brazil nut sector and the limited financial resources of both these partners and the Safenut project, a more extensive participation of Peru and Bolivia in the Safenut events has not been possible.

For efficiency of time and resources, the training courses usually have been organized in conjunction with project meetings/workshops.

3.4.1- Training course in AFPA agar plate methodology for the identification of aflatoxin producing fungi in Brazil nuts (A.4.1.)

☞ Training course in AFPA agar plate methodology

A three days training course on the identification and quantification of *Aspergillus flavus/parasiticus* by using the validated standard AFPA agar plate methodology (Method No. 177, Nordic Committee on Food Analysis) took place at EMBRAPA, Rio Branco, state of Acre, Brazil on 25-27 October 2006 (Photos 1). This method is simple, low cost and rapid (48 h) compared to other conventional agar plate methods.

Photos 1: Training course in AFPA agar plate methodology, October 2006

This course was organized by NFA with the local support of EMBRAPA Acre, CIRAD and MAPA, and offered to the Safenut partners in order to make the method available for data collection of the project specific objective 2, and to key stakeholders of the Brazil nut sector as a safety management tool. It was free of charge for the participants who had to pay only the travel expenses as necessary.

Thirteen participants joined the course: 11 from Brazil (EMBRAPA Acre, LACQSA-MG and LANAGRO-PA / MAPA, SEAPROF), 1 from the Brazil nut processing industry Tahuamanu SA, Bolivia and 1 from CIRAD (Annex 9).

The course was led by two members of NFA (Pernilla Johnsson and Ann Gidlund) and consisted of an introductory lecture and hands on practice in a laboratory.

The lecture, which was also available for participants joining the ELISA/LFD course, dealt with the following aspects:
- Introduction to food mycology with specific emphasis on aflatoxin producing moulds,
- Description of the AFPA selective medium including advantages, disadvantages, strengths, weaknesses, and interpretation of results,
- Laboratory procedures in mycological methodology including dilution and direct plating, identification, quantification and isolation of *Aspergillus flavus/ A. parasiticus,*
- Storage techniques for isolates,
- Safety considerations.

Laboratory practices included:
- Demonstration of pre-grown AFPA-plates and practice on calculating quantitative results,
- Demonstration of direct plating of Brazil nuts,
- Dilution and plating of a freeze dried sample containing *Aspergillus flavus / A. parasiticus* and of a real nut sample,
- Microscopy of different fungal species with a focus on species that may be confused with *Aspergillus flavus/ A. parasiticus* on AFPA.

Documents that were developed for the course were:
1. A checklist for equipment that is needed to carry out the analyses,
2. A text covering the same aspects as the lecture (see above),
3. Protocols for dilution plating of freeze dried and real samples,
4. Protocol for direct plating of food samples,
5. Recipes for media preparation,

6. Sample records for documentation of analytical methodology and results.

Documents 2-6 were translated to Portuguese and distributed to all participants at the beginning of the course. The lecture is available on the Safenut website.

☞ Production of reference materials

Freeze dried samples of fungal mixture in glass vials (containing *Aspergillus flavus*, *A. niger*, *A. tamarii* and *Cladosporium cladosporoides*) and pure cultures of *A. flavus*, *A. parasiticus*, *A. niger*, *A. tamarii* and *Eurotium amstelodami* were produced and checked by NFA and distributed to the laboratories of microbiology of EMBRAPA Acre and LANAGRO-PA/MAPA (more than 10 vials per laboratory) for the use as internal reference material in mycological analysis of the field samples.

☞ Ring-tests for fungal analyses

Ring-tests for fungal analyses were organized by NFA for the laboratories of microbiology of EMBRAPA Acre and LANAGRO-PA/MAPA in charge of the identification and quantification of *Aspergillus flavus/parasiticus* in collected Brazil nut samples (project specific objective 2).

Such ring-tests consisted in analyzing a freeze dried fungal mixture produced by NFA by using the AFPA agar plate method. They have been carried out regularly since November 2006 by NFA and EMBRAPA Acre, whereas the LANAGRO-PA/MAPA participated the first time in March 2007 due to renovation work on their laboratory.

Since the quantitative content of the freeze dried samples of fungal mixture was statistically certified by NFA, the ring-tests enabled the evaluation and comparison of laboratory skills between the laboratories of EMBRAPA Acre and LANAGRO-PA/MAPA. It was continuously concluded that both laboratories had gained a sufficient precision in their analyses before the onset of data collection within the project specific objective 2.

3.4.2- Training courses in ELISA (Enzyme Linked Immunosorbent Assay) and Lateral Flow device (LFD) for aflatoxin analyses in Brazil nuts (A.4.2.)

In total, three training courses, focusing on ELISA and LFD use, were organized in Brazil by CSL and R-Biopharm AG with the support of Brazilian local teams and CIRAD. As for the mycology course on AFPA agar plate methodology, they were offered to the Safenut partners and key stakeholders of the Brazil nut sector as a safety management tool, and were free of charge for the participants who had to pay only the travel expenses as necessary.

First training course in ELISA and LFD kits

A two days training course in rapid ELISA and LFD assays for aflatoxin analyses in Brazil nut samples took place at EMBRAPA, Rio Branco, state of Acre, Brazil on 25-26 October 2006, i.e. at the same period than the training course on AFPA agar plate methodology (Photos 2).

This course was organized by CSL and R-Biopharm with the local support of EMBRAPA Acre, CIRAD and MAPA.

The attendance had to be limited to 8 participants: 7 came from Brazil (EMBRAPA Acre; CGAL-DF, LACQSA-MG, LANAGRO-MG and LANAGRO-PA / MAPA) and 1 from the Brazil nut processing industry Tahuamanu SA, Bolivia (Annex 10).

Photos 2: Training course in rapid ELISA and LFD assays, October 2006

The course was led by CSL (John Banks) and R-Biopharm (Dan Kaplan, Argentinean representative). It consisted of two lectures which were also available for participants joining the mycology course, followed by practical demonstrations and hands on use of the immunoassay kits for aflatoxin analyses with instructions and training materials translated in to Portuguese.

The first lecture by John Banks was a general overview and introduction to immunochemistry and the second by Dan Kaplan which is available on the Safenut website was on the specific assays being used on the course and the principles behind them.

Participants were trained in different assays ranging from the traditional and generally laboratory based ELISA through to rapid systems that can be used on site in the field without specialist equipment: the LFD plus the Aflacard (a flow through device). The following R-Biopharm kits were used for the training:

- The RIDASCREEN®FAST Aflatoxin SC,
- The RIDA®QUICK Aflatoxin LFD,
- The Aflacard.

Second training course in ELISA and LFD kits

A two days training course in rapid ELISA and LFD assays for aflatoxin analyses in Brazil nuts took place at LANAGRO-PA/MAPA, Belém, state of Pará, Brazil on 24-25 September 2007 in order to complement the course that took place on October 2006 for the implementation of both methods in the laboratories of EMBRAPA Acre and LANAGRO-PA/MAPA (Photos 3).

The course was organized and led by R-Biopharm Brazil (Guilherme Andrade) and CSL (John Banks) with the local support of LANAGRO-PA/MAPA, LANAGRO-MG/MAPA and CIRAD for its organization. It was offered to EMBRAPA and MAPA partners involved in the aflatoxin analysis (Annex 10) and also counted with the participation of Kelly Marcelino and Luiz Mascaretti, ALKA Tecnologia em Diagnósticos (www.alka.com.br). The course gave instruction and hands on experience in ELISA and LFD kits. Training materials were developed and distributed.

Photos 3: Training course in rapid ELISA and LFD assays, September 2007

Third training course in LFD kit

A one day training course in rapid LFD assay, as the method of choice for aflatoxin analyses in Brazil nuts, took place at LANAGRO-PA/MAPA, Belém, state of Pará, Brazil on 5 November 2008 (Photos 4), at the same period than the training courses in good practices, final progress meeting and workshop.

Photos 4: Training course in rapid LFD assay, November 2008

The course was organized and led by R-Biopharm AG Argentina (Dan Kaplan) and Brazil (Luiz Mascaretti) with the support of CSL, LANAGRO-PA/MAPA, LANAGRO-MG/MAPA and CIRAD for its organization. It was offered mainly to key stakeholders of the Brazil nut sector (8 participants from processing industries, including 1 from Peru and 1 from Bolivia, and 3 federal food inspectors from MAPA), with the aim to set up the method in the Brazil nut production area and one processing plant, and to some Safenut partners (Annex 10). The course was initiated by a demonstration of Brazil nut sample preparation (homogenization, slurry) carried out by LANAGRO-PA/MAPA (Mauricio Araujo), and then gave instruction and hands on experience in LFD kit. Training materials were developed and distributed.

Feedback was positive, but raised many questions as to implementation and the leverage points along the Brazil nut production chain. Brazil nut processing industries were identified as a primary user. These industries, exposed to LFD and how it could monitor for aflatoxins, expressed an

interest in its use, noting it could effect positive behavioural change within the production chain towards improved quality. Preliminary discussions were initiated as to where and how a LFD based quality control system might be implemented, focusing on the use of a LFD at the point of receipt of Brazil nuts from Amazonian communities or middlemen.
Further work is needed to demonstrate proof-of-principle use of LFD in effecting quality, noting that its use would need to be based around a Hazard Analysis Critical Control Point (HACCP)-styled quality scheme that provided traceability to the gathering stage. Project specific objective 2 provided useful information and recommendations on the nature of "best practice" in the harvesting of Brazil nuts.

3.4.3- Training courses in good practises in the Brazil nut production chain (A4.3.)

Two training courses in Brazil nut good practices were organized in the state of Pará on 12 November 2008 (about 30 participants from Brazil nut processing industries including 1 from Peru, ADE-Para and MAPA services) and in the state of Acre on 21 November 2008 (6 participants from extension services and EMBRAPA). These courses were built on the new knowledge from the Safenut project (A2.2, A2.3, and A.2.4) and other relevant data available from other projects or from literature.
In the state of Pará, the course was organized in conjunction with the ConforCast project seminar on "Sampling plans in Brazil nuts", 10-12 November 2008 with the support of LANAGRO-MG/MAPA and led by CIRAD (Catherine Brabet) and NFA (Monica Olsen), whereas in the state of Acre, the course was organized and led by CIRAD (Catherine Brabet) and EMBRAPA.

NFA gave one lecture concerning aflatoxin-producing fungi and the risk for human health and described how the models could be used to estimate the probability of exceeding different maximum levels, such as the current European legislation, the Codex level, with different storage time of Brazil nuts. CIRAD gave one lecture on the Codex Brazil nut good practices updated by the Safenut results.

- ☞ *A CD containing the PowerPoint presentations will be sent to STDF along with this final report.*

The courses were highly interactive with a strong contribution from all participants in discussions on the good practices to be implemented in the Brazil nut production chain.
The awareness of the critical points in the Brazil nut production chain for fungal growth and aflatoxin production have been extensively improved at all levels of the production chain.

In addition, within the framework of a training program promoted by ASBRAER (Associação Brasileira das Entidades Estaduais de Assistência Técnica e Extensão Rural) in partnership with MAPA and EMBRAPA, four training courses on Brazil nut good practices were offered in 2008 to extension technicians in Rio Branco (13-15 July, 35 participants), Manaus (22-24 September, 35 participants), Marabá and Santarém (10-15 November, 17 and 18 participants, respectively). During the courses organized in Marabá and Santarém, the Safenut final conclusions and recommendations on Brazil nut good practices were disseminated and discussed.

Another training course, as "Field Day", is planned in July 2009 in order to disseminate in a larger scale the updated Brazil nut good practices to the productive sector and extension technicians.

3.4.4- Development of a project specific website (A4.4.)

Safenut specific website

A Safenut specific website (http://www.stdf-safenutproject.com/) was designed in 2006 by CIRAD with the support of a Brazilian student (Sarah Biruel), and sent to STDF for prior authorization to put it on the internet.
The objective of this website is to contribute to the dissemination of project information and results, and relevant information related to the topic.

The website which was translated in English, Portuguese and Spanish includes:
- An overview of the Safenut project (funding, duration, region of execution, background, objectives, activities, partners, contacts),
- Information on the Safenut workshops, meetings and training courses,
- Project publications & other relevant bibliography.

Once the Safenut results are published, they will be placed on the project website.

An intranet with restricted access to Safenut partners was also created during the second year project to have a confidential space to facilitate the exchange of project working documents (project reports, laboratory procedures, etc.), advances and outputs.

The Safenut website was updated during the duration of the project. At the end of the project, CIRAD will continue to maintain and update it on own financial resources.

Project room on the NFA web page

NFA has created during Year 1 a project room on its web page (www.slv.se/safenut) which access is restricted to the Safenut partners by individual username and pin code.
The objective of this project room is to keep a confidential space for the Safenut partners to up- and down-load project working documents, advances and outputs.
By using this project room, video films describing particular parts of the mycological methods were made available for the partners performing the analyses.

3.4.5- Scientific and specific sector publications (A.4.5.)

Scientific publications in international peer-reviewed journals (Annex 11)

Olsen M, Johnsson P, Gidlund A, Ågren P, Thim A-M, Lindblad M, **2009**. Modelling the growth and aflatoxin production of different strains of aflatoxigenic fungi isolated from Brazil nuts (*in preparation, internal report delivered to CIRAD in June 2008*).

Johnsson P, Lindblad M, Thim A-M, Vargas E. A., Medeiros N.L., Brabet, C, Quaresma de Araújo M, Olsen M, **2008**. Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts. World Mycotoxin Journal 1 (2) 127-137.

Olsen M, Johnsson P, Möller T, Paladino R, Lindlblad M, **2008**. *Aspergillus nomius*, an important aflatoxin producer in Brazil nuts? World Mycotoxin Journal 1 (2), 123-126. (*NFA supported work connected to Safenut project*)

Oral presentations in congresses and meetings

Brabet C., **2008**. Avaliação da eficácia das praticas atuais para controle de ocorrência de aflatoxinas na cadeia produtiva da castanha-do-brasil. Seminário "Plano de amostragem para castanha-do-brasil, Projeto ConforCast, 10-12 November 2008, Belém-PA, Brazil

Olsen M, **2008**. Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts. Oral presentation (no abstract but the handouts of the poster were distributed) at the Scientific and Government Affairs Committee meeting during the Annual International Nut and Dried Fruit Council Foundation, 9-11 May 2008, Santiago, Chile (Cost covered by INC)

Olsen M, **2008**. Presentation of the Safenut project at the meeting of the SPS-working group, Swedish Ministry of Agriculture, 24 May 2008, Stockholm, Sweden (Cost covered by NFA)

Olsen M., **2007**. Aflatoxins and the Brazil nut production chain. XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, 21-25 May 2007, Istanbul, Turkey (Cost covered by NFA). Presentation of the Safenut project as part of this plenary lecture.

Olsen M, **2007**. Aflatoxins in the Brazil nut production chain**.** International Commission on Food Mycology workshop, 4-6 June 2007, Key West, USA (same presentation as in IUPAC in May 2007) (Cost covered by NFA)

Olsen M, **2007**. Presentation of background and expected results of Safenut project at a meeting with STDF (Melvin Spreij) and SIDA (the Swedish development cooperation agency: www.sida.se), 14 September 2007, Stockholm, Sweden (similar presentation as the above) (Cost covered by NFA)

Brabet C., **2006**. The STDF Brazil nut project. Meeting on "Interfaces cooperativas para pesquisa de interesse mundial em segurança de alimentos – Os sistemas Embrapa/MAPA e a rede SafeFoodera", Embrapa Headquarter, 13 April 2006, Brasilia-DF, Brazil

- ☞ *For the list of oral presentations of Safenut partners at project meetings and workshops, see the program of these events (Annexes 15, 17, 19-22)*
- ☞ *A CD containing the PowerPoint presentations will be sent to STDF along with this final report.*

Posters presented in congresses (Annex 12)

Iamanaka B. T., Taniwaki M. H., Calderari T. O., Brabet C., Palacios-Cabrera H. A., **2008.** Moisture Sorption Isotherms of Brazil Nuts at Different Temperatures. Safenut final workshop, 6-7 November 2008, Belém-PA, Brazil (CIRAD *supported work connected to Safenut project*)

Johnsson P., Lindblad M., Thim A. M., Jonsson N., Vargas E. A., Medeiros N. L., Brabet C., Quaresma de Araújo M., Olsen M., **2008**. Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts. 30th Mycotoxin Workshop, 28-30 April 2008, Utrecht, NL (Cost covered by NFA)

Banks J., Hasnip S., Anderson S., Colyer A., Luebbe W., Reck B., **2007**. Rapid immunoassays for aflatoxins in Brazil Nuts. IXth International Conference on Agri-Food Antibodies (Society of Food and Agriculture Immunology), 10-13 September 2007, Vettre (Near Oslo) Norway.

Banks J., Hasnip S., Anderson S., Colyer A., Luebbe W., Reck B., **2007**. Rapid immunoassays for aflatoxins in Brazil Nuts. XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, 21-25 May 2007, Istanbul, Turkey

Student reports

Leite F.M.N., **2008**. Fungos aflatoxigênicos na castanha-do-brasil sob as condições da floresta e de armazenagem comunitária no Acre. Dissertação (Mestrado em Agronomia) – Pró-Reitoria de Pesquisa e Pós-Graduação, Universidade Federal do Acre, Rio Branco-Acre, 2008, 97 p.

Devillers O., **2007**. Caractérisation et influence des pratiques de production sur la contamination par les aflatoxines de la filière noix du Brésil dans l'état de l'Acre (Brésil). Rapport de stage CIRAD/ENSAR, Décembre 2006-Mai 2007, 55p.

Leaflet on the Safenut project (Annex 13)

This leaflet was produced in English, Portuguese and Spanish. It was uploaded on the Safenut website and distributed by NFA (Monica Olsen) and CSL (John Banks) at the XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, 21-25 May 2007, Istanbul, Turkey.

Articles on the Safenut project and activities published on Internet and in specific journals (Annex 14)

CIRAD, **2009**. Noix du Brésil : exporter à nouveau vers l'Europe/Brazil nuts: resuming exports to Europe. Actualités/News on the homepage of CIRAD website, 6 May 2009. (http://www.cirad.fr/fr/index.php and http://www.cirad.fr/en/index.php)

INC, **2008**. Safenut project. Final workshop « Effective aflatoxin management in the Brazil nut production chain for recovering and consolidating export markets". (http://www.nutfruit.org/inc-projects?url=safenut_nov08)

Petithuguenin E.L., **2008**. Castanha-do-brasil: chega ao fim o projeto SafeNut. França Flash n° 65, 10 de outubro de 2008. (http://www.cendotec.org.br/ffpartes/ff65-2safenut.html). Published also in Spanish (http://www.cendotec.org.br/ffpartes/franciaf65-2safenut.html).

Ivarsson C., **2008**. EU regulatory measures harm a precious natural resource: The Amazon rain forest and its indigenous population. Food Marketing and Technology, February 2008, p 26-29.

Ivarsson C., **2008**. EU regulatory measures harm Brazil nut trade. The Cracker, January 2008, No 1, p 3-4.

CIRAD, **2006**. Maîtriser la qualité de la noix du Brésil. Electronic Journal of Cendotec, N° 75, March 2006, p. 3 (http://www.cendotec.org.br/be/be75.pdf)

EMBRAPA Acre, **2006.** Pesquisadores testam métodos para análise e identificação de aflatoxina. Noticias of the EMBRAPA Acre website, 23/11/2006 (http://www.cpafac.embrapa.br/)

EMBRAPA Acre, **2006.** Setores do agronegócio da castanha-do-brasil participam de Workshop Internacional. Noticias of the EMBRAPA Acre website, 09/07/2006 (http://www.cpafac.embrapa.br/)

TV and radio programs

In February 2008, three short TV programs on the Brazil nut production chain and actions developed by the Safenut project were divulgated during the local TV News "Noticias da Aldeia", Rio Branco, state of Acre.

☞ *A CD containing these TV programs will be sent to STDF along with this final report.*

EMBRAPA members have also been interviewed by a local radio, Program called “Roots of Land”, about “Contamination of Brazil nut by aflatoxins”.

Further scientific and specific publications on information and outputs generated by the Safenut project will be delivered after the end of the project, in particular:

- Scientific publications in international peer-reviewed journals on the results obtained within the project specific objectives 1 and 2;
- Specific publications for the Brazil nut sector: publication of guides and articles in technical journals;
- Oral presentations and/or posters in congresses. The Safenut partners planned to participate in the following two events in 2009: 47° SOBER congress (http://www6.ufrgs.br/sober47/), 26-30 July, Porto Alegre-RS, Brazil and ISM conference (http://www.ism2009.at/), 9-11 September, Tulln/Vienna, Austria.

3.5. Specific objective 5: To strengthen the public-private dialogue and partnership in the Brazil nut sector

3.5.1- Kick off meeting and first workshop (A5.1)

☞ Kick off meeting held in Rio Branco, State of Acre, Brazil, 12-14 July 2006

A three days kick off meeting was organized in the Amazon region by CIRAD and NFA for project launching with a local logistic support of EMBRAPA Acre. This first meeting between key representatives of all the project partners aimed to plan and organize the execution of the Safenut activities by the different partners, including administrative, financial and scientific issues.

The program and list of participants are presented in Annex 15 and 16, respectively.

The kick off meeting started by a presentation of the Safenut project, objectives and expected outputs of the meeting by the general coordinator and of the different partner participants and institutions in order to know each other better.
The organization and planning of the project activities were then thoroughly discussed in working groups formed according to the Safenut specific objectives (Photos 5), by using the tool Lots® (http://www.lots.mindo.com/EN/) which was brought into the project and presented during the kick off meeting by Monica Olsen, NFA.
The outputs and conclusions of the working groups were discussed in plenary session by all the partners, and a detailed work plan was elaborated for each project specific objective, including the activities to be carried out (what), methodology (how/where), expected outputs, human resources (who) by considering competence and relationships, chronogram (when), follow-up (list of check points to ensure the good progress of the activities) and identified risks.

The administrative and financial rules to be respected by all the partners for project running and management were also discussed and clarified during the kick off meeting, as well as the financial and scientific reports (guidelines and deadlines).
The project detailed work plan and rules were disseminated within the partner consortium as part of a Safenut action plan which resulted from the use of the tool Lots® during the kick off meeting.

The kick off meeting was followed by two days (15-16 July 2006) of field visits organized for the Safenut partners in the Brazil nut processing unit of COOPEACRE, Brasiléia, state of Acre. Unfortunately however, because of problems of access, a visit to Brazil nut production area was not possible.

Specific objective 1:
Marie-Gabrielle Piketty, Isabelle Vagneron (CIRAD)
Ricardo Raski, Ariane Thomazini (CCRC-SDA, SFA-AC/MAPA)
Marcio Muniz (EMBRAPA Acre)

Specific objective 2:
Catherine Brabet (CIRAD)
Pernilla Johnsson (NFA)
Otavio Durans (SFA-PA/MAPA)
Cleísa Cartaxo (EMBRAPA Acre)

Specific objective 3:
John Banks (CSL)
Guilherme Andrade (R-Biopharm AG Brazil)
Eugenia Vargas, Adriana Chagas (LANAGRO-MG, CGAL-SDA/MAPA)
Rivadalve Gonçalves (EMBRAPA Acre)

Photos 5: Safenut kick off meeting - Working groups

☞ International first workshop on "Prevention and Control of Brazil Nut Contamination by Aflatoxins" held in Rio Branco, State of Acre, Brazil, on July 10-11 2006

This workshop (Photos 6), which was held in the auditorium of the EMBRAPA Acre, preceded the Safenut kick off meeting. It was organized by CIRAD and NFA in collaboration with EMBRAPA Acre and MAPA, and with the financial support not only of the STDF program but also of the INC (International Nut and Dried Fruit Council).

C. D. Bazan (Candela Peru)

E. Vargas (MAPA Brazil)

Photos 6: Safenut first workshop

The program is presented in Annex 17.

More than 60 people participated at the workshop coming from the three main Brazil nut producing countries (Brazil, Bolivia, Peru) and European countries as partners of the Safenut project (France, Sweden and United Kingdom). The participants attending the workshop belonged to the scientific community (universities and research centres), the private sector (associations of Brazil nut producers/extractivists, processors and exporters), the government authorities and their services (technical assistance, rural extension, etc.) and non-government organizations.

The program included the following sessions:

- Two sessions with oral presentations on "Aflatoxin occurrence and its impact on Brazil nut production in the Amazon Region" (Session 1) and "Prevention and control of aflatoxins in the Brazil nut production chain" (Session 2).
  Six speakers as non direct Safenut partners (2 from Peru, 3 from Bolivia and one from Brazil) were invited and financed by the project:
  - Carlos Diego Bazán, quality control and R&D manager, Candela Peru, Lima, Peru
  - Miguel Chavez Pinchi, professor, UNAMAD (Universidad Nacional Amazónica de Madre de Dios), Puerto Maldonado, Peru
  - Brigido Orellana Camacho, Brazil nut producer, COINACAPA (Cooperativa Integral Agroextractivistas Campesinos de Pando Ltda), Pando, Bolivia
  - Jorge Cardenas Kanoth, Brazil nut producer, ACEERM (Asociación Campesina de Extractivistas Ecológicos de la Reserva Manuripi), Manuripi, Bolivia
  - Rodrigo Ramallo, forest engineer, Tahuamanu SA, Cobija, Bolivia
  - Aguimar Vasconcelos Simões, Technical manager, AGROCON- Projects and consulting in agro-ambient businesses, Manaus-AM, Brazil

  The other speakers were partners of the Safenut project.

- A session of group discussions:
  Three groups were formed for discussing the following topics:
  1. Brazil nut harvesting, transportation and storage. Which are the major obstacles for an efficient aflatoxin management system? (rapporteur Catherine Brabet, CIRAD - General coordinator)
  2. Brazil nut processing and aflatoxin control: cases of successful aflatoxin reduction (rapporteur Eugenia Vargas, MAPA)
  3. Collaboration and networking between private and public sector. Which role can the STDF Brazil nut project play? (rapporteur Ricardo Raski, MAPA)

- A poster session on the topic of the prevention and control of aflatoxin in Brazil nuts.

A talk was given by R-Biopharm AG on the rapid ELISA and LFD assays for aflatoxin analysis being brought in to the project, with an exhibition of the kits.

The workshop facilitated discussions and exchanges of information and experience on:
- the current situation for the Brazil nut sector in the three main Amazon producing countries (Brazil, Bolivia, Peru);
- the research works and other actions implemented, in progress or needs for controlling Brazil nut contamination by aflatoxins,
- the incentives for good management program for Brazil nuts.

It was also an opportunity to stimulate and reinforce the dialogue and collaboration between the private and public sectors, and for the Safenut partners to identify and get to know key stakeholders of the Brazil nut sector, in particular those from Peru and Bolivia.
The participation of key partners from the three main Brazil nut producing countries (Brazil, Bolivia, Peru) aimed to ensure a regional impact in the Amazon.

The outputs of the workshop, which were very useful for the Safenut kick off meeting, were synthesized in the Safenut action plan as "Present Situation: What has happened, where are we today and what insights have we gained? What do we believe about the future?".

3.5.2- First progress meeting and open meeting (A.5.2)

A two days progress meeting (Photos 7) was organized in Belém, State of Pará, 26-27 September 07 with the participation of key representatives of all the project partners (Annex 18).
This meeting which was initially planned by month 13 was postponed in order to make sure that enough scientific data would be available for fruitful discussions, as well as the key representatives of all the Safenut partners.

Photos 7: Safenut first progress meeting

The project results and future plans until the end of the project were presented by the different Safenut partners and discussed within each specific objective. The administrative and financial rules to be respected by all the partners for project running and management were also reminded by the Safenut general coordinator (Annex 19).
As a result of the progress meeting, the Safenut action plan was updated and disseminating to the project partners.

The progress meeting was preceded by two days (24-25 September) of further training course in ELISA and LFD methods for the Safenut partners (see 3.4.2), and followed by one day of open meeting (28 September) with the private sector (most of the Brazil nut processing industries of the state of Pará) and other Brazilian institution supporting the Brazil nut sector. This meeting also counted with the participation of a representative of the INC – International Nut and Dried Fruit Council Foundation (http://www.nutfruit.org/) and Besana industry (Dr Cameon Ivarsson, Director of Napasol AG - http://www.napasol.com/). The INC had already provided a financial support for the organization of the Safenut first workshop in July 2006.

The open meeting (Photos 8) provided an opportunity to exchange and discuss relevant information and experiences on Brazil nuts, and in particular to present the Safenut project and first results to the private sector in the state of Pará as well as experience of other research projects related to the topic (Annex 20). It also contributed to strengthen the public-private dialogue.

The progress and open meetings were organized by CIRAD with the support of NFA and the local team of MAPA in Belém-Pará, and held at SFA-PA (Superintendência Federal de Agricultura do estado do Pará)/ MAPA.

Photos 8: Safenut open meeting

3.5.3- Final progress meeting and workshop (A.5.3)

A two days international final workshop on "Effective aflatoxin management in the Brazil nut production chain for recovering and consolidating export markets" was held in Belém, State of Pará, Brazil on 6-7 November 2008 (Photos 9). This workshop which program is presented in Annex 21 was organized by CIRAD with the support of NFA and local Brazilian teams of MAPA and EMBRAPA.

Photos 9: Safenut final workshop

About 60 persons participated at the final workshop from the Brazil nut private sector (producers/extractivists and industries) of Brazil, Bolivia and Peru, research centres and universities, governments authorities and their services. The workshop also counted with the participation of a representative of the European Commission (Mr Frans Verstraete, DG SANCO) and members of the Technical Group on Food Additives and Contaminants (GTFAC) of the Codex Alimentarius Committee of Brazil (CCAB).
The invitation and participation of Mr Frans Verstraete was very much appreciated by the participants of the Safenut final workshop and the possibility to combine this workshop with the seminar of the ConforCast project on sampling plans in Brazil nuts (10-12 November 2008) gave the possibility for Mr Verstraete to participate in both. In this way the European Commission was given an important possibility to understand the complexity of the Brazil nut production chain and the problem of aflatoxin formation which is valuable for different issues concerning the trade of Brazil nuts in the future.

The final workshop allowed to:
- Disseminate and report all the results and conclusions generated by the Safenut project to the regional and international community;
- Present the actions and results of other key R&D projects for Brazil nut aflatoxin prevention and monitoring;
- Present the current state of the Brazil nut production chain and aflatoxin control in the other producing countries (Bolivia and Peru);
- Discuss how the results of the Safenut project can be incorporated into the Brazil nut production chain;
- Facilitate the dialogue and collaboration between the private and public sectors.

It was very appreciated by all the participants, in particular the Brazil nut private sector that acknowledged the work done by the Safenut project. This sector had a very good participation and contribution at both Safenut and ConforCast events (they were mobilized for 6 days).

The final workshop was preceded by two days of final progress meeting for the Safenut partners (3-4 November 2008) and one day training course in LFD kit (5 November 2008) (see 3.4.2).
The final progress meeting allowed presenting and discussing all the results and conclusions of the Safenut project among partners and finalizing the oral presentations for the final workshop. The program and list of participants are given in Annex 22.

4. MEASURABLE PROJECT IMPACTS

☞ **Reinforcement of the private-public dialogue and partnership in the Brazil nut sector**

The organization of the Safenut workshops (first workshop in July 2006 and final workshop in November 2008) and open meeting (September 2007) have contributed to an improved dialogue on Brazil nut issues between the private and public sectors with high participation of both sectors in the debates in plenary session and working groups during these events.

The cooperation between the Brazil nut sector and public institutions was strengthened through the involvement of key stakeholders of the Brazil nut production chain (associations of producers/extractivits; processing industries) in the Safenut activities (field participative research).
Other public-private partnerships have started in Brazil after the Safenut final workshop, for example to conduct works on Brazil nut dehusking and sorting machines. CIRAD is also in contact with a Peruvian Brazil nut processing industry to develop research activities for improving product quality.

☞ **Regional impact in the Amazon**

A particular attention was given by the Safenut partners to disseminate project outputs to key stakeholders not only from Brazil but also from Peru and Bolivia for ensuring a significant regional impact.

This was achieved through the participation of key Peruvian and Bolivian stakeholders in the Safenut workshops and training courses in AFPA agar plate methodology for the isolation and quantification of aflatoxin producing fungi, rapid ELISA and LFD methods for aflatoxin analysis and Brazil nut good practices.
In total 7 Peruvian and Bolivian participants (mainly Brazil nut producers and processing industries) were funded by the Safenut project in order to be able to attend the project workshops and training courses (2 from Peru and 3 from Bolivia in July 2006 ; 1 from Peru and 1 from Bolivia in November 08).
The workshops facilitated and strengthened the exchange of scientific and technical information and experiences on Brazil nut issues between the three main Brazil nut producing countries, as well as the dialogue and debate on research results and their application in the Brazil nut production chain and on further research needs.

However, because of the difficulties in identifying the right Peruvian and Bolivian partners involved in the Brazil nut sector and the limited financial resources of both these partners and the Safenut project, a more extensive participation of Peru and Bolivia in the Safenut events has not been possible.

The dissemination of Safenut project information and results to the Peruvian and Bolivian countries was also achieved through the Safenut website and scientific publications. This will continue after the end of the project through website updating and publication of new scientific and technical documents.

☞ **International impact in the Brazil nut sector**

The participation of Dr Cameon Ivarsson (Director of Napasol AG - http://www.napasol.com/), as representative of INC - International Nut and Dried Fruit Council Foundation (http://www.nutfruit.org/)) in the Safenut open meeting on 28 September 2007 led to an article on the subject written by Dr Ivarsson and published in both The Cracker (January 2008) and Food Marketing and Technology (February 2008). This also supported a global awareness of the Safenut project which will hopefully create a future platform for recovering export markets.

The Safenut project got very positive feedback from the INC meeting in Santiago, Chile, 9-11 May 2008. The chairman of the Scientific Committee, Mr Pino Calcagni, gave a lot of credits to Safenut also in the plenum meeting even though Brazil nuts are very small in the total nut industry. There were more than 450 participants, most of them from exporters and importers but also some from the production side. Several participants, especially among producers, expressed their interest on the approach of the Safenut project.

Through the invitation and participation of Mr Frans Verstraete, from DG SANCO of the European Commission, in the Safenut final workshop in November 2008, the European Commission was given an important possibility to understand the complexity of the Brazil nut production chain and the problem of aflatoxin formation which is valuable for different issues concerning the trade of Brazil nuts in the future.

The participation of NFA in meeting with SIDA and The Swedish Ministry of Agriculture has led to awareness of how the Safenut project has realised some of the intensions of Article 9 of the SPS agreement. The Ministry of Agriculture and NFA disseminated information about Safenut as an example of technical support to developing countries during the International Seminar on Setting Food Safety Standards.

☞ **Improvement of the laboratory capacity and skills in the Amazon region for Brazil nut sample reception, preparation, storage and analyses**

The Safenut project provided trainings and continuous technical and methodological support to the project local teams and laboratories in the state of Acre (EMBRAPA) and Pará (LANAGRO-PA/MAPA) for Brazil nut sample reception, preparation, storage and analyses (water activity and aflatoxin-producing fungi) in order to attend the Safenut activities and requirements.

This included:

- Adequacy of the laboratory infrastructure and facilities
  The Safenut project stimulated renovation work and changes in the laboratory lay-out, in particular to avoid Brazil nut sample contamination, as well as allowed the purchase and installation of equipment for Brazil nut sample preparation (balance, meat grinder, dough mixers and blenders), and water activity analysis (water activity measurer).
  The general coordinator would like to highlight the continuous technical support of LANAGRO-MG/MAPA for the adequacy of laboratories, and the good cooperation of the coordinator of LANAGRO-PA/MAPA, Francisco Airton Nogueira, where there were no conditions to receive and prepare Brazil nut samples at the beginning of the Safenut project.

- Improvement of laboratory skills for Brazil nut sample preparation
  A continuous technical support was given by LACQSA/LANAGRO-MG/MAPA for Brazil nut sample preparation, with the organization of two training courses at both LANAGRO-PA/MAPA (9-10 July 2007) and EMBRAPA Acre (11 July 2007).

- Improvement of laboratory skills for fungal analysis
  A training course in AFPA agar plate methodology for the isolation and quantification of aflatoxin producing fungi in Brazil nuts was organized by NFA in October 2006 for the Safenut partners performing the analyses. The analytical quality of the laboratory of microbiology of both EMBRAPA Acre and LANAGRO-PA/MAPA was evaluated and improved by NFA through the organization of ring-tests by using the AFPA agar plate methodology and internal reference material, and technical recommendations. In particular, video films describing some

parts of the mycological methods were made available for the Safenut partners on the project room created by NFA on the internet.

- Elaboration of detailed protocols for Brazil nut reception, handling, preparation and analysis (water activity and aflatoxin-producing fungi)
  These protocols were provided to the Brazilian local teams of both EMBRAPA Acre and LANAGRO-PA/MAPA.

The technical knowledge of LANAGRO-PA/MAPA and EMBRAPA Acre has greatly improved due to Safenut project's actions. The new skills have already been used in other projects at EMBRAPA Acre and MAPA and can be adapted to other commodities as well.

Even if the laboratory capacity and quality controls must be improved, both EMBRAPA Acre and LANAGRO-PA/MAPA have now their laboratory implemented with adequate conditions to receive, prepare, and store Brazil nut samples for aflatoxin and fungal analysis (Photos 10). The implementation of these laboratories within the Safenut project has a strategic importance since it constitutes a first stage to have in the long term laboratories for aflatoxin and fungal analysis in the Amazon region at the service of the Brazil nut sector.

EMBRAPA Acre

LANAGRO-PA/MAPA

Photos 10: Implemented adequate laboratories for Brazil nut sample preparation at EMBRAPA Acre and LANAGRO-PA/MAPA

As a consequence of the Safenut project, two Brazilian partners had the opportunity to visit NFA in Sweden and CIRAD in France to be trained in techniques for fungal analysis and identification:

- Julio Cesar Garcia, technician, LACQSA/LANAGRO-MG/MAPA, 15 days (14-27 September 08) at the Microbiology Division of NFA, Uppsala, Sweden: Training in the identification of aflatoxin-producing fungal species isolated from the Safenut Brazil nut samples while examining and counting the AFPA plates (morphological and physiological characterization). This study was suggested by Monica Olsen with the objective to check if the strains that were used for the model work carried out at NFA are similar to the ones found in the Brazil nut production chain, and consequently check if the models developed are valid in all cases.
  This training course has contributed to the implementation of the mycological activities carried out by LANAGRO-MG.

- Daniela Matias de C. Bittencourt, researcher, EMBRAPA Acre, about 2 months (8/09/08-31/10/08) at CIRAD, Montpellier, France: Training in the application of the PCR-DGGE method for studying the evolution of the mycoflora along the Brazil nut production chain

(molecular approach). This study was suggested by CIRAD. The DNA of the mycoflora (total mycoflora and aflatoxin-producing fungal strains) was extracted from Brazil nut samples and fungal cultures at EMBRAPA Acre. The DNA samples were then amplified by PCR and analyzed by DGGE at CIRAD. This technique aims to generate DGGE profiles of the mycoflora at different points of the Brazil nut production chain.

These trainings were implemented after agreement of the Safenut partners and STDF. All the extra costs were paid by other resources of CIRAD, NFA and the Brazilian partners (and not the Safenut budget).

☞ **Skill improvement of the Safenut partners and key stakeholders of the Brazil nut sector for aflatoxin detection by rapid analytical methods**

Two training courses in rapid ELISA and LFD methods for aflatoxin analysis in Brazil nuts were organized in the states of Acre (25-26 October 2006 – 7 participants from EMBRAPA and MAPA and one from Bolivia) and Pará (24-25 September 2007 – 5 participants from EMBRAPA and MAPA), mainly for the Safenut partners.

A training course in LFD method was organized in the state of Pará (5 November 2008) mainly for key stakeholders of the Brazil nut sector (8 participants from Brazil nut processing industries including 1 from Peru and 1 from Bolivia; 3 federal food inspectors from MAPA) and for some Safenut partners (3 participants from EMBRAPA Acre and LACQSA/MAPA).

Training materials were produced and are available on the Safenut website.

☞ **Improved knowledge of key stakeholders on Brazil nut good practices for aflatoxin control along the production chain**

Two training courses in Brazil nut good practices were organized in the state of Pará (12 November 2008 – about 30 participants from Brazil nut processing industries including 1 from Peru, ADE-Para and MAPA services) and Acre (21 November 2008 – 6 participants from extension services and EMBRAPA). These courses were highly interactive with a strong contribution from all participants in discussions on the good practices to be implemented in the Brazil nut production chain. The awareness of the critical points in the Brazil nut production chain for fungal growth and aflatoxin production have been extensively improved at all levels of the production chain.

In addition, within the framework of a training program promoted by ASBRAER (Associação Brasileira das Entidades Estaduais de Assistência Técnica e Extensão Rural) in partnership with MAPA and EMBRAPA, four training courses on Brazil nut good practices were offered in 2008 to extension technicians in Rio Branco (13-15 July, 35 participants), Manaus (22-24 September, 35 participants), Marabá and Santarém (10-15 November, 17 and 18 participants, respectively). During the courses organized in Marabá and Santarém, the Safenut final conclusions and recommendations on Brazil nut good practices were disseminated and discussed.

☞ **Improved capacity to predict aflatoxin contamination for more effective risk management**

The statistical models developed within the Safenut project can be used in practice to:
- predict aflatoxin levels based on mould levels,
- evaluate the effect of storage humidity on the risk of high aflatoxin levels,
- estimate how fast the risk of high aflatoxin levels increases under field conditions.

☞ Contribution to the Codex discussions and works on aflatoxin contamination in Brazil nuts

NFA and CIRAD participated in *ad hoc* working groups (WG) during the ConforCast project seminar (10-12 November 2008) concerning the Codex work on aflatoxins in Brazil nuts. The WG included member from the Brazilian delegation and the European Commission (Mr Frans Verstaete) and the discussion elucidated several of the specific circumstances included in the Brazil nut production such as the extreme heterogeneous distribution of aflatoxin in Brazil nuts and the subsequent difficulties in sampling.

NFA has also supported the work of the Brazilian delegation concerning the Discussion Paper on Aflatoxin Contamination in Brazil nuts (Codex Committee on Contaminants in Food, CCCF) and suggested important changes to the Codex Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Tree Nuts (CAC/RCP 59 -2005, REV.1-2006) which were supported by the outcomes of the Safenut project (copied to Michael Roberts, WTO on 8 December 2008).

The recommendations of the current Codex Code of Practice for aflatoxins in tree nuts concerning the drying and storage in the extrativist communities are built on the assumptions that the nuts can be dried to a safe level by "spreading them out in thin layers, in open air, on clean surfaces, above ground level, and exposing them to sun drying and/or to a natural air circulation, with a regular turn" in the rain forest area, but the Safenut project has showed that this is not the case.

According to the report of the 3rd session of the CCCF in March 2009 (ALINORM 09/32/41, http://www.codexalimentarius.net/web/archives.jsp?lang=en), the Committee agreed to initiate new work on the revision of the Code of Practice for the Prevention and Reduction of Aflatoxin in Tree Nuts (additional measures for Brazil nuts) taking into account the results and recommendations of the Safenut project. If the Commission approves, the Proposed Draft Revision prepared by the Delegation of Brazil will be circulated for comments at Step 3 and consideration at the 4th Session of CCCF in 2010. Adoption at Step 5/8 can be expected by 2010.

5. RECOMMENDED FOLLOW-UP ACTIONS

The Safenut project contributed to the identification of the critical points/factors for fungal growth and aflatoxin contamination in the Brazil nut production chain and the update of existing manuals of good practices, as well as to the identification of the socio-economical constraints for the adoption of good practices by the stakeholders and the formulation of organizational and incentive strategies. In addition, the project strengthened the training of Brazil nut stakeholders both on good practices and rapid aflatoxin analytical methods for toxin prevention and monitoring.
Despite this important progress, actions are still necessary to effectively recover and consolidate the Brazil nut market export, in particular in Europe for in-shell nuts.

The follow-up actions identified by the Safenut project are:

☞ **Research activities to test and validate the quality management system recommended by the Safenut project for aflatoxin prevention and monitoring in the Brazil nut production chain**

The Safenut project demonstrated that the practices currently implemented in the Brazil nut production chain based on the recommendations of the existing guidelines on good practices, in particular the Codex ones, are not effective for reducing aflatoxins in unshelled nuts below the European tolerated limits, without Brazil nut drying to a safe moisture content (water activity less than 0.7 in order to prevent fungal growth and aflatoxin contamination) within 10 days after collection and adequate storage conditions to avoid moisture re-absorption.
This means that within 10 days after collection, the unshelled Brazil nuts must be dried in the extractivist communities or transported and dried in the processing industries.

Based on such considerations, the following research actions were identified:
- Testing simple and low-cost dryers (using solar, air –convection-, gas or pods as heat sources) in the extractivist communities, soon after collection of pods in the forest, to assess the technical and economical feasibility of these equipments to dry unshelled nuts to a safe moisture content;
- Testing the effectiveness of the rotating dryers used in the processing industries to dry unshelled Brazil nut to safe moisture content;
- Testing the effect of storage conditions in the extractivist communities and processing industries on moisture re-absorption by Brazil nuts dried to safe moisture content. In particular, the maximum storage time should be defined according to storage conditions;
- Evaluate the conditions of Brazil nut maritime transport in containers, between Brazil and the port of destination in Europe where official aflatoxin control are performed, and their effect on fungal growth and aflatoxin contamination. The development of special container prototypes may be necessary with the aim to avoid condensation and moisture re-absorption by Brazil nuts dried to safe moisture content.

It is also recommended that the current quality control system used by most processing plants for evaluating the percentage of “bad” and “good” Brazil nuts through visual inspection after broken the unshelled nuts is further developed and validated. By doing so, this method may be used as a tool for decision if a lot can be used for production of in-shell nuts or if it should be shelled and sorted to eliminate bad nuts. Bad nuts are likely to contain very high levels of aflatoxins according to the results of the Safenut project and ConforCast project (www.agricultura.gov.br under the ConforCast logo).

Finally, the Safenut project has identified the LFD (Lateral Flow Device) assay as the most appropriate rapid method for total aflatoxin analysis in Brazil nuts for a screening purpose at both field and laboratory levels, and the Brazil nut processing industries as primary users, for monitoring aflatoxin contamination in the production chain. Further work is needed to:

- Confirm where and how a LFD based quality control system might be implemented along the Brazil nut production chain. Preliminary discussions were initiated within the Safenut project focusing on the use of LFD at the point of receipt of Brazil nuts in the processing industry from Amazonian communities or middlemen;
- Demonstrate proof-of-principle use of LFD in effecting quality, noting that its use would need to be based around a Hazard Analysis Critical Control Point (HACCP)-styled quality scheme that provided traceability to the gathering stage.

For quantitative aflatoxin measurement, especially at low concentrations (less than 4 ppb of total aflatoxins), ELISA or HPLC would be necessary to check if aflatoxin B1 levels are below 2 ppb.

☞ **Extension activities for a larger-scale dissemination of Brazil nut good practices and LFD method to the key stakeholders**

- Larger-scale dissemination of effective and sustainable Brazil nut good practices, in particular to producers/extractivists (most of the communities are still not aware of good practices for reducing aflatoxin contamination).
  The training activities in Brazil nut good practices carried out within the Safenut project have been limited (two trainings organized in the states of Acre and Pará for the Brazil nut sector) because the project has prioritized the identification of critical points/factors and good practices as a prerequisite (collection and analysis of a larger number of Brazil nut samples during two harvest periods and implementation of a more comprehensive laboratory experimental study in Sweden than originally scheduled in the Safenut proposal). In addition, research activities are still needed to test and validate the Brazil nut good practices recommended by the Safenut project.

- Larger-scale dissemination of the LFD method for monitoring aflatoxin contamination
  Half of the Brazil nut processing industries of the states of Acre and Pará participated in the training course on LFD organized by the Safenut project. This training must be extended to the other industries in Brazil.
  In addition, technical support to the Brazil nut processing industries must be provided, as necessary, for the effective implementation of a validated aflatoxin monitoring system along the Brazil nut production chain by using the LFD assay.

The training courses on good practices and LFD method organized by the Safenut project also benefited Brazil nut processing industries from Peru and Bolivia. These courses must also be extended in both these countries for a greater regional impact.

☞ **Stimulate the adoption of good practices by the Brazil nut private sector through incentive instruments**

Economical incentives:
The Safenut project highlighted the need to develop mechanisms for price differentiation of Brazil nuts according to product quality (premium price) to offset the additional production costs related to the implementation of good practices. A premium price should be paid by European importers and benefits distributed among the actors, until producers/extractivists. According to the Safenut socio-economic results (project specific objective 1), since 2005, the price paid by the European Community for unshelled Brazil nuts is the highest compared to the other export markets. Nevertheless, most of the producers are not paid according to product quality.

Regulatory incentives:

Maximum limits of aflatoxins:

Although Europe is paying the highest price since 2005, the Brazil nut sector is currently discouraged to export in-shell nuts to Europe because it is difficult to respect the European aflatoxin standards which are the strictest worldwide, especially for Brazil nuts which is supplied entirely from extractivist production (and not cultivated as other tree nuts). Consequently, the stakeholders do not want to risk having their consignments rejected.
In addition, other export markets for in-shell Brazil nuts have emerged in countries where regulation for aflatoxins is less strict. The existence of these alternative markets may make difficult the implementation of good practices in the Brazil nut production chain to reduce aflatoxin levels to the European standards.

In that context, there is a need for a worldwide regulatory aflatoxin levels in Brazil nuts, based on scientific evidence, aiming at the protection of human health with a minimum economical impact on international trade.

In light of the conclusions of the JECFA risk assessments associated with aflatoxins in tree nuts, the Codex Alimentarius Commission (CAC) adopted in 2008 maximum total aflatoxin levels of 10 ppb and 15 ppb in almonds, pistachios and hazelnuts, respectively ready-to-eat and for further processing.
On the request of the Delegation of Brazil, the Codex Committee on Contaminants in Foods (CCCF) agreed to start new work on Draft Maximum Levels for Total Aflatoxins in Brazil Nuts destined for further processing and ready-to-eat, both in-shell and shelled nuts. According to the report of the 3rd session of the CCCF in March 2009 (ALINORM 09/32/41), the Committee agreed to return the Proposed Draft Maximum Levels to Step 2/3 for redrafting by the Delegation of Brazil, comments and consideration by the next session of the Committee.

Official control methods for aflatoxins in unshelled nuts

In the case of in-shell Brazil nuts, rotten kernels (i.e. the ones most likely contaminated by aflatoxins) can only be identified once they are taken out of the shell. It has also been demonstrated in a study conducted in Sweden (Marklinder *et al.*, 2005[1]) that consumers will discard the rotten nuts because of its appearance and strong smell. European official controls for aflatoxins are done on the in-shell nuts and therefore may include rotten kernels which contribute to high aflatoxin levels of the consignment. In addition, the ConforCast project has shown that the shells contribute significantly to the aflatoxin concentration in the whole nut, even when the nut is visually classified as good nut (www.agricultura.gov.br under the ConforCast logo). These results were presented during the 3rd session of the CCCF in Rotterdam, March 23-28, 2009.

Organizational incentives:

- Development of a consortium for Brazil nuts that will reinforce the dialogue and partnership among the different stakeholders and between the private and public sectors, and support research initiatives, marketing, etc.

- Identification of Brazil nut production areas close to the processing industries, with good practices and infrastructures, in order to deliver and dry the unshelled nuts in the industries

[1] Marklinder I., Lindblad M., Gidlund A., Olsen M., 2005. Consumers'ability to discriminate aflatoxin-contaminated Brazil nuts. Food Additives and Contaminants, January 2005, 22 (1): 56-64.

within 10 days after pod falling. The development of an integrative system in which producers are under contract to the industries was discussed to enable the implementation of the recommended good practices for unshelled nuts.

☞ **Diversification of the Brazil nut products to aggregate value**

Today, there is a growing willingness of the Brazil nut industry to develop and commercialize products with added value and less aflatoxin contamination risks, such as shelled nuts and derived products. This approach must be accompanied by a strengthening of policies to support local Brazil nut processing.

6. INDIVIDUAL PARTNER FINAL REPORTS

INDIVIDUAL PARTNER FINAL REPORT

PARTNER: CIRAD – FRANCE

CIRAD team

Catherine Brabet, General coordinator, PhD Food science
Marie-Gabrielle Piketty, PhD socio-economist
Isabelle Vagneron, PhD, socio-economist

Management activities

As the general coordinator of the Safenut project, CIRAD was in charge of the overall administrative and financial management, the planning and follow-up of the scientific activities in conjunction with the scientific coordinator (regular contacts with Monica Olsen) for ensuring the execution of the work program, the progress reporting to STDF and the timely and appropriate problem solving.
The Safenut general coordination was performed by Catherine Brabet, with the support of CIRAD services, Montpellier, France specialized in financial, administrative and legal issues.

The management activities concerned:

The formalization and commitment of the Safenut partnership
- The general coordinator prepared contracts of technical and financial cooperation between CIRAD and the other project partners by following the model of the contract signed by CIRAD and WTO. The contracts were then reviewed by the CIRAD administrative and legal services before sending them to the Safenut partners for signature.
- In order to solve the problems and delays encountered for the signature of the contracts with the Brazilian partners (EMBRAPA and MAPA), the general coordinator has had several contacts with the Brazilian partners, CIRAD legal services and STDF, meetings of the general coordinator and Brazilian partners were organized in Brasilia-DF and technical notes were prepared and sent to the Brazilian partners for clarifying their doubts on contract issues.

Fund transfer to the Safenut partners
- CIRAD transferred to all the Safenut partners 50 % and 35 % of their total budget once the contracts of technical and financial cooperation were signed and 50 % and 35 % of the total Safenut budget received from WTO.
- Due to the delay in the signature of the contracts with EMBRAPA and MAPA and consequently in the fund transfer to these partners, CIRAD assumed directly the expenses of the Brazilian partners as necessary in order not to compromise the execution of the project activities. The advanced amounts were invoiced to the Brazilian partners as supporting documents for their accounts, and deduced from the first fund transfer to these partners (i.e. in September and November 2007, respectively for EMBRAPA and MAPA).

Safenut rules to be respected by all the partners
The rules of project running and management, including the STDF and WTO ones, were presented by the general coordinator to the whole consortium members during the Safenut kick off meeting, July 2006 and the first annual progress meeting, September 2007.

Development of a Safenut action plan
The general coordinator participated in the writing and updating of the Safenut action plan.

Planning, organization and follow up of the Safenut scientific and dissemination activities
As general coordinator, CIRAD has been responsible for the follow up of the Safenut scientific activities in collaboration with the scientific coordinator (regular contacts of the general coordinator with Monica Olsen through emails and skype-meetings) and for the timely and appropriate problem

solving in order to ensure the execution of the work program. CIRAD also supported actively the planning and organization of the Safenut activities in conjunction with the other project partners.
In particular, CIRAD organized and participated in several meetings in Brazil (Belém, state of Pará ; Rio Branco, state of Acre ; Belo Horizonte, state of Minas Gerais ; São Paulo-SP) for the planning, organization and follow up of the field and laboratory technical activities (project specific objective 2), as well as the socio-economic activities (project specific objective 1) (Annex 1).

Support for material purchase in Brazil
The general coordinator purchased equipment and consumables for EMBRAPA and MAPA from Brazilian suppliers located outside the Amazon region.

Collaboration with the Brazil nut private sector as focus group of the Safenut project
- The general coordinator supported the Brazilian partners for making the contacts and formalizing the collaboration with the Brazil nut private sector (cooperatives and associations of producers/extractivits, processors/exporters) in the states of Acre and Pará in order to involve them in the activities of the Safenut project.
- The general coordinator also developed and maintained the collaboration with key Bolivian and Peruvian partners in order to ensure the Safenut regional impact in the Amazon.

Selection of students and consultants for Safenut activity support
The general coordinator selected the following students and consultants, and was in charge of solving the administrative issues for formalizing their participation in the project (visa, training agreement, contract of consultancy, etc.):
Students:
- Janaína Deane de Abreu Sá Diniz, Brazilian PhD student from UnB (Universidade de Brasilia) for supporting the socio-economic activities in the state of Pará (project specific objective 1);
- Olivier Devillers, French engineering student from ENSAR (Ecole Nationale Supérieure Agronomique de Rennes, France) for supporting the activities A2.1 and A2.2 in the state of Acre (project specific objective 2),
- Sarah Biruel, Brazilian student from the Colégio Politécnico Bento Quirino for the Safenut website design and implementation,
- César Augusto Piedrahíta Aguirre, Colombian student from UNICAMP-FEA (Universidade Estadual de Campinas-Faculdade de Engenharia de Alimentos) for the Safenut website Spanish translation.

Consultants:
- Douglas Storto, Brazilian engineer, for supporting the sample preparation and analysis in the state of Pará.
- Hugo Gimenes de Lima, website designer, for the Safenut website updating and intranet implementation.

Progress reporting to STDF

Scientific reports
The general coordinator prepared all the status, progress, annual and final scientific reports. These reports were reviewed by the scientific coordinator (Monica Olsen) before sending them to STDF.

Financial reports
The general coordinator participated actively in the preparation of the financial reports of CIRAD and the Brazilian partners (EMBRAPA and MAPA), and organized meetings with the Brazilian partners on financial matters, in particular to give instructions for completing their reports.
All the Safenut financial reports were reviewed and validated by the CIRAD financial service before the signature by the project partners and sending to STDF.

Request for Safenut project extension
The general coordinator prepared a request for extension of the Safenut project with an explanation of the reasons for such an extension and the new timetable proposed for the implementation of the project pending activities and the delivery of expected outputs and outcomes. A project extension of 6 months (i.e. until 30 November 2008) was agreed by the STDF Working Group in November 2007.

Scientific activities performed within the different project specific objectives

Project specific objective 1

A.1.1. Technical and socio-economical diagnostic of the Brazil nut production chain in the Brazilian States of Acre and Pará
A1.2: Analysis of the major constraints and opportunities in the Brazil nut production chain for aflatoxin control.
A1.3: Formulation of organizational and incentive strategies for a sustainable and safe Brazil nut production chain

CIRAD contributed to the execution of the activities of project specific objective 1 through the participation of its two socio-economist researchers, Marie-Gabrielle Piketty out posted in Brazil at USP (Universidade de São Paulo) and Isabelle Vagneron based at CIRAD headquarters, Montpellier, France. Both of them participated in the preparation of a detailed work plan for this specific objective, as part of the Safenut action plan, during the project kick off meeting.

Marie-Gabrielle Piketty coordinated the implementation of the field surveys, as well as data analysis and synthesis, and she greatly contributed to the production of the final report entitled "Dinâmicas, processos e atores da cadeia produtiva da castanha-do-brasil nos estados do Acre e do Pará, Brasil". She organized different meetings of the socio-economic team in Brazil (Rio Branco, state of Acre ; Belém, State of Pará ; São Paulo-SP) for the follow up, planning and organization of the activities of project specific 1 (Annex 1).

The general coordinator also supported the implementation of the activities of project specific objective 1 through:
- The constitution of the Brazilian team to execute the socio-economic activities in the state of Pará (field surveys, data analysis and synthesis). This included the justification and formalization of the participation of Jair Carvalho dos Santos, researcher in socio-economy from EMBRAPA Belém, to the Director of this research unit, as well as the identification and formalization of the participation of Janaína Deane de Abreu Sá Diniz, Brazilian PhD student from UnB (Universidade de Brasilia);
- The elaboration of the questionnaires applied to the different actors of the Brazil nut production chain (producers/extractivists, intermediaries, agro-industries) in both states of Acre and Pará;
- The participation in the meeting of the socio-economic team organized in São Paulo in May 2007;
- The review of the two reports produced by the socio-economic team "Situação atual das legislações brasileiras e internacionais relacionadas a comercialização da castanha-do-brasil" and "Dinâmicas, processos e atores da cadeia produtiva da castanha-do-brasil nos estados do Acre e do Pará, Brasil".

Project specific objective 2:

A.2.1: Identify existing or set up Brazil nut production systems following recommended code of practices

The general coordinator supported the constitution of the multi-disciplinary team in both states of Acre and Pará (selection of students and consultants), as well as participated in the selection of the Brazil nut production chain in each state and the construction/verification of the Brazil nut Flow Diagrams through field visits and meetings with the key stakeholders and Safenut Brazilian teams.

A.2.2: Collection of data (*A. flavus/parasiticus*, aflatoxins, water activity, moisture content, relative humidity, temperature, insect infestation)

The general coordinator participated in:
- The definition of the sampling plans and the procedures for sample handling, preparation and water activity analysis in collaboration with the Safenut partners. She wrote in Portuguese all the detailed protocols and forms to be filled and joined to the collected samples, and sent them to the Safenut partners involved in the project specific objective 2;
- The improvement of the laboratory capacity of EMBRAPA Acre and LANAGRO-PA/MAPA for sample preparation and water activity analysis through several visits and meetings in both states of Acre and Pará;

The general coordinator also supported the Brazil nut sample collection along the selected production chains through several field visits in the states of Acre and Pará.

A2.4: Formulate recommendations to update the existing manual of safety and quality in Brazil nuts

The general coordinator reviewed, compiled and organized all the field data from Acre and Pará (temperature and relative humidity at the sampling points, water activity, total fungi and aflatoxin-producing fungi, aflatoxins), and sent them to NFA for statistical treatments. She also checked and corrected if necessary all the calculations of the number of colony forming units (cfu) of the fungi.
The general coordinator visited NFA in Sweden during two 1-week visits at the end of the Safenut project (in August and September 2008) for the analysis and interpretation of the field results, and the formulation of conclusions/recommendations for updating the existing Code of Practices of the Codex.

Project specific objective 3

A3.1: Adapt and validate existing rapid ELISA brought in to the project for aflatoxins in Brazil nuts.
A3.3 Adapt and validate existing rapid, on-site Lateral Flow Device (LFD) brought in to the project for aflatoxins in Brazil nuts

The general coordinator followed up the implementation of the evaluation of the rapid ELISA and LFD assays by CSL and R-Biopharm AG. She participated in the discussions among the Safenut partners for the elaboration of the second fit for purpose work plan, and reviewed the two technical reports produced by CSL and R-Biopharm AG.
The general coordinator also prepared a work plan proposal for additional tests agreed among Safenut partners during the second progress meeting (September 2007) to complete the evaluation of the ELISA and LFD kits. She also discussed and validated the comprehensive protocol produced by the Brazilian team of LACQSA/LANAGRO-MG/MAPA based on the CIRAD work plan proposal (see above) validated by CSL and R-Biopharm AG.

A3.2: Set up in Brazil a rapid ELISA for aflatoxins in the laboratory, the Brazil nut production area and one processing plant.
A3.4: Set up in Brazil a rapid on-site LFD for aflatoxins in the laboratory, the Brazil nut production area and one processing plant, and compare with HPLC and best other immunoassays (e.g. ELISA)

The general coordinator supported the organization of the three training courses in rapid methods for aflatoxin analysis in Brazil nuts (see below).

Dissemination activities (project specific objective 4)

A4.1: Training course in AFPA agar plate methodology for the identification of aflatoxin producing fungi in Brazil nuts

The general coordinator participated in this training course led by NFA in Rio Branco, state of Acre, and supported its local organization (contribution in the identification of participants, the translation in Portuguese of training materials and AFPA lecture during the training).
She also provided link between NFA and Brazilian partners for methodological support on AFPA agar plate methodology and the execution of the ring-tests for fungal analyses.

A4.2: Training courses in ELISA and Lateral Flow device (LFD) for aflatoxin analyses in Brazil nuts

The general coordinator supported the local organization of the three training courses in ELISA and LFD led by CSL and R-Biopharm in Brazil (contribution in the identification of participants, sending of invitations, logistic organization…).
She also participated in a short theoretical training course in ELISA and LFD organized in December 2006 at R-Biopharm AG Brazil, Jundiai, state of São Paulo, together with the French student Olivier Devillers.

A4.3: Training courses in good practices in the Brazil nut production chain

The general coordinator supported the local organization of the two training courses in good practices given in Belém, state of Pará (12 November 2008) and in Rio Branco, State of Acre (21 Novemver 2008), and she gave one lecture concerning recommended good practices in the Brazil nut production chain for aflatoxin prevention and control based on the Safenut results, at each training course.

A4.4: Development of a project specific website

CIRAD has designed, implemented and updated the Safenut website available in English, Portuguese and Spanish with the support of local students and consultants.

A4.5: Scientific and specific sector publication

Scientific publications in international peer-reviewed journals

Johnsson P, Lindblad M, Thim A-M, Vargas E. A., Medeiros N.L., Brabet. C, Quaresma de Araújo M, Olsen M, 2008. Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts. World Mycotoxin Journal 1 (2) 127-137.

Oral presentations in congresses and meetings

- Brabet C., 2008. Avaliação da eficácia das praticas atuais para controle de ocorrência de aflatoxinas na cadeia produtiva da castanha-do-brasil. Seminário "Plano de amostragem para castanha-do-brasil, Projeto ConforCast, 10-12 November 2008, Belém-PA, Brazil
- Brabet C., 2006. The STDF Brazil nut project. Meeting on "Interfaces cooperativas para pesquisa de interesse mundial em segurança de alimentos – Os sistemas Embrapa/MAPA e a rede SafeFoodera", Embrapa Headquarter, 13 April 2006, Brasilia-DF, Brazil

CIRAD also gave oral presentations at the project meetings and workshops (see the program of these events in Annexes 15, 17, 19-22).

Posters presented in congresses

- Johnsson P., Lindblad M., Thim A. M., Jonsson N., Vargas E. A., Medeiros N. L., Brabet C., Quaresma de Araújo M., Olsen M., **2008**. Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts. 30th Mycotoxin Workshop, 28-30 April 2008, Utrecht, NL (Cost covered by NFA)
- Iamanaka B. T., Taniwaki M. H., Calderari T. O., Brabet C., Palacios-Cabrera H. A., **2008.** Moisture Sorption Isotherms of Brazil Nuts at Different Temperatures. Safenut final workshop, 6-7 November 2008, Belém-PA, Brazil (CIRAD *supported work connected to Safenut project*)

Leaflet on the Safenut project

The general coordinator produced a leaflet in English, Portuguese and Spanish. This leaflet was uploaded on the Safenut website.

Articles on the Safenut project and activities published on Internet and in specific journals

- CIRAD, 2009. Noix du Brésil : exporter à nouveau vers l'Europe/Brazil nuts: resuming exports to Europe. Actualités/News on the homepage of CIRAD website, 6 May 2009. (http://www.cirad.fr/fr/index.php / http://www.cirad.fr/en/index.php)
- Petithuguenin E.L., 2008. Castanha-do-brasil: chega ao fim o projeto SafeNut. França Flash n° 65, 10 de outubro de 2008. (http://www.cendotec.org.br/ffpartes/ff65-2safenut.html). Published also in Spanish (http://www.cendotec.org.br/ffpartes/franciaf65-2safenut.html).
- CIRAD, 2006. Maîtriser la qualité de la noix du Brésil. Electronic Journal of Cendotec, N° 75, March 2006, p. 3 (http://www.cendotec.org.br/be/be75.pdf)

TV programs

In February 2008, CIRAD participated in the short TV programs on the Brazil nut production chain and actions developed by the Safenut project that were divulgated during the local TV News "Noticias da Aldeia", Rio Branco, state of Acre.

Participation in project events and meetings (project specific objective 5)

CIRAD organized in collaboration with the other Safenut partners and participated in the following project events and meetings:

* **A.5.1. Kick off meeting and first workshop held in Rio Branco, state of Acre, Brazil, July 2006:**
  - Participation of Catherine Brabet, Isabelle Vagneron and Marie-Gabrielle Piketty.
  - The general coordinator prepared a synthesis of the workshop results and conclusions for the INC (International Nut and Dried Fruit Council) which sponsored the event.

* **A.5.2. Progress and open meetings**
- First annual progress meeting, Belém, state of Pará, 26-27 September 2007:
  Participation of Catherine Brabet and Marie-Gabrielle Piketty
- Second annual progress meeting, Belém, state of Pará, 3-4 November 2008
  Participation of Catherine Brabet and Marie-Gabrielle Piketty
- Open meeting, Belém, state of Pará, 28 September 2007
  Participation of Catherine Brabet and Marie-Gabrielle Piketty

* **A.5.3. Final workshop, Belém, state of Pará, 6-7 November 2008**
Participation of Catherine Brabet and Marie-Gabrielle Piketty

For these events and meetings, CIRAD contributed with:
- The elaboration of the program and list of participants;
- Invitation sending to the participants (workshops and open meeting);
- The elaboration of the workshop program folder, banner and band;
- The organization of the logistic (auditorium and room reservation, simultaneous translation, coffee break, cocktail, hotel reservation, travel organization of Peruvian and Bolivian partners, etc.).

CIRAD also acted as speaker, rapporteur and/or moderator (see programs in Annexes 15, 17, 19-22), and contacted INC, FAO and the European commission for their participation at the final workshop. Mr Frans Verstraete from DG SANCO of the European Commission was present at this workshop.

The general coordinator also participated in a meeting with STDF in Geneva on the 4 September 2007 concerning the progress of the Safenut project.

Co-supervision of students / Training of researchers

The general coordinator participated in the supervision of Olivier Devillers, French engineering student from ENSAR (Ecole Nationale Supérieure Agronomique de Rennes), December 06-May 07, and Felicia Maria Nogueira Leite, Brazilian MSc student from UFAC (Universidade Federal do Acre) / SEAPROF (Secretaria de Extensão Agroflorestal e Produção Familiar), January 07-November 08, for the execution of the activities A2.1, A.2.2 and A.2.4 in the state of Acre (project specific objective 2).

The general coordinator and Marie-Gabrielle Piketty participated in the supervision of Janaína Deane de Abreu Sá Diniz, Brazilian PhD student from UnB (Universidade de Brasilia), June-November 08 for the execution of the socio-economic activities - Field surveys in the state of Pará, and data analysis and synthesis ; Updating of the legislation for Brazil nut commercialization (project specific objective 1).

The general coordinator also supervised Sarah Biruel, Brazilian student, and César Augusto Piedrahíta Aguirre, Colombian student, respectively for the Safenut website design and Spanish translation, and Hugo Gimenes de Lima, website designer, for the Safenut website updating and intranet implementation.

CIRAD also arranged a 2-months visit at CIRAD, Montpellier, France (September-October 2008) for one researcher from EMBRAPA Acre (Daniela Matias de C. Bittencourt) in order to train in the PCR-DGGE method developed by CIRAD for studying the evolution of the mycoflora along the Brazil nut production chain. The visit was financed by other resources of CIRAD (and not the Safenut project).

INDIVIDUAL PARTNER FINAL REPORT

PARTNER: NFA – SWEDEN

NFA team:

Monica Olsen, Scientific Coordinator, PhD Biologist
Pernilla Johnsson, Microbiologist
Mats Lindblad, PhD Microbiologist
Ann Gidlund, Microbiologist
Paula Ågren, Biomedical Scientist
Anna-Maria Thim, Chemist
Marianne Nyberg, Technician

Tasks and activities performed within the framework of the different project specific objectives

Management activities

NFA has had regular contacts through e-mails and SKYPE-meetings with the Project coordinator to solve different issues during the years such as follow-up, planning and progress reporting of the scientific activities. During the Kick-off meeting in July 2006, NFA led the detailed planning of the project by using the tool LOTS® which resulted in an action plan for the project. The action plan has been up-dated during the project time.

NFA has created during Year 1 a project room on the internet available for all organisations, by individual username and pin code (www.slv.se/safenut), to up- and down-load documents used in the project. By using this project room, video films describing particular parts of the mycological methods were made available for the partners performing the analyses.

NFA has supported the Project coordinator in planning of the International Workshops in 2006 and 2008 and the Kick-Off meeting in 2006, and the annual progress meeting in Belém in Sep 2007. Contacts have been made by NFA with INC (http://www.nutfruit.org/) and Cameon Ivarsson (http://www.napasol.com/) participated in the open meeting in Sep 2007. For the final meeting in Nov 2008, FAO and the European Commission were contacted and Mr Frans Verstraete, from DG SANCO of the European Commission, participated in the final international workshop in November 2008.

Scientific activities

A.2.2: Collection of data (*A. flavus/parasiticus*, aflatoxins, water activity, moisture content, relative humidity, temperature, insect infestation)

Year 1

The NFA made a preliminary selection of which points in the Brazil nut flow diagrams (BnFd) that should be sampled for mould and aflatoxin content and environmental factor registration in February 2007. BnFd:s were acquired from EMBRAPA in Jan 2007.

Preliminary plans for sample handling were also elaborated. Instructions for sample preparation and analysis for microbiological analysis already existed as a result of the training course in microbiological analysis (see A.4.1).

A final agreement on sampling points and sample handling was made during a meeting between the project partners in Brazil in the beginning of March 2007.

Year 2 (until Nov 2008)
The NFA has given continuous support to the teams in Pará and Acre concerning the analyses of aflatoxin producing fungi in the Brazil nuts samples and reviewed the results of the internal control of the labs to quantitatively estimate the concentrations of these fungi in the samples by using the reference material previously provided by NFA.

NFA has used the results from Pará to evaluate the predictive model developed in A.2.3.

Furthermore, the data from the field trials in Acre and Pará of Year 1 and 2 were extensively studied together with the Coordinator in the end of the project and NFA performed all the modelling/predicting and statistical work, and illustrations of A.2.2 which were presented at the final workshop in Nov 2008. During this work the Coordinator visited NFA, Sweden, during two 1-week visits.

NFA has developed a protocol with photo illustrations to enable the laboratories in Pará and Acre to isolate fungal strains for further examinations to species level. Laboratory material and packing material was sent to the lab in Pará. A limited number of strains, approx. 20, were sent to Sweden in Aug 2008 and all the strains were identified to *A. flavus*. These strains were also used for training of a staff member from one of the Brazilian partners (MAPA) who visited NFA during 2 weeks in Sep 2008.

A.2.3: Development of a simple predictive model for aflatoxin and fungi production in the Brazil nut production chain

Seventeen kilos of Brazil nuts were collected in a community of an association of producers in the state of Pará and brought into Sweden in the beginning of March 2007. A storage experiment with storage of Brazil nuts in a respirometer was started 15 March according to plan. Samples were taken on a regular basis for analyses of the growth of the aflatoxin producing fungi. The samples were also be analyzed for aflatoxins by HPLC at the NFA. An improved HPLC method for aflatoxin analysis in Brazil nuts was developed by NFA and validated for Brazil nuts. The storage experiments were finalized in the end of June 2007. The storage experiment was statistically analyzed and a full project report was delivered in Nov 2007. From this material a scientific article has been written and published in The World Mycotoxin Journal (see below).

A second study was initiated to verify the robustness of the models developed and was agreed by the Coordinator and STDF. The additional study was performed in March to April 2008 and a report has been sent to the Coordinator and STDF. The main conclusion from this study is that the results produced in the previous report are robust and can be used to predict and set the maximum storage time of Brazil nuts, to avoid levels above the maximum limit, under conditions allowing fungal growth such as in the rain forest or in the storage facility of the community or processing plant. The models was used together with data from the field to the updated and validated good practices in the Brazil nut production chain at the prospect of the training course in good practises in the Brazil nut production chain (A4.3).

The aflatoxin-strain used in the storage experiment was previously isolated from Brazil nuts imported to Europe in another project and tested for aflatoxin-production. The aflatoxin analyses showed that this strain was a good producer of both B and G aflatoxins and hence it was assumed that this isolate was an *Aspergillus parasiticus*. However, closer examination and with confirmation by CBS in the Netherlands we have now showed that this is an *Aspergillus nomius.* This species may earlier been misidentified and has not been regarded as an important aflatoxin-producer in food commodities. Two more strains from this earlier project have been identified as *Aspergillus nomius.* This work, which is not a part of SafeNut, has also been published in the World Mycotoxin Journal

(see below). It was indicated that *A. nomius* may be an important aflatoxin producer in Brazil nuts. During the final workshop in Nov 2008 this finding was also confirmed by unpublished data of Dr M Tanawaki from Instituto de Tecnologia de Alimentos, Campinas, Brazil.

Summary and Conclusions from the scientific activities:

- Aflatoxin levels could be predicted from mould levels. There were high accuracy using laboratory data, and regional differences using field data
- The increase in probability of exceeding different aflatoxin maximum limits with time was described, based on field data.
- The models can be used in practice to:
  - evaluate the effect of storage humidity on the risk of high aflatoxin levels
  - predict aflatoxin levels based on mould levels
  - estimate how fast the risk of high aflatoxin levels increases under field conditions
- The current Codex Code of Practice for Aflatoxin in Tree nuts (CAC/RCP 59 -2005, REV.1-2006) needs to be up-dated. The current recommendations concerning the drying and storage in the communities are built on the assumptions that the nuts can be dried to a safe level by sun drying in the rain forest area, but this project has showed that this is not the case.

Dissemination activities

A.4.1: Training course in AFPA agar plate methodology for the identification of aflatoxin producing fungi in Brazil nuts

A training course in AFPA agar plate methodology for the identification of aflatoxin producing fungi in Brazil nuts was organized by the NFA in October 2006 at EMBRAPA-Acre the 25 to 27 October 2006. The course was free of charge for the participants but participants had to pay for board and lodging. Invitations were sent in September. Sixteen participants, 15 from Brazil and 1 from Bolivia, joined the course. The course consisted of an introductory lecture and of hands on practice in a laboratory. The lecture, which was also available for participants joining the ELISA/LFD course (Specific objective 3), dealt with the following aspects:

- Introduction to food mycology with specific emphasis on aflatoxin producing moulds,
- Description of the AFPA selective medium including advantages, disadvantages, strengths, weaknesses, and interpretation of results,
- Laboratory procedures in mycological methodology including dilution and direct plating, identification, quantification and isolation of *Aspergillus flavus/ A. parasiticus,*
- Storage techniques for isolates,
- Safety considerations.

Laboratory practices included:

- Demonstration of pre-grown AFPA-plates and practice on calculating quantitative results.
- Demonstration of direct plating of Brazil nuts.
- Dilution and plating of a freeze dried sample containing *Aspergillus flavus/ A. parasiticus* and of a real nut sample.
- Microscopy of different fungal species with a focus on species that may be confused with *Aspergillus flavus/ A. parasiticus* on AFPA.

Documents that were developed for the course were:

1. A checklist for equipment that is needed to carry out the analyses
2. A text covering the same aspects as the lecture (see above).
3. Protocols for dilution plating of freeze dried and real samples
4. Protocol for direct plating of food samples
5. Recipes for media preparation
6. Sample records for documentation of analytical methodology and results

Documents 2 – 6 were translated to Portuguese (by EMBRAPA) and distributed to all course participants at the beginning of the course. The lecture is available at the course web site. Freeze dried samples of fungal mixture in glass vials (see below) and pure cultures of *A. flavus*, *A. parasiticus*, *A. niger*, *A. tamarii* and *Eurotium amstelodami* were produced and checked by the NFA and given to the partners performing the mycological analyses of the field samples (more than 10 vials per laboratory).

Freeze dried fungal mixture

Quality checks for analyses

The NFA has organized ring-tests for fungal analyses for partners that participate in the scientific activities in the project. Such ring-tests have been carried out regularly since November 2006 by the NFA, MAPA and EMBRAPA by analyzing a freeze dried fungal mixture containing the species *Aspergillus flavus*, *A. niger*, *A. tamarii* and *Cladosporium cladosporoides*. The freeze dried samples were produced by the NFA for the use as internal reference material in the SafeNut project. Since the quantitative content of the freeze dried samples was statistically certified by NFA, the ring-tests enabled the evaluation and comparison of laboratory skills between laboratories. It was continuously concluded that both EMBRAPA and MAPA had gained a sufficient precision in their analyses before the onset of specific objective A.2.2 (see above).

Co-supervision of students/researchers

NFA has participated in a supervision of a French student, Olivier Devillers, concerning especially the mycological analyses at EMBRAPA Acre (Jan-May 2007).

NFA also arrange a 2-weeks training visit for one researcher from MAPA (Julio Garcia, Belo Horizonte) in Sep 2008 to train among other things examination of the *Aspergillus* strains received from Pará and references strains. The visit started with repeating mycological nomenclature, preparation of microscopical slides, producing single-spore cultures, and storage. The visit was not financed by the SafeNut budget and was free of charge from NFA.

A4.2: Training courses in ELISA and Lateral Flow device (LFD) for aflatoxin analyses in Brazil nuts

NFA participated and supported the two days training course in both ELISA and LFD kits which was organized at LANAGRO-PA/MAPA, Belém-Pará on 24-25 September 2007.

A4.3: Training course in good practises in the Brazil nut production chain

NFA participated in the first training course in good practices given on the 12 Nov 2008 in Belém, Pará. NFA gave one lecture concerning aflatoxin producing fungi and the risk for human health and described how the models could be used to estimate the probability of exceeding different maximum levels, such as the current EU legislation, the Codex level, with different storage time of Brazil nuts.

A4.5: Scientific and specific sector publications

Oral presentations in congresses and meetings

- Olsen M, 2006: Aflatoxins: formation and effects on public health and international standards. Prevention and control of Brazil nut contamination by aflatoxins. International workshop 10-11 July, 2006, Rio Branco, Brazil.
- Olsen M, 2007: Aflatoxins and the Brazil nut production chain . IUPAC Istanbul May 21-25, 2007 (Cost covered by NFA)

- Olsen M: Aflatoxins in the Brazil nut production chain**.** International Commission on Food Mycology workshop, June 4-6, Key West, USA (same presentation as in IUPAC in May 2007) (Cost covered by NFA)
- Olsen M: presentation of background and expected results of SafeNut project at a meeting with STDF (Melvin Spreij) and SIDA (the Swedish development cooperation agency: www.sida.se), Sep 14, 2007, Stockholm. (Similar presentation as the above) (Cost covered by NFA)
- Olsen M: Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts (poster presentation). The 30th Mycotoxin Workshop, Utrecht, NL 28-30 April 2008 (Cost covered by NFA)
- Olsen M: Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts. Oral presentation (no abstract but the handouts of the poster were distributed) at the Scientific and Government Affairs Committee meeting during the Annual International Nut and Dried Fruit Council Foundation, Santiago, Chile 9-11 May 2008 (Cost covered by INC)
- Olsen M: Presentation of the SafeNut project at the meeting of the SPS-working group, Swedish Ministry of Agriculture, Stockholm 24 May 2008. (Cost covered by NFA).
- Olsen M: Rapid method for fungal monitoring in Brazil nuts (method, quality assurance and training course). SafeNut International Workshop, Belém, Brazil 6-7 Nov 2008
- Lindblad M: Development of a predictive model for aflatoxin production and fungal growth in the Brazil nut production chain. SafeNut International Workshop, Belém, Brazil 6-7 Nov 2008.
- Olsen M: Technical recommendations for the prevention and control of aflatoxins in the Brazil nut production chain. SafeNut International Workshop, Belém, Brazil 6-7 Nov 2008.
- Olsen M: Introduction to aflatoxin producing fungi and the risk for human health. ConforCast International meeting, Belém, Brazil, 10-12 Nov 2008 (Cost covered by ConforCast project)

Scientific publication

1. Johnsson P, Lindblad M, Thim A-M, Vargas E. A., Medeiros N.L., Brabet, C, Quaresma de Araújo M and Olsen M, 2008: Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts, World Mycotoxin Journal 1 (2) 127-137.

2. Olsen M, Johnsson P, Gidlund A, Ågren P, Thim A-M and Lindblad M, 2009: Modelling the growth and aflatoxin production of different strains of aflatoxigenic fungi isolated from Brazil nuts (*in preparation, internal report delivered to CIRAD in June 2008*).

3. Olsen M, Johnsson P, Möller T, Paladino R and Lindlblad M, 2008: *Aspergillus nomius*, an important aflatoxin producer in Brazil nuts? World Mycotoxin Journal 1 (2), 123-126. (*NFA supported work connected to SafeNut project*)

Summary and Conclusions from the dissemination activities

- The mycological training has led to the fact that there are now staff in labs of both Acre and Pará capable of identifying and quantifying the amount of aflatoxin producing fungi in Brazil nuts and other commodities
- These new skills have already been used in other projects at EMBRAPA Acre and MAPA and can be adapted to other commodities as well.
- The work of the SafeNut project have been acknowledge by stakeholder organisations and the awareness of the critical points in the Brazil nut production chain have been extensively improved at all levels of the production chain.

Participation in project events and meetings

A5.1: Kick off meeting, and first workshop with the participation of Bolivian and Peruvian key partners

NFA participated in the Workshop and the project kick-off meeting in July 2006. NFA contributed with ideas for the meeting, seeking successfully a sponsor for the meeting, acting as moderator and speaker during the workshop.
During the Kick-off meeting NFA led the detailed planning of the project by using the tool LOTS® (http://www.lots.mindo.com/EN/) which resulted in an action plan for the project.

A5.2 Progress meeting

NFA staff participated actively in the meetings held in Bélém and Orixíminá in early March 2007. These meetings settled the final sampling points and procedures for A.2.2.

NFA participated, upon request of the Coordinator, in a meeting with STDF in Geneva on the 4 Sep 2007 concerning the progress of the SafeNut project.

NFA participated in the progress meeting 24-28 Sep 2007 in Belém and the results of the "A.2.3: Development of a simple predictive model" was presented (previously sent to Coordinator).

NFA participated in the progress meeting 3-5 Nov 2008 in Belém. The final presentations (as presented during the International workshop 6-7 Nov 2008 were presented and discussed together with all other work. Final inclusions of the last data were done and support was given by NFA to the Coordinator and the other partner's presentations.

A.5.3. Final workshop

NFA supported the Coordinator in the planning of the two day final workshop which was held in Belém 6-7 November 2008 and gave 3 different presentations during the workshop. NFA prepared the minutes of the final discussion of the workshop.

Measurable project impacts during the project period (as distinct from project outputs)

- NFA has supported the work of the Brazilian delegation concerning the Discussion Paper on Aflatoxin Contamination in Brazil nuts (Codex Committee on Contaminants in Food, CCCF) and suggested important changes to the Codex Code of Practice for Aflatoxin in Tree nuts (CAC/RCP 59 -2005, REV.1-2006) which were supported by the outcome of the SafeNut project (*copied to WTO and CIRAD in 8 Dec 2008*).

- NFA participated in *ad hoc* working groups during the ConforCast meeting 10-12 Nov 2008 concerning the Codex work on aflatoxin in Brazil nuts. The WG included member from the Brazilian delegation and the European Commission (Mr Frans Verstaete) and the discussion elucidated several of the specific circumstances included in the Brazil nut production such as the extreme heterogeneous distribution of aflatoxin in Brazil nuts and the subsequent difficulties in sampling.

- The invitation and participation of Mr Frans Verstraete was very much appreciated by the participants of the SafeNut project and the possibility to combine this project meeting with the one of ConforCast project gave the possibility for Mr Verstraete to participate in both. In this way the European Commission was given an important possibility to understand the complexity of the Brazil nut production chain and the problem of aflatoxin formation which is valuable for different issues concerning the trade of Brazil nuts in the future.

- The participation of NFA in meeting with SIDA and The Swedish Ministry of Agriculture has led to awareness of how the SafeNut project has realised some of the intensions of Article 9 of the SPS agreement. The Ministry of Agriculture and NFA disseminated information about SafeNut as an example of technical support to developing countries during the International Seminar on Setting Food Safety Standards.

- The SafeNut project got very positive feedback from the INC meeting in Santiago 9-11 May 2008. The chairman of the Scientific Committee, Mr Pino Calcagni, gave a lot of credits to SafeNut also in the plenum meeting even though Brazil nuts are very small in the total nut industry. There were more than 450 participants, most of them from exporters and importers but also some from the production side. Several participants, especially among producers, expressed their interest on the approach of the SafeNut Project.

- The involvement of Dr Cameon Ivarsson in the SafeNut annual meeting in Sep 2007 led to an article on the subject written by Dr Ivarsson and published in both The Cracker (Jan 2008) and in Food Marketing and Technology (Feb 2008).

- The involvement of INC in the open meeting of SafeNut in Sep 2007 supported a global awareness of the SafeNut project which will hopefully create a future platform for recovering exports markets. The open meeting and the participation of INC also contributed to an improved dialogue between the public and private sector.

INDIVIDUAL PARTNER FINAL REPORT

PARTNER: CSL – UNITED KINGDOM
with R-Biopharm AG as sub-contractor

Technical Summary

The implementation of a quality-critical point in the production chain of brazil nuts for control of aflatoxin can leverage quality attributes upwards to the harvesting of nuts.

The efficacy of on-the-market R-Biopharm AG aflatoxin ELISA and Lateral Flow Device (LFD) detections systems for monitoring aflatoxin in brazil nut samples was evaluated with respect of current EU regulations that recognises four categories of aflatoxin tolerance dependent on use, sorting or other physical treatments prior to direct consumption and methods of measuring total (B1 and G1 aflatoxins mainly) or B1 aflatoxin.

ELISA based methods were shown to detect at the lowest EU threshold levels for total (4ppb) and B1 (2ppb) aflatoxin. LFD devices were sensitive to 4ppb total aflatoxin. Some variability in ELISA data was observed, but in the main a 95% probability for prediction in ELISA to HPLC value was proven. The variability of aflatoxin detection obtained from different laboratories (Ring testing) was not assessed.

This study is aimed at finding the best-fit application for use within the production chain, from Amazonian collectors, to middlemen and millers that can leverage a quality improvement. For these stakeholders some of the key requirements are ease-of-use, speed of result and low investment and running cost. In looking at these aspects between the test devices the LFD devices have a clear advantage over the ELISA methods

Based on the above information consideration was given to placing detection devices within the production to market chain, with the expectation of leveraging quality-awareness upwards to the point of collection. In this context it was thought that based on ease of use and rapidity of results (fitness-for-purpose) the LFD devices were overall the most appropriate and could easily be used by millers or middlemen. It is estimated that the LFD would require only modest investments in infrastructure and human resources.

Millers exposed to LFDs and how they could monitor for aflatoxin expressed an interest in their use, noting it could effect positive behavioural change within the production chain towards improved quality. Preliminary discussions were initiated with millers as to where and how a LFD based quality control system might be implemented, focusing on the use of a LFD at the point of receipt of brazil nut from Amazonian communities or middlemen. Further work is needed to demonstrate proof-of-principle use of a LFD in effecting quality, noting that its use would need to be based around a Hazard Analysis Critical Control Point (HACCP)-styled quality scheme that provided traceability to the gathering stage. Specific Objective 2 provided useful information and recommendations on the nature of 'best practice' in the harvesting of Brazil nuts.

Training in the use of the detections systems to national scientist of Brazil and other stakeholder of the brazil nut production chain was provided.

Project overview

Four main objectives were identified:

1. Specific objective 1: Characterization of the Brazil nut production chain, and formulation of organizational and incentive strategies for safety control

2. Specific objective 2: Validation of recommended good practices in the Brazil nut production chain for aflatoxin control

3. Specific objective 3: Validation and implementation of a rapid aflatoxin surveillance system for use along the Brazil nut production chain

4. Specific objective 4: Knowledge and technology transfer to the key stakeholders

5. Specific objective 5: To strengthen the public-private dialogue and partnership in the Brazil nut sector

CSL contribution was mainly against Objective 3, 4 and 5.

List of members of the partner team:

Central Science Laboratory:

- John Banks
- Julian Smith
- CSL Statistical team

R-Biopharm AG

- Pat Taylor
- Dan Kaplan
- Luis Mascaretti
- R-Biopharm AG support staff

Tasks and Activities performed during the reporting period

Management Activities

CSL submitted annual reports as requested. CSL and R-Biopharm AG staff attended the main management planning meetings. For efficiency of time and resources project management meetings were linked with Specific Objectives 4 (Knowledge transfer) and 5 (Strengthening Dialogue). In total CSL and R-Biopharm AG staff visited Brazil four times over the course of the project, including the final workshop.

Tasks and activities

CSL, in partnership with R-Biopharm AG, was mainly tasked with Specific Objectives 3 (3.3 person mths) and 4 (2 person mths).

Specific objective 3: Validation and implementation of a rapid aflatoxin surveillance system for use along the Brazil nut production chain

Two validation activates were completed, with the research on both occasion undertaken at R-Biopharm AG, Germany. Two technical reports were provided to the Safenut general coordination.

These studies aimed to validate fit-for-purpose detection assays for aflatoxins in brazil nut samples, comparing ELISA[2] and LFD[3] formats, against the standard HPLC method (for second study only). The detection limits were to be considered with respect of current EU legislation for levels of aflatoxin permissible within nut samples. These thresholds are summarised in Table 1. The assessments aimed to determine if detection was reliable at aflatoxin concentrations applicable to the EU legislation. It was not the purpose of the study to determine the upper and lower end-point for aflatoxin detection. For this reason the results are discussed in relation to the EU thresholds and not absolute levels.

The results from Study 1 and 2 are summarised in Table 2 and, for Study 2, Table 3. Low levels of variability in detection within replicate samples were observed.

Table 2: Summary on suitability for use of Aflatoxin detection devices for total and B1 aflatoxin using various R-Biopharm AG devices

EU thresholds for aflatoxin (See Table 1)	**ELISA**			**LFD**
	RIDASCREEN® FAST Aflatoxin	**RIDASCREEN® FAST Aflatoxin SC**	**RIDASCREEN® Aflatoxin B1 30/15**	**RIDA QUICK Aflatoxin**
5.0µg/kg (B1)	Not tested	Not tested	Yes	No tested
10.0µg/kg (Total aflatoxin)	Yes	Yes	Not tested	Yes
2.0µg/kg (B1)	Not tested	Not tested	Yes	Not tested
4.0µg/kg (Total aflatoxin)	Yes	Yes	Not tested	Yes

These data conformed with the industry's' expectation for the devices. Note that HPLC and ELISA provide quantitative data, whereas LFDs are effectively semi-quantitative, determining minimum detection levels at set incubation time intervals (see Table 4). Detection values for ELISA and HPLC showed strong equivalence, however, some variability in data was observed for HPLC and ELISA (see Table 3). In general greater variability was seen with ELISA data. Statistical analysis on the relationship between HPLC and ELISA in the main supported 95% confidence limits in prediction. As a trend the ELISA kits over estimate concentration at low levels and under estimate at high levels of aflatoxin.

Project activities provided training in the use of the LFD and ELISA methods (see Specific Objective 4). However, no systematic analysis for ring testing the methods amongst laboratories was undertaken. This would be a useful activity to consider in establishing inter-lab variability and the development of appropriate Standard Operating Procedures.

[2] ELISA devices provide multiple wells for individual assessments. The devices are capable of processing many samples at a time; typically an ELISA plate has 96 wells. A positive result is determined by a colour change, with quantification achieved by comparison to a positive control of known concentration.

[3] Lateral Flow Devices provide a single test format only. A positive result is recorded by the observation of a line that shows the test has been undertaken successfully (positive control) and a second line if aflatoxin is detected.

Table 4: Specifications of the ELISA and LFD assays

Name of test kit	Principle / Format	Incubation time (min)	Technical equipment and result interpretation
RIDASCREEN®FAST Aflatoxin	ELISA 48 well MTP[1]	15	Photometer quantitative measurement
RIDASCREEN®FAST Aflatoxin SC, FGIS Certificate	ELISA 48 well MTP	15	Photometer quantitative measurement
RIDASCREEN® Aflatoxin B_1 30/15	ELISA 96 well MTP	45	Photometer quantitative measurement
RIDA®QUICK Aflatoxin	LFD[2] 20 single tests	reading at 4, 8, 16 min	Visual result interpretation Semi-quantitative measurement

[1] MTP = microtiter plate

[2] LFD = lateral flow device

This study is aimed at finding the best-fit application for use within the production chain, from Amazonian collectors, to middlemen and millers that can leverage a quality improvement. For these stakeholders some of the key requirements are ease-of-use, speed of result and low investment and running costs. In looking at these aspects between the test devices the LFD devices have a clear advantage over the ELISA methods that required relatively sophisticated analytical equipment and trained staff. Largely, for LFD use the extraction phase presents the main investment by users. Accordingly, in considering the potential targeted users of the detection systems (most likely millers or middlemen) of the methods tested the LFD approach was considered the most appropriate for use within the brazil nut industry.

Specific objective 4: Knowledge and technology transfer to the key stakeholders

In total three training activities were carried out in Brazil under the project objective, focusing on ELISA and LFD use. Stakeholders for the training were identified by the project coordinator and Brazilian partners. The workshops and training provided by CSL and R-Biopharm AG demonstrated the capacity for using ELISA and LFD within a laboratory and processing plant in Brazil. The capacity building elements of these activities transferred this functionality to the local Brazilian partners.

During the project extension a training activity was provided during the final workshop (convened for scientist and representative of millers and Amazonian nut gathering communities, amongst others) that was specifically aimed at the use of the LFDs as the method of choice. Feedback was positive, but raised many questions as to implementation and the leverage points along the brazil nut production chain. Millers were identified as a primary user. To address some of these areas a questionnaire for brazil nut millers was designed to structure available data obtained under Specific Objective 2 and attain additional data as required. Coming at the end of the project has meant insufficient time was available to see this activity to completion.

The implementation of a quality-critical point along the production chain can leverage quality outcomes upwards to the harvesting of nuts, resulting in an improved quality of product. However, additional effort is needed to demonstrate proof-of-principle in the use of LFDs in effecting quality. Examples from other systems suggest that the use of the LFD would need to be based about a Hazard Analyze Critical Control Point (HACAP)-styled quality scheme that provided traceability to the gathering stage. Specific Objective 2 provided useful information and recommendations on the nature of 'best practice' in the harvesting of Brazil nuts.

The following lectures, training/workshop activities and scientific publications were delivered by CSL and R-Biopharm AG under the Safenut project:

Lectures
- Kick-off meeting July 2006

Training:
- ELISA and LFD training course October 2006, EMBRAPA, Acre, Rio Branco. 8 participants, 2-day training.
- ELISA and LFD training and workshop September 2007, Belém; approximately 5 participants
- LFD training, Belém, Nov 2008; approximately 14 participants

Publications as Posters::
- Rapid immunoassays for aflatoxins in Brazil Nuts. XIIth International IUPAC Symposium on Mycotoxins and Phycotoxins, May 21-25, 2007. Istanbul.
- Rapid immunoassays for aflatoxins in Brazil Nuts. IXth International Conference on Agri-Food Antibodies (Society of Food and Agriculture Immunology) 10-13 September 2007, Vettre (Near Oslo) Norway.

Specific objective 5: To strengthen the public-private dialogue and partnership in the Brazil nut sector

CSL and R-Biopharm AG played an active role under SO5, participating fully in all key meetings. CSL and R-Biopharm AG agreed to participate in the no-cost extension of the project.

Table 1: Summary of maximum permissible levels for aflatoxin in nuts under EU legislation

Category	**Contaminant**	**Product**	**Maximum level**	**EU or UK legislation**	**Sample method**	**Reference analysis method**
1	Aflatoxin B1	Nuts to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in food stuffs	5.0 μg/kg	Regulation (EC) 1881/2006	Regulation 401/2006/EC	Regulation 401/2006/EC
2	Aflatoxin B1+B2+G1+G2	Nuts to be subjected to sorting, or other physical treatment, before human consumption or use as an ingredient in food stuffs	10.0 μg/kg	Regulation (EC) 1881/2006	Regulation 401/2006/EC	Regulation 401/2006/EC
3	Aflatoxin B1	Groundnuts and nuts and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	2.0 μg/kg	Regulation (EC) 1881/2006	Regulation 401/2006/EC	Regulation 401/2006/EC
4	Aflatoxin B1+B2+G1+G2	Groundnuts and nuts and processed products thereof, intended for direct human consumption or use as an ingredient in foodstuffs	4.0 μg/kg	Regulation (EC) 1881/2006	Regulation 401/2006/EC	Regulation 401/2006/EC

Table 3: Summary of Study 2 results comparing fit-for-purpose use of ELISA and LFD devices for detection of total and B1 aflatoxin in Brazil nut samples of varying total and B1/G1 content

Level	Sub sample	HPLC						Deduced values from HPLC				ELISA									LFD	
		Total Aflatoxin			B1 Aflatoxin			G1 aftaoxin		Ratio G1:B1		RIDASCREEN®FAST Aflatoxin			RIDASCREEN®FAST Aflatoxin SC			RIDASCREEB®Aflatoxin B1 30/15			RIDA®QUICK Aflatoxin	
		Conc (ppb)	Mean	CV (%)	Conc (ppb)	Mean	CV (%)	Conc (ppb)	Mean	Ratio	Mean	Conc (ppb)	Mean	CV (%)	Conc (ppb)	Mean	CV (%)	Conc (ppb)	Mean	CV (%)	Conc (ppb)	Mean
1	1.1	2.3	2.2	5.3	0.9	0.9	2.9	1.4	1.2	1.6	1.3	3.1	3.0	10.0	4.1	4.3	12.3	1.4	1.2	15.2	<4	< 4
	1.2	2.2			0.9			1.3		1.4		2.6			4.2			1.1			<4	
	1.3	2.0			0.9			1.1		1.2		3.3			3.9			1.0			<4	
	1.4	2.1			1			1.1		1.1		2.9			5.1			1.3			<4	
2	2.1	7.6	7.4	3.0	4.2	4.1	3	3.4	3.4	0.8	0.8	9.2	9.2	23.1	10.8	10.7	12.9	4.8	5.1	5.8	4-10	4-10
	2.2	7.1			3.9			3.2		0.8		7.9			10.0			5.1			4-10	
	2.3	7.5			4.1			3.4		0.8		12.2*	-8.2	-10.8	12.5			5.5			4-10	
	2.4	7.5			4.1			3.4		0.8		7.5			9.3			5.0			4-10	
3	3.1	28.1	27.9	3.9	12.7	12.6	6.7	15.4	15.4	1.2	1.2	30.0	32.7	15.6	33.5	33.3	3.3	13.7	13.9	6.7	>20	>20
	3.2	26.5			11.4			15.1		1.3		34.1			31.8			13.0			>20	
	3.3	29.2			13.4			15.8		1.2		39.3			34.4			13.7			>20	
	3.4	27.9			12.8			15.1		1.2		27.4			33.5			15.2			>20	
4	4.1	35.1	31.6	12.0	29.9	26.9	10.4	5.2	4.8	0.2	0.2	56.6	49.1	12.5	51.8	50.7	6.2	17.8	17.4	7.5	>20	>20
	4.2	27.1			23			4.1		0.2		42.2			48.4			15.5			>20	
	4.3	34.3			29.1			5.2		0.2		51.0			48.0			18.4			>20	
	4.4	29.9			25.4			4.5		0.2		46.6			54.7			18.0			>20	
5	5.1	37.0	35.3	9.7	12.6	11.9	12.1	24.4	23.3	1.9	2.0	31.3	30.8	10.4	39.3	39.8	9.6	35.8	33.7	5.1	>20	>20
	5.2	38.0			13			25.0		1.9		33.3			36.6			33.4			>20	
	5.3	35.7			11.9			23.8		2.0		32.5			45.3			31.6			>20	
	5.4	30.3			10.2			20.1		2.0		26.2			38.1			33.8			>20	
6	6.1	282.2	273.9	3.8	72.3	70.3	3.7	209.9	203.6	2.9	2.9	259.0	230.3	12.9	312.9	308.3	2.3	138.9	123.1	9.8	>20	>20
	6.2	266.8			68.4			198.4		2.9		240.7			298.8			120.8			>20	
	6.3	283.5			72.8			210.7		2.9		189.1*	-244.1	-5.6	314.3			109.7			>20	
	6.4	263.2			67.8			195.4		2.9		232.5			307.3			123.1			>20	

* G1 is calculated from the Total aflatoxin minus B1 aflatoxin (may include aflatoxins other than G1)

INDIVIDUAL PARTNER FINAL REPORT

PARTNER: MAPA – BRAZIL

Ministério da
Agricultura, Pecuária
e Abastecimento
GOVERNO FEDERAL

MAPA team

LACQSA - Laboratory for Quality Control and Food Safety:
Eugenia Azevedo Vargas, Coordinator, Chemist
Eliene Alves dos Santos, Chemist
Júlio César Garcia, Agronomist
Luciana de Castro, Pharmacyst
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Diolanda Fernandes de Sousa, Chemist
Cristina Neres da Silva, Chemist
Nilton Giovani de Almeida, Technician

LANAGRO/PA - National Agricultural Laboratory in Pará State
Francisco Airton Nogueira, Veterinarian
Raimundo Dantas Brito, Agronomist
Mauricio Quaresma de Araújo, Chemist
Nilce Limeira Medeiros, Agronomist
Poliana Carla Góes de Souza, Agronomist

SFA/PA - Federal Superintendence of Agriculture in Pará State
José Carlos Barroso, Agronomist
Gilson Pedrosa dos Santos, Technician
Otavio Durans, Agronomist

SDA - Secretariat of Agricultural Defense
Luzia Maria Souza, Agronomist
Ricardo Kobal Raski, Agronomist

Tasks and activities performed within the framework of the different project specific objectives

Management activities

MAPA has continually supported the project's coordination and partners to solve issues regarding the planning and the progress reporting of the scientific activities. MAPA has also helped the project's coordinator in the organization of the annual and final workshops.

Scientific activities

Specific objective 1: Characterization of the Brazil nut production chain, and formulation of organizational and incentive strategies for safety control

MAPA has collaborated to the collection of data and production of the following two reports:

- "Situação atual das legislações brasileiras e internacionais relacionadas à comercialização da castanha-do-Brasil" (Luzia Maria Souza, SDA).

- "Dinâmicas, processos e atores da cadeia produtiva de castanha-do-brasil nos estados do Acre e do Pará, Brasil" (Ricardo Kobal Raski, SDA).

José Carlos Barroso (SFA-PA) has facilitated the contacts with the Brazil nut private sector in the state of Pará to carry out the socio-economic field surveys in this state.

Specific objective 2: Validation of recommended good practices in the Brazil nut production chain for aflatoxin control

Sampling

SFA-PA/MAPA has collected a total of 148 Brazil nut samples (forest and processing plant) in Pará state. Field trips were organized in order to collect the necessary samples in the forest (95). Sampling was carried out according to Commission Regulation EC 401/2006 (European Commission, 2006) and considering four different steps in the forest:

1) Sampling and selection of Brazil nut pods inside the "*castanhais"* (production areas);
2) Piled Brazil nut pods and nut selection;
3) Dried Brazil nuts and
4) Community barns.

In the processing plant, SFA-PA/MAPA has collected 53 Brazil nut samples according to Commission Regulation EC 401/2006 (European Commission, 2006).

Fungal Isolation and Colony Counts

Under NFA's supervision, the microbiology team from LANAGRO-PA has quantitatively estimated the concentrations of aflatoxin producing fungi isolated from the collected Brazil nut samples. The technical protocols were passed through the training course *Analysis of aflatoxin-producing molds by Aspegillus-Flavus-Parasiticus-Agar (AFPA),* organized by the NFA's team and held in Acre from 25 to 27 October 2006. The analytical protocol consisted in dilute plating Brazil nut sample extracts, inoculating in the selective medium AFPA (*Aspergillus-Flavus-Parasiticus-Agar*) and colony counting aflatoxin producing colonies.

Sample Preparation

LACQSA/LANAGRO-MG has given continuous technical support to Pará and Acre teams regarding sample preparation, storage and dispatch procedures. Two training courses have been organized: one in Pará (9-10 July 2007) and the other in Acre (11 July 2007). The two training courses followed a protocol based on LACQSA's SOP-009 *Sample preparation, storage and disposal* (MAPA, 2008a).

The mycotoxin team from LANAGRO-PA was responsible for the preparation of all Brazil nut samples collected in the Pará state. Initially, the lab had to carry out some tests to adjust sample preparation and storage procedures to the project's needs. Sample preparation only started after all tests have been finished.

Aflatoxin Analyses

LACQSA/LANAGRO-MG has determined aflatoxins B_1, B_2, G_1 and G_2 (MAPA, 2008b) in 315 samples of Brazil nuts collected in Acre and in 215 samples collected in Pará, resulting in a total of 530 samples and 2120 determinations. The samples have been analysed with a quantitative method using immunoaffinity columns clean-up, determination and quantification by high performance

liquid chromatography with Kobra Cell derivatization - Fluorescence detector. The clean-up step was executed with an automatic sample processor (solid phase extraction) ASPEC Xl – Gilson. The recovery results were deemed as acceptable and the Brazil nut sample results were only accepted when the analytical quality control check complied with the Regulation EC No 401/2006, i.e., the recoveries obtained for the Brazil nut spiked samples should be within the range of 70 to 110%. If the quality control check presented a result out of this range, the analytical batch was supposed to be re-analysed.

Total aflatoxin concentrations varied from *non-detected* (< 0.06 µg/kg) up to 1939.40 µg/kg for samples collected in Pará and from *non-detected* (< 0.06 µg/kg) up to 2410.66 µg/kg for samples collected in Acre. The complete analytical results were provided to the Safenut general coordination and partners.

Specific objective 3: Validation and implementation of a rapid aflatoxin surveillance system for use along the Brazil nut production chain

Method Validations:

LACQSA/LANAGRO-MG has collaborated in the revision of the work plan proposed by CSL to validate R-Biopharm's aflatoxin immunoassay kits. This revision has considered a contamination level of 4 ppb and a preliminary test with direct injection in HPLC of the extract prepared according to the kit's manual, using FAPAS reference samples.

LACQSA/LANAGRO-MG has also elaborated a fully comprehensive protocol named *Evaluation of the performance of the 03 different R-Biopharm Kits in the estimation of aflatoxins BG levels in Brazil nuts - STDF PROJECT* (MAPA, 2008c) to validate/verify the following immunoassay kits:

– RIDASCREEN®FAST Aflatoxin (R5202) - An ELISA kit with the full set of controls (standards)
– RIDASCREEN Aflatoxin B1 30/15 (R1211) - An ELISA kit with a single control (standard)
– RIDA®QUICK Aflatoxin (R5204) - A rapid Lateral Flow Device or LFD

This validation protocol proposed by LACQSA/LANAGRO-MG included a preliminary study in which reference materials purchased from FAPAS (aflatoxins in tree nuts) and homogeneous Brazil nut samples (blank and naturally contaminated) would be analysed both by the R-Biopharm kits and by HPLC method, in quintuplicate. Aliquots of sample extracts (extracted as stated by the manufacturer's protocols) would be directly injected into HPLC and the results compared with the ones generated by the kits and the HPLC reference method. If the results were not comparable, the R-Biopharm would be contacted and the study interrupted until the difference is clarified.

The main objectives of the validation protocol were:

- To evaluate linearity, RSD, cross reactions and false positive and negative results;
- To evaluate the detections limits;
- To correlate the HPLC and kits' results for naturally contaminated Brazil nuts samples;
- To confirm the kits' characteristics according to the manufacture claim.

LACQSA/LANAGRO-MG was not able to implement the R-Biopharm rapid aflatoxin immunodiagnostic kit validations due to the delay in the approval of the protocol by CSL and R-Biopharm and also because the necessary validation measures could not be effectively implemented within the project's time frame.

Dissemination activities

Scientific publications

P. Johnsson, M. Lindblad, A. M. Thim, N. Jonsson, **E. A. Vargas**, **N. L. Medeiros**, C. Brabet, **M. Quaresma de Araújo** and M. Olsen: Growth of aflatoxigenic moulds and aflatoxin formation in Brazil nuts, World Mycotoxin Journal, Volume 1, Number 2, May 2008.

Participation in project events and meetings

MAPA has collaborated to the organization of all events held in Brazil, including the final project workshop in Pará.

MAPA's team has also participated in the following events:

Event	Title	Period	Participants
International Workshop	Prevention and control of Brazil nut contamination by aflatoxins	10-11 July 2006	Eugenia Azevedo Vargas Ricardo Kobal Raski Luzia Maria Souza Maurício Quaresma de Araújo
Meeting	SafeNut Project kick-off meeting	12-14 July 2006	Eugenia Azevedo Vargas Ricardo Kobal Raski Luzia Maria Souza Maurício Quaresma de Araújo
Training Course	Rapid immunodiagnostic methods for the detection of aflatoxins in Brazil nuts	25-26 October 2006	Eliene Alves dos Santos Giovana A. Amaral Gonçalves Maurício Quaresma de Araújo
Training Course	Analysis of aflatoxin-producing molds by Aspegillus-Flavus-Parasiticus-Agar (AFPA)	25-27 October 2006	Júlio César Garcia Poliana Carla Góes de Souza
Training Course	Rapid immunodiagnostic methods for the detection of aflatoxins in Brazil nuts	24-25 September 2007	Eliene Alves dos Santos Giovana A. Amaral Gonçalves Maurício Quaresma de Araújo
Meeting	First annual progress meeting	26-27 September 2007	Eugenia Azevedo Vargas
Meeting	Meeting with the private sector	28 September 2007	Eugenia Azevedo Vargas
International Workshop	Final Workshop	6-7 November 2008	Eugenia Azevedo Vargas Júlio César Garcia Eliene Alves dos Santos Thais Alves de Sá Giovana A. Amaral Gonçalves Maurício Quaresma de Araújo Nilce Limeira Medeiros José Carlos Barroso Gilson Pedrosa dos Santos Ricardo Kobal Raski Luzia Maria Souza

Measurable project impacts in the reporting period (as distinct from project outputs)

The technical knowledge of LANAGRO-PA and EMBRAPA Acre has greatly improved due to SafeNut project's actions. Their lab teams have received hands-on training and continuous technical support in order to achieve the project's demands. LANAGRO-PA had their mycotoxin lab finally implemented and is able now to carry out most of the tasks related to the mycotoxin analyses (sampling, sample preparation, kit analysis, etc). EMBRAPA Acre also had their mycotoxin lab implemented and improved. These two labs have a strategic importance in the Brazil nut chain since they are the only mycotoxin labs located in the Amazon region.

LACQSA/LANAGRO-MG had the opportunity to send a technician (Julio Cesar Garcia) for a two-week training course on the identification of aflatoxin producing molds in NFA, Sweden. It was a hands-on training course in which the aflatoxigenic isolates were identified by species level. The course was held at the Microbiology Division of NFA from 15 to 26 September 2008. This training course has contributed to the implementation of the mycological activities carried out by LANAGRO-MG.

References

EUROPEAN COMMISSION - Commission Regulation EC No 401/2006 of 23 February 2006 - Laying down the methods of sampling and analysis for the official control of the level of mycotoxins in foodstuffs. Official journal of European Union, 9-03-2006, 12p.

MAPA - Ministério da Agricultura, Pecuária e Abastecimento (2008a). LACQSA/LANAGRO-MG - Laboratório de Controle da Qualidade e Segurança Alimentar. Procedimento Operacional Padrão (POP 009, rev. 09) Preparo, armazenamento e descarte de amostras, Belo Horizonte, Brasil, 15 p.

MAPA - Ministério da Agricultura, Pecuária e Abastecimento (2008b). LACQSA/LANAGRO-MG - Laboratório de Controle da Qualidade e Segurança Alimentar. Procedimento Operacional Padrão (POP 055, rev. 02) Determinação de aflatoxinas B_1, B_2, G_1 e G_2 por cromatografia líquida de alta eficiência e em camada delgada, Belo Horizonte, Brasil, 18 p.

MAPA - Ministério da Agricultura, Pecuária e Abastecimento (2008c). LACQSA/LANAGRO-MG - Laboratório de Controle da Qualidade e Segurança Alimentar. Validation Protocol: Evaluation of the performance of the 03 different R-Biopharm Kits in the estimation of aflatoxins BG levels in Brazil nuts - STDF PROJECT. Belo Horizonte, Brasil, 28p.

INDIVIDUAL PARTNER FINAL REPORT

PARTNER: EMBRAPA - BRAZIL

EMBRAPA team (from EMBRAPA Acre and Belém)

Researchers:
- Cleísa Brasil da Cunha Cartaxo, MSc Post-harvest Technology, Coordinator of EMBRAPA activities until March 2007;
- Joana Maria Leite de Souza, MSc. Food Technology, Coordinator of EMBRAPA activities from March 2007;
- Lúcia Helena de Oliveira Wadt, Ph.D. Plant Genetics and Breeding – Forest Management;
- Rivadalve Coelho Gonçalves, Ph.D. Phytopathology;
- Virgínia de Souza Álvares, Ph.D. Post-harvest physiology;
- Jair Carvalho dos Santos, Ph.D. socio-economy.

Technicians/Assistants:
- Márcio Muniz Albano Bayma, Technician (Economist);
- Francisco de Salles, Research Assistant;
- Roberto Vieira Sampaio, Research Assistant;
- Ailson Madruga, Research Assistant;
- Elden Paiva, Research Assistant;
- Josivaldo da Silva Saraiva, Research Assistant;
- Hailton Melo de Araújo, Research Assistant;
- John Lennon Mesquita Catão, Laboratory Assistant;
- Aldeci Oliveira, Field Assistant;
- Antoninho Izidório Petik, Field Assistant;
- Raimundo Bezerra Macedo, Field Assistant;
- Diva Motta Gonálves, Journalist;
- Dorila Motta Gonçalves, Technician (Economist);
- Raimundo Nonato Oliveira Costa, Driver;
- Abelardo Vareda Guimarães, Driver;
- Claudir Vezu, Driver;
- Renato Telles do Nascimento, Driver.

Students:
- Olivier Devillers, BSc student in agronomy, ENSA Rennes, France, December 2006-May 2007;
- Felícia Maria Nogueira Leite, SEAPROF (Secretary of Extension and Production in Acre), MSc student in agronomy (Federal University of Acre), January 2007- November 2008;
- Sarita Maria de Azevedo, BSc student (Scientific Initiation Scholarship), November 2007-May 2008;
- Idaiane Lira Costa, undergraduated student (Scientific Initiation scholarship), June-November 2008;
- Juliana Milan, Zoology technician (Scientific Scholarship), July 2007;

Workers:
- Célio Pereira dos Santos
- Francisco Jorge Araujo da Silva
- Francisco Soares de Melo
- Ilma Gomes Salvatierra Zelada
- Lilia Gomes de Freitas
- Maria das Graças Lima Domingos
- Maria de Nazaré Menezes
- Maria Dorinete Freire Pereira
- Maria Laelia da Paixão Alves

Tasks and activities performed within the framework of the different project specific objectives

Management activities

Embrapa Acre managed project development in Acre through activities such as: acquisition of laboratory materials and equipment; negotiation of the purchase of Brazil nuts with one producers' cooperative for use in experiments and microbiological and aflatoxin analyses; shipment of samples for aflatoxin analyses to the Laboratory of Quality control and Food Safety in Belo Horizonte, Minas Gerais (LACQSA/MAPA); trip solicitations and internal requests of vehicles for field collections, in addition to the payment of staff involved in the project, including scholarship recipients, Brazil nut collectors, the field team, and others.

Embrapa Acre accompanied and participated in local meetings as part of scientific coordination visits, presenting materials and information to aid in project advancement, in addition to visiting field sites. Members of Embrapa Acre's team maintained regular contact with the general project coordinator and other external team members to develop appropriate sampling methods and laboratory analyses.

Scientific activities

Specific Objective 1: Characterization of the Brazil nut production chain and formulation of incentive strategies for safety control

First year (2007)

Data on commercialized volume, price and destination of Brazil nuts extracted from Acre were obtained through on-line databases with the objective of elaborating a preliminary technical document that contained information on all stages of the Brazil nut production chain.

Second year (2008)

During the first trimester 2008, field surveys were conducted and questionnaires applied to Brazil nut collectors, intermediaries and agro-industries in the cities of Sena Madureira, Rio Branco, Xapuri and Brasiléia, to determine the practices along the production chain. Information collected included: materials used to collect, store and transport the product, forms of production drainage and main marketing channels. In the table below, the number of interviewees in each s of the production chain is indicated.

Segment in the production chain	Number of questionnaires applied
Agro-industry/association	4
Intermediate agents	13
Institution (CONAB)	1
Total	**18**

To compare the production systems used in the region, the technical coefficient values of the traditional production system were updated and new data collected on the technical coefficient values of the improved production system, i.e. with use of good extractive practices. The study on the improved system was performed in July 2008 in the association of producers 1 where a technical meeting was organized with the participation of 13 extrativists along with agricultural technicians, researchers and other actors involved in Brazil nut production. The objective of this

meeting was to obtain data on production for a standard situation with minimal possible distortion of information, always attempting collection of parameterized and median data of the region.

The collection of data on prices for the commercialization of inputs and equipment used in the activity was performed from July to August 2008 in the local market of Brasiléia and Epitaciolandia.

Specific objective 2: Validation of good practices recommended in the Brazil nut production chain for aflatoxin control

Collection of data (temperature and relative humidity at sampling points, nut water activity, total fungal contamination and contamination by Aspergillus flavus/Aspergillus parasiticus)

First year (2007)
To define study sites, communities which already applied good extractive practices (GEP) recommended by existing guidelines published by Embrapa, the Food Safety Program and Codex Alimentarius, were identified by Embrapa Acre and SEAPROF teams.
The sampling plan was discussed in meetings with project partners and actors of the Brazil nut production chain, after defining the different stages of the production chain with the communities.

During the first year, Brazil nut samples were collected from April to November 2007, based on the European Commission Regulation n º 401/2006, in the following stages: selection (unselected, selected and discarded Brazil nuts), drying (selected and discarded Brazil nuts), storage for different time periods (0, 15, 30, 60 and 90 days of permanence in communal storage) and in the processing plant (during the final selection and storage).
Throughout sampling, temperature and relative humidity at sampling points were registered by a digital thermo hygrometer.
All collected samples were transported to Embrapa Acre's Microbiology Laboratory, in Rio Branco, to be homogenized, peeled and triturated, and to undergo the analyses of water activity, total fungal contamination and contamination by Aspergillus flavus/A. parasiticus. Water activity was determined by using a Pawkit model from Decagon. Fungal counting was performed on AFPA medium specific for A. flavus / A. parasiticus. In addition to these analyses, samples were frozen and sent to the Laboratory of Quality Control and Food Safety (LACQSA/MAPA) in Belo Horizonte, Minas Gerais, to determine levels of aflatoxins B1, B2, G1 and G2 by using High Performance Liquid Chromatography (HPLC).
During the first year, 53 global samples were collected in the forest and communal warehouses of the associations of producers 1 and 2, in Acre, and 16 samples in a processing plant.

Second year (2008)
Brazil nut samples were collected from January to August 2008, based on the European Commission Regulation n º 401/2006, in the following stages: pod collection (0-5 days, 15-20 days, and 2 months, separately), nut selection (unselected, selected and discarded nuts), drying (selected and discarded nuts), storage for different time periods (0, 15, 30, 60 and 90 days of permanence in communal storage areas) and in the processing plant (storage before drying, selected and discarded nuts after selection and drying, final storage).

Throughout sampling, temperature and relative humidity at sampling points were registered by a digital thermo hygrometer.
All collected samples were transported to Embrapa Acre's Microbiology Laboratory, in Rio Branco, for analysis of water activity, total fungal contamination, contamination by Aspergillus flavus/A. parasiticus and aflatoxins (B1, B2, G1 and G2) as described in the methodology of year 1.

During the second year, 164 samples were collected in the forest and communal warehouse of the association of producers 1, and 34 samples in a processing plant.

Conclusions

Based on our analyses, along with data of aflatoxin levels supplied by LACQSA, the main conclusion of this study is that the most critical stages in the Brazil nut production chain are drying and storage.

The drying process used in the extractivist communities (natural aeration) was ineffective in reducing Brazil nut water activity to safe levels in relation to fungal growth and mycotoxin contamination.

In terms of storage, contamination was much greater in nuts stored in bags for more than 30 days in the communal warehouse. It is recommended that the transport of nuts to processing plants occurs as quickly as possible.

In summary, the good extractive practices adopted by the community in this study are ineffective for attending aflatoxin levels allowed by the European legislation.

Dissemination activities

Co-organization and participation in trainings

Training for counting aflatoxin-producing moulds by using specific AFPA medium and for aflatoxin analysis in Brazil nuts by using ELISA and LFD methods

- Rio Branco, AC – 2006: training for fungal analysis by using specific AFPA medium and for aflatoxin analysis by using ELISA and LFD kits took place at Embrapa Acre and consisted in theory and laboratory practices;
- Belém, PA – 2007: members of team were trained on ELISA and LFD kits for aflatoxin analyses in Brazil nuts;
- Belém, PA: during the Final Project Workshop in 2008, the team members were trained on LFD kit for aflatoxin detection in Brazil nuts.

Technical meeting for project result spreading:

Rio Branco, AC – November 2008: a technical meeting was organized at SEAPROF (Secretaria de Agricultura Familiar e Extrativismo do Estado do Acre) with the participation of extension technicians for a presentation and discussion of the final project results.

Courses on Good Practices in Brazil nut production: in the scope of a training program for extension technicians, four 24 hour technical courses on good practices in Brazil nuts were organized by ASBRAER (Associação Brasileira das Entidades Estaduais de Assistência Técnica e Extensão Rural) in partnership with MAPA and EMBRAPA. The courses took place in:

- Rio Branco: 35 technicians
- Manaus: 35 technicians
- Marabá: 17 technicians
- Santarém: 18 technicians

Scientific publications

As a result of the project, there was an MSc thesis of Felícia Maria Nogueira Leite, student in Vegetal Production of the Acre Federal University (UFAC):

Leite, Felícia Maria Nogueira. Fungos aflatoxigênicos na castanha-do-brasil sob as condições da floresta e de armazenagem comunitária no Acre. Dissertação (Mestrado em Agronomia) – Pró-

Reitoria de Pesquisa e Pós-Graduação, Universidade Federal do Acre, Rio Branco – Acre, 2008, 97p.

Articles resulting from this thesis are in preparation, as well as other articles on results from the first and second year projects.

It is also planned to present data from the socio-economic studies at the 47° Congress of SOBER (Sociedade Brasileira de Economia Administração e Sociologia Rural) to be held in Porto Alegre /RS, 26-30 July 2009 (http://www6.ufrgs.br/sober47/):
Bayma, Marcio M. A; Santos, Jair Carvalho de; Piketty, Marie Gabrielle. Análise comparativa entre os sistemas de produção extrativista tradicional e melhorados de castanha-do-brasil ocorrentes na Regional do Alto Acre/AC.

Technology diffusion

An international workshop was organized in Rio Branco/AC, on 10-11 July 2006, with the participation of production chain agents, technicians and students from Peru, Bolivia and Brazil, where project strategies and partner's roles were presented.

During a technical visit at the community selected for sample collection, project members were interviewed by a local TV station about the Safenut project and the importance of the study of fungal growth and aflatoxin contamination in the Brazil nut production chain. Participated in this interview: Joana Maria Leite de Souza, Felícia Maria Nogueira Leite, Virgínia de Souza Álvares, Catherine Brabet and Francisco Soares de Melo, a Brazil nut producer.
The members have also been interviewed by a local radio, Program called "Roots of Land", about "Contamination of Brazil nut by aflatoxins", in February 2008.

The final Safenut workshop took place in Belém/PA, on 6-7 November 2008. There were researchers from the partner countries, technician, as well as agents of Para and Acre's production chains.

Supervision of students/trainees

During the project, scholarship holders were trained on the identification of filamentous moulds, preparation of slurry for analysis, determination of nut's water activity and temperature. The trained scholarship holders had intensively supported the project activities during the field collections and lab activities.
Embrapa Acre and CIRAD's researchers supervised the student Olivier Devillers, during the mycological analysis, in 2007. In 2008, the students Sarita Maria Borges, David Aquino Costa and Idaiane Lira Costa, from UFAC and UNINORTE, respectively, participated of this training program.

Project Impacts

Project results strengthened the inter-institutional arrangement in the State of Acre, for research and technical extension on Brazil nut matters. The inter-institutional arrangement, LPA-Brazil nut, which is composed of partners as WWF, UICN, Sebrae, Embrapa and Cooperacre, argues on subjects such as: Brazil nut certification, handling of product in driers and warehouses in extractivist community. The group develops radio programs in order to disseminate Good Extraction Practices among extractivist producers.

In the same way, other challenges had come out in order to guarantee the continuity of research on nut contamination by aflatoxins. New projects are being articulated/executed to complement the extensive work performed by the Safenut Project, as for example the project "Post-harvest handling of Brazil nut for communitarian organizations" and a network between several organizations in the State of Acre, such as WWF, Sebrae, IUCN, Seaprof and Embrapa for spreading the aspects of product quality to Brazil nut producers and consumers.

A Field Day is planned in July 2009 for spreading research results on Brazil nuts, including those of the Safenut project, to the productive sector and government technicians.