APPLICATION FOR AN STDF PROJECT GRANT
Theme 2:
"Capacity building for public and private organizations, notably with respect to market access"

Date of submission: 14 December 2005

1. Project 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.
2. Requesting government/agency or private body	**MAPA, Brazil** Ministério da Agricultura, Pecuária e Abastecimento / Ministry of Agriculture, Livestock and supply Contact: Dr Marcelo Bonnet - mbonnet@agricultura.gov.br
3. Collaborating government (s)/agency	**CIRAD, France** Centre de coopération internationale en recherche agronomique pour le développement / International Cooperation Centre in Agronomic. Research for Development Contact : Dr. Catherine Brabet – catherine.brabet@cirad.fr **NFA, Sweden** National Food Administration Contact: Dr. Monica Olsen - monica.olsen@slv.se **CSL, United Kingdom** Centre Science Laboratory Contact: Dr. John Banks - j.banks@csl.gov.uk **EMBRAPA, Brazil** Empresa Brasileira de Pesquisa Agropecuária / Brazilian Agricultural Research Corporation Contact: Dr Lucia Wadt - lucia@cpafac.embrapa.br See Appendix 1: Description, role and team members of the project partners (p. 8)
4. Overall project management	**General coordination** by **Dr. Catherine Brabet, CIRAD, France** **General scientific coordination** by **Dr. Monica Olsen, NFA, Sweden** See Appendix 2: Overall project management (p. 17)
5. Project objectives Attach description of project background and rationale	With the aim to reduce and control aflatoxin contamination in the Brazil nut production chain to levels that attend the international sanitary standards, in particular the stricter European regulation, the Brazilian government and its executive services, research institutes, universities, and Non-Governmental Organizations among others initiated a series of actions in partnership with the stakeholders of the Brazil nut production chain. These actions mainly concern: - Research work to identify the Critical Control Points (CCP) and factors for aflatoxin contamination from the Brazil nut collection to the processing units. **Further investigation needs to be performed 1) to identify and confirm these CCP and factors all along the Brazil nut production chain 2) to estimate the probability of aflatoxin contamination at the different stages of the production chain through the establishment of the critical limits and the development of predictive models for aflatoxin production and fungal growth;**

	- The definition and publication of good practices and the development of post-harvest technologies (prototypes of warehouses, washing and drying equipment). **However, the effectiveness of these recommended good practices and technological alternatives have not been tested yet on a scientific-basis**, - Training courses in good practices for their implementation in the Brazil nut production chain as well as in sampling methods according to the European Directive 98/53/EC for aflatoxin inspection and sanitary certification, **but these activities of extension are still in their early stages and consequently they need to be reinforced.** In addition, **there are no rapid, robust and low-cost on site aflatoxin analytical methods that can be used throughout the Brazil nut production chain to monitor aflatoxin contamination**. Also there are no such methods to aid the implementation of HACCP (Hazard Analysis and Critical Control Point) type control schemes based on preventive and correctives measures. Currently, Brazil nut inspection is performed prior to export. Finally, **the conditions of Brazil nut production and commercialization, as well as the production chain organization which vary significantly between the producing regions, are still poorly defined and documented.** The present project will address the shortcomings indicated above. **General objective:** The overall objective of the project is to validate and transfer to the key stakeholders a sustainable and effective safety management system for reducing and controlling the occurrence of aflatoxins along the Brazil nut production chain. This work is aimed at enhancing the capacity to meet the international sanitary standards, in particular the recent stricter European regulations, for recovering and consolidating export market access, to protect human health and prevent deforestation of the Amazonian forest. **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. See Appendix 3: Project background and rationale (p. 18)
6. Project activities Itemise main elements here and attach a detailed work plan, dissemination plan and evaluation plan	- Describe the current conditions of Brazil nut production and commercialization in the Brazilian States of Acre and Para, and their constraints and opportunities for aflatoxin control. - Formulate propositions to improve the organization and to provide better incentives for the development of a sustainable Brazil nut production chain.

	- Validate and update existing recommendations of good practices in the Brazil nut production chain for aflatoxin control through in-field case studies and the development of a simple predictive model for aflatoxin and fungi production. - Adapt and validate rapid methods brought in to the project for aflatoxin analyses in Brazil nuts - e.g. Enzyme Linked Immunosorbent Assay (ELISA) and Lateral Flow Device (LFD)-, and implement them in Brazil in the laboratory, the Brazil nut production area and one processing plant. - Set up training courses in ELISA and LFD for aflatoxin analyses in Brazil nuts, AFPA agar plate methodology for the identification of aflatoxin producing fungi and good practices in the Brazil nut production chain for the project participants and key stakeholders. - Disseminate project information and results through different information systems (website with details & outputs from the project ; scientific and specific sector publications, etc). - Organize project meetings and workshops (kick off meeting and first workshop, progress meeting and final workshop). See Appendix 4: Detailed work plan, dissemination plan and evaluation plan (p. 25)
7. Private/public sector co-operation Detail the arrangements for public/private sector co-operation, if any, in the project	The Brazilian project partners, MAPA and EMBRAPA, as well as the governments of the States of Para and Acre as project co-participants, have been developing a full partnership with the different key stakeholders of the Brazil nut production chain. EMBRAPA has been working in collaboration with the main cooperatives and associations of Brazil nut producers of the extractive reserves of the state of Acre (CAPEB, CAEX, COOPERACRE, COOPERIACO) within the framework of research projects and extension activities. MAPA in 2004 signed an agreement of technical cooperation with the Association of Brazil nut Exporters of the State of Para aiming at the implementation of a laboratory for aflatoxin analysis and to improve the quality of Brazil nuts. The project will benefit from these existing arrangements for public/private sector co-operation to conduct field participatory research involving the key stakeholders of the Brazil nut production chain (extractivists, processors, exporters, etc) to attend the project Specific objectives 1 and 2, and to organize training courses for knowledge and technology transfer to these key stakeholders (Specific objective 4). The project will also contribute to strengthen the private/public sector dialogue and co-operation in the Brazil nut area through the organization and implementation of project meetings and workshops with the participation of the two sectors (Specific objective 5). R-Biopharm AG (RBAG) has a long and proven experience in the development and the production of rapid tests for mycotoxin detection/screening. Through the cooperation of this company, rapid Enzyme Linked Immunosorbent Assay (ELISA) kits and recently developed one step, on-site and robust Lateral Flow Devices (LFD), that only takes a few minutes to perform, for total aflatoxins will be brought in to the project. The cooperation of RBAG will also entail the testing and technical transfer of these assays by means of hands on training sessions at laboratories and therefore to the Brazil nut production chain in Brazil.

8. Partner institutions involved If appropriate, identify STDF partner institutions who will be involved and describe the nature of that involvement	There is no STDF partner institutions directly involved in the project. Nevertheless, some project participating members have been actively working in the Codex Committee on Food Additives and Contaminants (CCFAC), in particular on the code of practice for aflatoxins in tree nuts: - Monica Olsen (NFA), in both the position as national delegate of Sweden and during her role as FAO JECFA secretary in 2005; - Luzia Maria Souza (CCRC-SDA), Eugênia Azevedo Vargas (LANAGRO-MG) and Ricardo Kobal Raski (DIPOV-SDA) from MAPA, as national delegates of Brazil. All are representatives in the specific technical group "Food Additives and Contaminants" of the Brazilian Committee of the Codex Alimentarius (CCAB).
9. Project outputs Specify outputs clearly and in detail and show the relationship to key STDF objectives including capacity enhancement, improved market access and trade opportunities, poverty reduction, linkages to country or regional program development priorities, public-private co-operation, innovativeness, demonstration effects, etc.	1. Current conditions of Brazil nut production and commercialisation in the Brazilian States of Acre and Para will be described along with the major constraints and opportunities for aflatoxin control. 2. Availability of information and analysis through reports on the full identification of production chains and the effectiveness of existing codes of practice will allow the output of a predictive model for the probability of aflatoxins in Brazil nuts. The model outputs combined with (1) above will lead to the reformulation of a Manual of Safety and Quality in Brazil Nuts. 3. Inexpensive and rapid assay for use in the laboratory and on-site throughout the production chain will be available to complement existing confirmatory and standard HPLC methods. These "new" methods will be adapted, developed and fit for purpose evaluation carried out specifically for and within the Brazil nut industry. The output of technical training will be carried out with key stakeholders in the Brazil nut industrial area. In addition to the assays for aflatoxins, analytical methods for the causative organisms (*A. flavus/A. parasiticus*) will be implemented in the Brazilian laboratories close to the Brazil nut production areas. 4. Training outputs will be knowledge & technology transfer to the key stakeholders. This will include training courses and material on of AFPA agar plate methodology, ELISA, LFD and good practices in Brazil nut production chain. Other outputs will include the implementation and maintenance of a project specific website and scientific and specific sector publications. Some of the latter will also be available on the website. 5. In addition to the outputs of (4) above, there will be a kick off meeting at the beginning, and progress meetings throughout the development of the project and a final workshop. These will include Brazilian, Bolivian and Peruvian key partners. Outputs from (1) and (2) will be pooled and the information made available will allow the management of Sanitary and Phytosanitary Standards (SPS). This will assist in the mandatory official controls required under international trading conditions to gain and maintain market access. Outputs from (3) will provide the means for ensuring outputs (1) and (2) are achieved and maintained in the future through an efficient monitoring tools. Outputs (4) and (5) will ensure that all information and methods arising from the project are available to the whole of the Brazil Nut industry from the jungle floor to the world markets. The project will increase the public-private dialogue and cooperation in standards

	development, implementation, domestic enforcement and export market strategy. It is envisaged that the project will increase the shared responsibilities between the public and private sector and increase their awareness of international food standards and how they can be met. Environmental and socio-economic factors will also be key beneficiaries from the outputs of this project. For example, decreased post-harvest losses due to aflatoxin contamination will improve earnings of the local people and help alleviate poverty. The health of the local population will also be protected through the consumption of uncontaminated Brazil nuts. Of ever increasing global concern is the preservation of the rain forests and outputs from the project that sustain the Brazil nut industry will also help sustain this critically important environmental asset.
10. Project impact Specify the expected impact the project will have on market access, the SPS situation and poverty reduction. Identify how the project will fit with existing bilateral or multilateral donor projects and programmes, examine the sustainability of the proposed action and, where possible, suggest where the project may be replicated.	The Brazil nut production chain is confronted with problems of contamination by high levels of aflatoxins. This is of major concern not only for public health but also in regard to economic losses and environment impact. Most of the collected Brazil nuts are subject to minimal processing for export either in shell or as a kernel, mainly to Europe and United States. The strengthening of European regulations on aflatoxin levels - Commission Regulation (EC) N° 1525/98 of 16 July 1998 and Decision 2003/493/EC of 4 July 2003 -, led to a drop in Brazilian exports to Europe of about 87 % for total Brazil nuts (in shell and kernels) between 2000 and 2004, and close to 92 % for Brazil nuts in shell (the main European market) between 2003 and 2004. The reduction of Brazil nut exports to Europe has affected the economy of the producing regions and caused the clearing of larger areas of the Amazonian forest by local populations forced to look for other economic resources. In addition, the rejected contaminated Brazil nut lots could be reintegrated in to the national markets and constitute a risk for the health of the Brazilian population through the consumption of the nuts themselves or nut based-products. In that context, through the validation and transfer to the key stakeholders of a sustainable and effective safety management system for reducing and controlling the occurrence of aflatoxins along the Brazil nut production chain, **the project foresees the following final impacts:** • Recover and consolidate Brazil nut export markets by meeting the international sanitary standards related to food aflatoxin maximum tolerance limits, in particular the recent stricter European regulations; • Protect and promote human health through the commercialization of safer Brazil nuts and by avoiding the reintegration of rejected contaminated lots in the national market of the producing countries. This applies to : - Consumers in the Amazon region and Brazil nut producing and importing countries, - Both raw nuts and processed products like flour and biscuits that are frequently consumed by school students in the Brazilian North region. • Alleviate the poverty of the local Amazon population through the reduction of Brazil nut post-harvest losses due to aflatoxin contamination, and consequently income generation. • Preserve the Amazon forest against deforestation and thus the biodiversity through the valorisation of the *Bertholletia excelsa* tree that depends on other species for pollination and fruit production. All the expected socio-economic and environmental project impacts are considered to be positive and will contribute to the sustainable development of the Amazon region.

The sustainability of the proposed action will be insured by :

1) the involvement in the project of:

- the key stakeholders of the Brazil nut production chain (cooperatives of producers, processors, association of exporters) :
  - Field participative research will be conducted for 1) testing, validating and updating as necessary the recommended good practices at a pilot scale, i.e. in a selected Brazil nut production system where the recommended good practices have been implemented and the agents demonstrated a high interest and motivation for Brazil nut quality improvement 2) implementing and validating rapid, low-cost and simple aflatoxin analytical methods in the production area and processing plant. This participative research will ensure that the safety management tools (good practices and rapid methods for aflatoxin analyses) are acceptable and compatible with the local socio-economic and environmental context. This approach will facilitate a better adoption/appropriation of the project results by the agents of the Brazil nut production chain.
  - Training courses in good practices and rapid methods for aflatoxin surveillance along the Brazil nut production chain will be organized for a larger group of agents both in the states of Para and Acre, including in-field demonstration sessions. The pilot group of agents will act as a leader and serve as an example for the other agents of the region for the adoption of the project results during and after the end of the project.

- The government of the states of Para and Acre as the local competent authority for the development of public policies, programmes and actions to improve Brazil nut quality:
  - Indirect financial support (infrastructure, transport, fuel, extension agents, etc.) will be provided by the local government for the execution of the project activities in the Brazil nut producing areas and the dissemination of the results;
  - Training courses in good practices and rapid methods for aflatoxin surveillance will be organized for the extension agents of the local government executive agencies to ensure knowledge and technology transfer continuity after the end of the project.
  - The dialogue and the co-operation between the public/private sector will be promoted and strengthened.

- The key Peruvian and Bolivian partners for a regional impact in the Amazon
  Participation in the project workshops and progress meeting as well as in the training courses. The final workshop will be dedicated to the dissemination of all the project results, with in-field demonstration sessions of both good practices and rapid methods for aflatoxin surveillance.

2) The capitalization of the project results through the publication of documents that will be available after the end of the project : updated and validated Code of Good Practices, Standard operating procedures for aflatoxin surveillance system, etc.

The project may be replicated in the other Brazil nut producing countries (Peru and Bolivia) and the safety management system / model may be used for other tree nuts.

11. Project inputs Specify total project cost. Attach detailed breakdown of proposed uses of funds.	**Total project cost : 826 219 US dollars** **75 % STDF contribution requested : 619 664 $ (USD)** See Appendix 5: Detailed budget (p.41)
12. Non-STDF contributions If appropriate specify any financial contributions expected from sources other than STDF.	The R-Biopharm AG company will, at their expense, bring rapid assays for aflatoxin analyses in to the project that will be evaluated and adapted on Brazil nuts and represents a contribution in kind. Assays used in the project for routine analysis of samples will be made available at competitive rates. The implementation and validation of a harmonized robust HPLC method to be used by the project participants for aflatoxin analyses will benefit from the financial support of a project approved in 2005 and coordinated by LANAGRO-MG of MAPA entitled "Analytical tools for training Brazil in the guarantee of the Brazil nut conformity related to aflatoxin hazards" – Project MCT/FINEP/1262/05. The execution of the in-field project activities will benefit of an indirect financial contribution (infrastructure, transport, fuel, extension agents, etc.) from the government of the States of Acre and Para, in particular the validation and transfer of the good practices in the Brazil nut production chain.
13. Timetable Show proposed commencement and conclusion dates (maximum project duration two years)	Project of a **full 2 years duration** See Appendix 6: Work planning (p. 43)

APPENDIX 1
Description, team members and role of the project partners

The partner consortium is based on the following considerations to ensure the success of the project.

- The consortium consists of :

  - **a core group of 5 partners** which request an STDF grant: 2 Brazilian partners (MAPA and EMBRAPA) and 3 European partners (CIRAD, NFA, CSL). Four of them (EMBRAPA, CIRAD, NFA, CSL) are involved in public research and technology transfer activities.

  - **a co-participant group** formed by the governments of the Brazilian States of Acre and Para and its executive services (Table 1), and the key stakeholders of the Brazil nut production chain – extractivist cooperatives, processing units, association of exporters – as focus groups of the project. These participants which do not request an STDF grant, will assist the core partner group in some activities and provide a counterpart mainly in kind to the project (infrastructure, transport, fuel, extension agents, etc.). Their involvement will ensure the project sustainability in the longer term.

  Table 1: Brazilian State government services of the project co-participant group

Brazilian State Government service	Brazilian state
SEPLANDS (Secretaria de Estado de Planejamento e Desenvolvimento Econômico Sustentável / Sustainable Economic Development and Planning State Service)	Acre
SEPROF (Secretaria de Produção Familiar e Extrativismo / Familiar Production and Extractivism Service)	Acre
SEATER (Secretaria de Assistência Técnica Rural / Rural Technical Assistance Service)	Acre
SAGRI (Secretaria Executiva de Agricultura / Agriculture Executive Service)	Para
ADE-Para (Agencia de Defesa Agropecuária do Estado do Para / Agriculture Defence Agency of the Sate of Para)	Para
EMATER-Para (Empresa de Assistência Técnica e Extensão Rural do Estado do Pará / Technical Assistance and Rural Extension Service of the State of Para)	Para
SMA (Secretarias Municipais da Agricultura / Agriculture Municipality Services) of the main Brazil nut producing municipalities	Acre and Para

- The partners offer not only a strong and complementary set of expertise, experience and research in the area of the project, but also the human and material resources needed by the project (see detailed description of each partner below). In particular, the project will benefit from the support of EMBRAPA-Acre and LANAGRO-Para/MAPA laboratories for fungi and aflatoxin analyses in the two selected Brazil nut producing States (Acre and Para).

- Historic and/or ongoing institutional and project-related scientific collaboration already exists among the partners as shown by the following table:

	CIRAD	NFA	CSL	MAPA	EMBRAPA
CIRAD	-	●		●	●
NFA	●	-	●	●	
CSL		●	-		
MAPA	●	●		-	●
EMBRAPA	●			●	-

- The strong articulation of the Brazilian partners with the key stakeholders of the Brazil nut production chain ensures their participation in the project, along with national universities and Non-Governmental Organizations (NGO) for project support and scientific exchanges as necessary.

- MAPA is the central competent authority in Brazil in charge of the public policy, programmes and actions to improve and guarantee Brazil nut quality and safety. Its Laboratory for Food Quality and Safety Analysis (LACQSA) which is involved in the project, is the only Brazilian laboratory recognized by the Commission Decision 493/2003/EC to perform aflatoxin analysis in Brazil nuts destined to export. This laboratory will provide a methodological support for Brazil nut sampling and aflatoxin analysis.

- EMBRAPA plays an important role in research and development policies within Brazilian agriculture and has a good experience of technology transfer to productive sector. EMBRAPA is also involved in a regional Brazil nut network "MAP" (**M**adre de Dios, Peru ; **A**cre, Brazil ; **P**ando, Bolivia) dedicated to the sustainable development of the Brazil nut extractive areas. This will facilitate the dissemination of project information and results at the regional level in the main Brazil nut producing countries.

- CIRAD has a long experience in coordinating international projects and working throughout Brazil, as well as in research fields that fit totally with the project objectives. Two senior scientists involved in the project are out posted in the country and a third one has a 7 year experience in Brazil. CIRAD acts as interface between research and academic institutes and the private sector for ensuring better partnership and integration of all stakeholder concerns.

- The NFA team is specialized in food mycology, and has an expertise on predictive modelling. As an European expert, Dr. Monica Olsen participated in the two FVO (Food and Veterinary Office) missions carried out in Brazil in 2003 and 2004 to assess the facilities and measures in place for the control of aflatoxin levels in Brazil nuts intended for export into the European Union. This will facilitate the integration of the European recommendations and the links with the European policy all along the project.

- The CSL team has a strong experience on rapid tests for aflatoxin determination.

- Partners are involved in European and/or national mycotoxin networks and cluster:
  - NFA, through Dr Monica Olsen, was involved in the European Mycotoxin Prevention Cluster (2000-2004), is member of the steering committee of the Mycoglobe network (2004-ongoing), and member of the International Commission on Food Mycology (ICFM).
  - CIRAD has also been invited to participate in the steering committee of the Mycoglobe network.
  - MAPA and EMBRAPA are involved in the Brazilian mycotoxin networks.

The role of the different partners and their complementarities are summarized in the following table:

Partners	Specific Role
CIRAD	General coordination. Scientific support for technical and socio-economic issues (agri-chain analysis, good practices, appropriate post-harvest technologies).
NFA	General scientific coordination. Scientific support for fungi identification and characterization. Links with European policy and mycotoxin clusters.
CSL	Scientific support and coordination with industry of the work on rapid immunoassays for aflatoxins.
MAPA	Support for the development and validation of quantitative and screening aflatoxin analytical methods. Field support for the execution of the project activities in the State of Para. Links with the Brazil nut chain stakeholders.
EMBRAPA	Field support for the execution of the project activities in the State of Acre. Links with the Brazil nut chain stakeholders.

Description and team members of the project partners

- **CIRAD, France - General coordinator of the project - www.cirad.fr**

CIRAD (Centre de coopération internationale en recherche agronomique pour le développement) is a public scientific institute specialized in development-oriented research on tropical agriculture, with the headquarter in Montpellier, France. The centre employs 1800 persons of which one third works outside of France. Food Quality and Safety is presently a high priority research topic for CIRAD through various projects dealing either with the global food quality management and risk analysis or traceability or food qualification and certification. CIRAD has a long experience in managing and contributing to many international research projects. The institute acts as interface between research and academic institutes and private sector for ensuring better partnership and integration of all stakeholder concerns. CIRAD maintains close relationship with the Brazilian project partners (EMBRAPA, MAPA) through official agreements of co-operation and has developed various successful research projects with them.
The CIRAD project team is multidisciplinary, consisting of fields of expertise highly relevant to the project activities (agri-chain analysis, food science and quality management, food technology). It will involve four senior scientists that belong to the CIRAD research units TROPIQUAL "Tropical Food Quality" (Drs. Brabet and Pallet) and NOMADE "Governance and Norms in Agricultural Markets" (Drs. Picketty and Vagneron). The TROPIQUAL research unit focuses on the characterization of food quality attributes and the main factors that affect them throughout the productive chain, the development of processing techniques for adding value to products, and the food safety guarantee and traceability. NOMADE research is aimed at promoting a better understanding of how international agricultural markets are regulated (*e.g.* traditional agricultural policies and international public and private standards), how such regulations are elaborated and what micro and macroeconomic impacts they have on developing countries
The general coordinator of the project (Dr. Brabet) as well as Dr. Picketty are currently out posted in Brazil, respectively at the UNICAMP (Universidade Estadual de Campinas), Campinas, State of São Paulo and USP (Universidade Estadual de São Paulo), São Paulo. Dr. Pallet that will be posted at CIRAD headquarters since 2006, has also a long 7 year experience in Brazil, particularly in fruit processing technologies.
In Brazil, the CIRAD project team participates in various on-going research projects developed in the Amazon region (biodiversity and valorisation of under-utilized fruits, integrated participative and sustainable management systems for forestry and agriculture resources, etc). A study on the Brazil nut production chain in the state of Amapa was also conducted and published in 2004. Dr. Brabet currently coordinates the field activities in Brazil of a European project on integrated mycotoxin management along cereal production chains, INCO-DEV MYCOTOX.

Permanent staff involved in the project

Name	Education level / Function	Fields of expertise for the project	Responsibilities / tasks in the project
Catherine Brabet	PhD / Senior scientist **Out posted in Brazil since 2002**	Food science Quality and safety management in food production chains Mycotoxins	**General coordination** Scientific and field support for technical issues Teaching in good practices
Dominique Pallet	PhD / Senior scientist **Out posted in Brazil from 1998 to 2005**	Food technology Specialist in fruit processing technologies	Scientific and field support for post-harvest technology issues
Marie-Gabrielle Picketty	PhD / Senior Scientist **Out posted in Brazil, since 2002**	Socio-economy	Scientific and field support for socio-economic issues Agri-chain analysis
Isabelle Vagneron	PhD / Senior Scientist	Economics	Scientific support for socio-economic issues Agri-chain analysis

- **NFA, Sweden - General Scientific Coordinator of the project - www.slv.se**

The National Food Administration (NFA) is the central supervisory authority for matters relating to food, including drinking-water. The NFA has the task of protecting the interests of the consumer by working for safe food of good quality, fair practices in the food trade, and healthy eating habits. The NFA is directly responsible to the Swedish government.
The aim of the work of the Research and Development Department is to increase knowledge in food related subjects. A central part of the work is risk assessment of chemical and microbiological hazards in food. Another area is analysis of foodstuffs and development of analytical methods for food control. The Microbiology Division is a part of this Research & Development Department.
The Microbiology Division is an accredited food laboratory (ISO 17025) and has a long experience of research in food mycology. Dr Olsen has worked for many years in the field of moulds and mycotoxins in food and feed. The laboratory (Dr Olsen) has recently coordinated a project within the Fifth framework program (OTA PREV, QLRT-1999-00433, homepage: www.slv.se/OTAPREV) concerning preventing ochratoxin A in cereals. Dr Olsen has also participated as national expert in two FVO (the EC Food and Veterinary Office) mission to Brazil in 2003 and 2004 concerning aflatoxins in Brazil nuts. Mrs. Gidlund has a good experience identifying fungi using both conventional and molecular methods. The group has recently developed a PCR methodology for aflatoxin producers. Dr Eric Danell is a new co-worker who has long experience of molecular methods for fungi and from collaboration with developing countries. Dr Lindblad is our expert on predictive modelling

Permanent staff involved in the project

Name	Education level / Function	Fields of expertise for the project	Responsibilities / tasks in the project
Monica Olsen	Ph.D / Senior scientist	Biology/Mycology	**General scientific coordination**
Eric Danell	Ph.D / Senior scientist	Microbiology/Mycology	Fungal methodology and teaching
Mats Lindblad	Ph.D / Senior scientist	Microbiology/Statistics	Modeling
Ann Gidlund	BMA / Technician	Mycology	Fungal identification and teaching

- **CSL, United Kingdom - www.csl.gov.uk**

The Central Science Laboratory (CSL) is an Executive agency of the Ministry of Agriculture Fisheries and Food (MAFF). In 1996, CSL moved to purpose built, 'state of the art' laboratory facilities at Sand Hutton near York. Here over 500 scientists work on a wide variety of fields relevant to the agriculture and food industry. Of particular relevance to this project is the long established expertise within the team in developing antibodies and immunoassays to a wide variety of analytes such as small molecules (e.g. antibiotics, pesticides and mycotoxins), micro-organisms, small peptides, viruses, insects etc. Coupled with that is the teams interest in mould ecology and the effect of that on the control of mycotoxin production. In addition, the team is also very experienced in the incorporation of antibodies into rapid (3 minutes), one step on-site systems such as those developed for potato viruses e.g. Pocket Diagnostic TM which is now in widespread use see web site: http://www.pocketdiagnostics.co.uk
The team at CSL has been developing antibodies, immunoassays and rapid formats since 1984 and has written several texts on the production of antibodies e.g. Kane, M. & Banks, J. N. (2000), Raising monoclonal antibodies, Chapter 3, pp 19-58 in Immunoassay: A Practical Approach, Gosling, J.P. (Ed.), Oxford University Press. The team is also a participant in a number of EU and SMT projects and COST actions.

Permanent staff involved in the project

Name	Education level / Function	Fields of expertise for the project	Responsibilities / tasks in the project
John Banks	PhD / Senior Scientist	Immunodiagnostics and mycology	Project leader for CSL input and technical transfer / training in assays
Chris Danks	BSc / Researcher	Immunodiagnostics Specialist in rapid field formats	Extraction and development testing of assays. Input in to technical transfer / training in assays
Victoria Tomkies	BSc / Researcher	Immunodiagnostics	Extraction and development testing of assays. Input in to technical transfer / training in assays
Sioban Ostoja-Starzewska	BSc / Researcher	Immunodiagnostics	Extraction and development testing of assays

Sub-Contractor:

CSL will sub-contract the **R-Biopharm AG (RBAG) company**, Germany - www.r-biopharm.de

RBAG will be bringing in to the project ELISA and rapid assays for aflatoxins in Brazil Nuts (representing a contribution in kind) and just as importantly they will play a major role in the training and technical transfer of these assays to the local Brazil Nut industries.
RBAG is a privately owned company located in Darmstadt with subsidiaries in USA and UK. Founded in 1988, RBAG which currently employs a staff of 90 people, is one of the worldwide leading companies to solve analytical and diagnostic questions for food safety and healthy nutrition for humans and animals. It researches, develops, and manufactures a wide range of tests for infectious diseases and the detection of a variety of substances in food and feed. RBAG has an excellent group of highly qualified scientist in developing a range of unique enzyme immunoassays for the detection of hormones, mycotoxins, antibiotics, food allergens, vitamins and adulterants in the food and in the agri-diagnostic field. RBAG achieved ISO 9001 Quality Standard Certification in 1996.
Dr Peter Schubert, head of research and development at RBAG, is an experienced manager in food analytical and clinical diagnostic projects, especially for the development and commercialization of immunological test kits.

The involvement of RBAG in the project is relevant for the following reasons:
- They have a good ELISA for total aflatoxin analysis that can be used in the project;
- The Lateral Flow Device (LFD) method is the most relevant for field use (rapid, one step, low-cost and reliable) and a prototype LFD for total aflatoxin analysis has recently been developed by RBAG which can be used successfully on Brazil nuts;

- To the best of the project partner knowledge, there are no other similar test kits for aflatoxins that can be brought in to the field;
- RBAG have the knowledge and expertise for training and support in the methods and also have an existing network (thought a company AM BRASIL CIENTÍFICA) in Brazil to assist in this aspect of the project.

An agreement of co-operation will be signed between the company and the other project partners. This agreement will specify among others that RBAG will not make any exclusive or non-competitive contracts signed.

Permanent staff involved in the project
Dr Peter Schubert, scientist in microbiology combined with molecular biological methods.
Dr Ulrike Immer, scientist in biology and specialist in the development of enzyme immunoassays.
Dr Bernhard Reck, scientist in biology and specialist in validation programs for enzyme immunoassays.

- **MAPA, Brazil - www.agricultura.gov.br**

The MAPA (Ministério da Agricultura, Pecuária e Abastecimento) is the central competent authority in Brazil in charge of the implementation of public policy, programmes and actions aiming to guarantee food quality and safety. In 1998-1999, it implemented the first project on Brazil nut monitoring for assessing the frequency and levels of contamination by aflatoxins. In order to attend the recent stricter European regulations on food aflatoxin maximum tolerance limits, the MAPA conducted a series of actions within the framework of its National Plan for Safety and Quality of Plant Products (PNSQV). These actions concern :

- The publication of the Normative Instruction N°13, 27 May 2004 on basic hygiene and handling measures in the Brazil nut production chain, the general requirements and basic criteria for Brazil nut sanitary certification and traceability in the processing stage, and the procedures for Brazil nut sampling and aflatoxin analyses;
- The organization of training courses in Brazil nut sampling according to the European Directive 98/53/EC for food inspectors, and in good practices for Brazil nut processing units;
- The signature of an agreement of technical cooperation with the Association of Brazil nut Exporters of the state of Para to implement a laboratory for aflatoxin analysis and improve Brazil nut quality.

The MAPA will support the implementation and execution of the project through the participation of its following branches : LACQSA/LANAGRO-MG and LANAGRO-PA, DIPOV-SDA, CCRC-SDA and SFA-Para.

The LANAGRO (Laboratório Nacional Agropecuário) constitutes MAPA' s laboratorial support for the activities of food inspection in Brazil.
The LACQSA/LANAGRO-MG (Laboratório de Controle de Qualidade e Segurança Alimentar) is a laboratory of the LANAGRO in the state of Minas Gerais. This laboratory which is under NBR/ISO/IEC Nº 17025 accreditation, is MAPA' s reference laboratory on mycotoxins. The LACQSA is in charge of the development and validation of reference analytical methods, the implementation of inter-laboratory programmes, the production of reference materials, and the audits of accredited laboratories, among others. It also coordinates and/or participates in training courses in mycotoxin analysis and in various national and international research projects. 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 LANAGRO-PA will also be involved in the project for the aflatoxin and fungi analysis in Brazil nuts in the state of Para.

The DIPOV-SDA (Departamento de Inspeção de Produtos de Origem Vegetal-Secretaria de Defesa Agropecuária) coordinates the activities of inspection and sanitary certification of plant products, creates part of the federal legislation related to its field of action, and coordinates working programs linked with the 27 SFAs (Superintendia Federal de Agricultura) representatives of MAPA throughout Brazil.

The CCRC-SDA (Coordenação de Controle de Resíduos e Contaminantes) coordinates the activities of food inspection for contaminants and residues.

The SFA-Para which is the representative branch of MAPA in the State of Para, executes the activities of Brazil nut inspection and sanitary certification (sampling, verification of good practices, etc) according to the directives of the MAPA regulation. It also participates in the organization and implementation of training courses in good practices and sampling methods for Brazil nut. The SFA-Para will support the field activities of the project in the State of Para and facilitate the articulation with the key stakeholders of the Brazil nut production chain, the government of the State of Para and the SMA (Secretarias Municipais de Agricultura) of the main Brazil nut producing municipalities for their participation in the project.

Permanent staff involved in the project

Name	Education level / Function	Fields of expertise for the project	Responsibilities / tasks in the project
DIPOV-SDA, Brasilia, Distrito Federal			
Marcelo Bonnet Alvarenga	PhD / Director of DIPOV	Microbiological food safety Predictive microbiology	Scientific support Legislation
Ricardo Kobal Raski	BSc / Federal agricultural inspector	Good practices in Brazil nut production chain Rural extension Food inspection	Proposal for legislation related to the subject. Logistic support between LACQSA and SFA-PA. Dissemination of project information and results to agricultural inspectors in SFA's of other Brazil nut producing States. Link with the Brazilian Committee of the Codex Alimentarius (CCAB)
Plant Programs-SDA, Brasilia, Distrito Federal			
Álvaro Eleutério Silva	PhD / Director of the SDA Plant Programs	Plant breeding	MAPA coordination Scientific support
CCRC-SDA, Brasilia, Distrito Federal			
Luzia Maria Souza	BSc / Federal agricultural inspector	Food safety	Support for aflatoxin prevention, control and monitoring in the Brazil nut production chain.
SFA-PA, Belém, Para state			
Otavio César Durans de Oliveira	BSc / Federal agricultural inspector	Good practices in Brazil nut production chain Brazil nut sampling, analyses and sanitary certification	Support for official procedures for Brazil nut sampling, analyses and sanitary certification
José Carlos Barroso Junior	BSc / Federal agricultural inspector	Brazil nut sampling, analyses and sanitary certification	Support for official procedures for Brazil nut sampling, analyses and sanitary certification
LACQSA/LANAGRO-MG, Belo Horizonte, State of Minas Gerais			
Eugenia Azevedo Vargas	MSc / Technical coordinator of LANAGRO-MG and technical responsible for LACQSA	Chemistry Food science	Methodological support for Brazil nut sampling and aflatoxin analyses
LANAGRO-PA, Belém, State of Para			
Mauricio Quaresma de Araújo	MSc / Federal agricultural inspector	Chemistry	Aflatoxin analysis

- **EMBRAPA, Brazil - www.embrapa.br**

EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária) is the Brazilian agricultural research institute linked to the MAPA. Its mission is to provide feasible solutions for the sustainable development of Brazilian agribusiness through knowledge and technology generation and transfer. EMBRAPA which employs 8619 persons, is present in almost all the states of Brazil through 37 research centers. It coordinates the National Agricultural Research System, which includes most public and privates entities involved in agricultural research in the country, and collaborates in national and international projects sharing knowledge and technology with 56 countries. The institute is presently working on strategies for reduction of post-harvest losses and food quality and safety. EMBRAPA supports the Brazilian Program of Safe Foods (PAS) which aims to guaranty the food safety and quality by using the principles of the HACCP (Hazard Analysis and Critical Control Points). One of the current research tasks deals with collaborative studies on mycotoxin control in plant products, including Brazil nuts, within the framework of EMBRAPA national research macro programmes.

The project will involve the EMBRAPA headquarter, Executive Direction in Brasilia, and the research centre EMBRAPA Acre located in the state of Acre which represents one of the largest Brazil nut producing regions.

EMBRAPA Acre has a huge experience working on Brazil nut in partnership with the government of the state of Acre, universities and other Brazilian institutes. The studies take place in extractive reserves, in collaboration with the families enrolled in this activity and the extractivist cooperatives and associations.
Since 2000, EMBRAPA Acre has been conducting research on the identification of the critical control points for aflatoxin contamination from the Brazil nut collection to processing units, the definition of good practices and the development of appropriate post-harvest technologies in order to improve and guarantee the nut quality and safety. It has also worked on the socio-economic characterization of the Brazil nut production chain, the management and ecology of the *Bertholettia excelsa* in the state of Acre and the development of added value Brazil nut based products (breads, biscuits, etc).
EMBRAPA Acre participates in the PAS for Brazil nut initiated in 2004, and has contributed to the elaboration of the "Manual of safety and quality for Brazil nut production" of the PAS-Farm sector. The research centre is currently involved in a project of extension for technicians and extractivist communities in good practices for Brazil nut. At the request of the productive sector, it will initiate another project on Brazil nut certification (2006-2008) in partnership with the government of the State of Acre, SEBRAE (Serviço Brasileiro de Apoio as Micro e Pequenas Empresas) and the Non-Governmental Organization CTA (Centro de Trabalhadores do Acre). The EMBRAPA Acre also participates in a project approved in 2005 and coordinated by the LANAGRO-MG, "Analytical tools for training Brazil in the guarantee of the Brazil nut conformity related to aflatoxin hazards", which aims to develop and validate analytical methods and sampling procedure, produce reference materials, organize inter-laboratory programme and training courses.
The laboratory of the EMBRAPA Acre presents the adequate infrastructure for supporting the project in the aflatoxin and fungi analysis in Brazil nuts.

The EMBRAPA headquarter in Brasilia will provide a support to the project through its Executive Direction which coordinates the EMBRAPA research centres in the Amazon region, and through the Information Technology Department for the dissemination of the project information and results. It will also act as the interface between the present project and the project of the Brazilian-Italian Biodiversity Program (PBBI – http://www.pbbi.org.br/site/home/default.php) "Model for the conservation and sustainable use of the biodiversity by traditional communities in the Amazon", including Brazil nuts.

Permanent staff involved in the project

Name	Education level / Function	Fields of expertise for the project	Responsibilities / tasks in the project
EMBRAPA Acre			
Lúcia Helena de Oliveira Wadt	PhD / Researcher	Genetics and plant improvement	Brazil nut tree management for sustainable production
Joana Maria Leite de Souza	MSc / Researcher	Food Technology	Good practices
Cleísa Brasil da Cunha Cartaxo	MSc / Researcher	Post-harvest technologies	Definition of contamination points Good practices Aflatoxin analyses
Dorila Silva O. Mota Gonzaga	BSc / Researcher	Rural extension Dissemination of technologies	Coordination of training courses for technicians and producers
Rivadalve Coelho Gonçalves	PhD / Researcher	Phyto-pathology	Fungi identification
Soraya Pereira da Silva	BSc / Journalist	Journalism	Dissemination of project information and results
Márcio Muniz Albano Bayma	BSc / Researcher	Socio-economy	Agri-chain study
Claudenor Pinho de Sá	MSc / Researcher	Socio-economy	Agri-chain study
EMBRAPA Headquarters, Executive Direction, Brasilia			
Herbert Cavalcante de Lima	PhD / Researcher	Food Science Post-harvest technology	Good practices and teaching Aflatoxin analyses and teaching Dissemination of project information and results

APPENDIX 2
Overall project management

The **General Coordination** of the project, including overall financial and administrative management, will be performed by Dr. Catherine Brabet of CIRAD, France, who will have overall responsibility for ensuring the efficient execution of the work programme, timely reporting, effective team working, and timely and appropriate problem solving. Dr. Brabet is out posted in Brazil at the UNICAMP (Universidade Estadual de Campinas) Campinas, State of São Paulo. She has a good experience in food quality and safety management applied to mycotoxin control. Dr. Brabet will be supported by Dr. Pallet located at CIRAD headquarters, Montpellier, France and CIRAD services specialized in financial, administrative and legal issues.

The **General Scientific Coordination** will be performed by Dr. Monica Olsen of NFA, Sweden who will assist the General Coordination in the follow-up, planning, progress reporting and result dissemination of the scientific activities.
Dr. Olsen has recently coordinated a 3½ year long project within the Fifth Framework Programme. The final report of the project (OTA PREV, www.slv.se/otaprev) was accepted during the Spring 2004. Dr Olsen has also participated in the European Mycotoxin Awareness Network (EMAN) which is a multidisciplinary group of 14 organisations that provide high quality scientific information and news about mycotoxins to industry, consumers, legislators and the scientific community, through its website (www.mycotoxins.org/). In addition, Dr Olsen has assisted FAO in the coordination of the JECFA 56 (Fifty-sixth meeting: Safety evaluation of certain mycotoxins in food. FAO Food and Nutrition Paper 74, 2001), which included the risk assessment of 7 mycotoxins.

Project and related information exchanges will be performed at two levels:

1. Between project coordinators and participants:
   Mostly by Internet services for day-to-day information exchange regarding project activities, decisions, scientific information, reporting, financial transactions, meetings, etc., supplemented by project meetings and workshops in the Amazon region.

2. Between the overall project coordinator and financiers (STDF partner institutions):
   Mostly regarding administrative and financial management matters and technical reports.

Specific management tasks

The intellectual property rights and information management will be managed with particular attention to respect of confidentiality and private information.
All the Brazil nut samples sent outside the Amazon region to be analysed will accomplish with all the necessary authorization from local authorities.

APPENDIX 3
Project background and rationale

Brazil nut production and commercialisation, and its environment and socio-economic impact in the Amazon region

Brazil nuts, also named Para nuts, are the edible seeds of wild, tall (30 to 60 m in height) and large (average diameter of 2 m in the base) trees of *Bertholletia excelsa*, native of the Amazon forest. The fruit pod, containing the nuts in a shell (15-35 nuts per pod), is a very hard and woody capsule with an average diameter of 15 cm and 1 kg weight, and it is located only near the top of the trees. Each tree produces around 500 pods per crop. The trees grow slowly, taking as long as 10 to 30 years before reaching a commercial production of fruit and they require a specific species of bee (and to a lesser extent bats) to pollinate the flowers. For new trees to establish, it is necessary for an animal, *Dasyprocta aguti* (a cat-sized brown rodent) capable of chewing through the fruit pod, to disburse the seeds..
Naturally, there is a low density of trees per unit of area in the jungle, with about 1 to 5 trees / hectare. Until now, only a few plantations of Brazil nuts have been developed and these have not been economically successful as wild trees fare much better than the cultivated orchards.

Brazil nuts are thus collected from wild trees in Bolivia, Brazil and Peru, representing one of the most important non-timber forest products of extractive exploration in the Amazon region. The fruit pods are gathered during the rainy season (from December to March) when they have fallen down from the trees and chopped open to obtain the nuts in shell. Most of the collected Brazil nuts are subject to minimal processing for export either in shell or shelled (as a kernel), mainly to Europe and United States of America ([1], [2]). The main processing steps concern selection, washing, drying, storage, pressure-sealing, shell cracking, vacuum packaging for shelled nuts ([3]).

During the last ten years, Brazil passed from the first to the second largest Brazil nut producer and exporter after Bolivia, with an annual production of about 25 000 tons in 2003. Amazonas, Acre and Pará are the main producing states, with more than 80 % of the national production, followed by Rondônia and Amapá ([4], [5]). Europe and United States represented the two major Brazilian export markets of Brazil nuts, mainly in shell, until 2003. Since 2004, the bulk of the Brazil nuts are exported to Bolivia exclusively in shell, followed by United States (mainly in shell) and Europe (mainly kernels) (Figure 1 and 2). The states of Pará and Amazonas are responsible for the Brazil nut exports to Europe and United States, with Para as the largest exporter to these destinations, while Acre concentrates the national exports to Bolivia.

The Brazil nut extractive exploitation has an important socio-economic and environmental role in the Amazon region. Significant part of the local population, of which many are impoverished, directly or indirectly depend on Brazil nut collection and commercialisation to the local or export markets which generates employment and income. Brazil nut extractivism also contributes to the socio-economic organization of large extractive areas, prevents rural migration and the deforestation of the rainforest ([6]). Brazil nuts have a high nutritional value (rich in proteins, fats, vitamins and selenium) and apart from their commercialisation, they are also an important source of food for the local families ([7]). For these reasons, they are considered as a special priority for sustainable development of the Amazon region.

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[1] Infoagro Bolivia - Castanha: www.infoagro.gov.bo
[2] Agência de noticias Brasil- Árabe: Brasil exporta mais de 90 % de sua produção de castanha-do-pará, fruto típico da Amazônia, 16/08/2004: www.anba.com.br/noticia.php?id=4075
[3] Manual de segurança e qualidade para a cultura da castanha-do-Brasil. CampoPAS, Série qualidade e segurança dos alimentos, Brasília, DF, 2004, 62 p.
[4] FAOSTAT : http://faostat.fao.org/
[5] IBGE, Diretoria de Pesquisas, Coordenação de Agropecuária, Produção da Extração Vegetal e da Silvicultura, 2003.
[6] Newing H., Harrop S., 2000. European Health Regulations and Brazil nuts: Implications for Biodiversity Conservation and Sustainable Rural Livelihoods in the Amazon. Journal of International Wildlife Law and Policy: 3(2): 109-124.
[7] Manual de segurança e qualidade para a cultura da castanha-do-Brasil. CampoPAS, Série qualidade e segurança dos alimentos, Brasília, DF, 2004, 62 p.

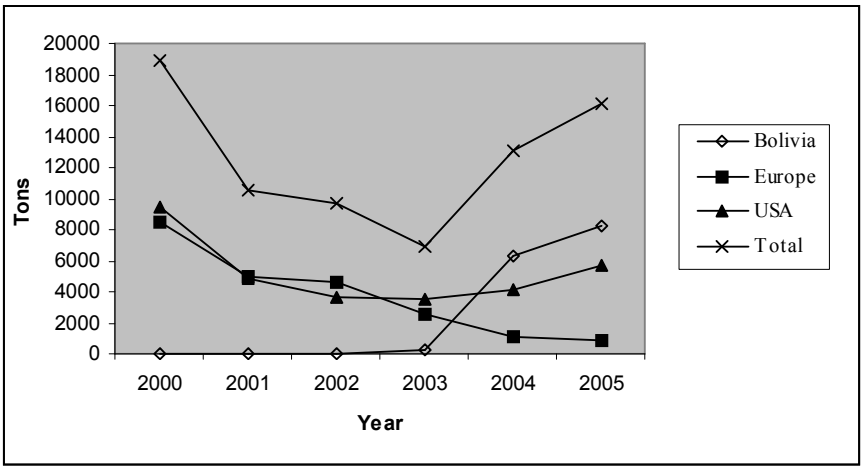


Figure 1: Brazil nut export trends in Brazil. Source: http://aliceweb.desenvolvimento.gov.br/alice.asp

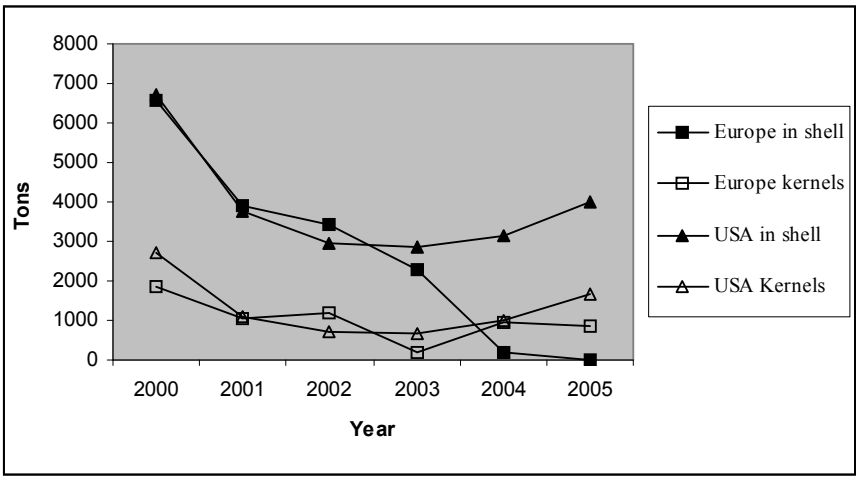


Figure 2: Brazilian export trends of Brazil nuts in shell and kernels to Europe and United States. Source: http://aliceweb.desenvolvimento.gov.br/alice.asp

Aflatoxin contamination of the Brazil nuts, and its consequences for the Amazon region and the local population

Brazil nuts are frequently contaminated by high levels of aflatoxins (B1, B2, G1, G2), which are toxic secondary metabolites produced by *Aspergillus flavus*/*A. parasiticus* when the nuts are kept under conditions that favour the development of these fungi. Official analysis of 500 Brazil nut samples, shelled (302) and in shell (198), collected in different sites of Brazil from 1998 to 2004 showed that 30 % of the samples were above 4 µg/kg and 14 % above 50 µg/kg for total aflatoxins, and 30,6 % were above 2 µg/kg and 10 % above 50 µg/kg for aflatoxin B1, with levels up to 5000 µg/kg ([8]). In shell Brazil nut samples showed significantly higher levels of contamination than shelled Brazil nuts. The Brazilian Ministry of Health authorizes maximum levels of 30 µg/kg for total aflatoxins in foods for human consumption ([9]).

[8] LACQSA/MAPA, 2005. Data on the occurrence of aflatoxins in Brazil nuts, in Brazil, from 1998-2004.
[9] MAPA -Ministério da Agricultura, Pecuária e Abastecimento. RDC 274 of 16 October 2002.

Such contamination is of major concern not only for public health because of the carcinogenic and genotoxic effects of aflatoxins in human beings, but also in regard to economic losses and environment impact. It constitutes the largest impediment to Brazil nut commercialisation, especially in Europe where the regulation on aflatoxin levels has become very restrictive.

In 1999, the European Commission established maximum levels of 4 µg/kg for total aflatoxins and 2 µg/kg for aflatoxin B1 in nuts and derived products for direct human consumption or as ingredient in food products and 10 µg/kg for total aflatoxins and 5 µg/kg for aflatoxin B1 in nuts subject to sorting or other physical treatment before their human consumption or use as ingredient ([10]). Since 2003, special conditions have been imposed on the import of Brazil nuts in shell originating or consigned from Brazil ([11]). This was due to an increased number of rejections of imported contaminated lots during 2001 and 2002 (Table 1) and shortcomings found in the Brazilian production chain and aflatoxin control systems ([12]). These conditions include certification of all consignments by the Brazilian Ministry of Agriculture ensuring that they were sampled and analysed by their Laboratory of Food Quality and Safety Control (LACQSA) in accordance with the Directive 98/53/EC. There is also a systematic additional aflatoxin control at the point of entry in Europe with strict conditions for sending back non-complying consignments to the country of origin or their destruction ([13, 14]).

Table 1: Data on rejections by European countries of contaminated Brazil nut lots imported from Brazil. Source PVA-PA (Posto de Vigilância Agropecuária do Estado do Para / Agriculture Surveillance Posts of the State of Para)

Year	European country	Expeditions	Quantity (tons)	US$
2001	Germany	7	189	178 955.92
	Italia	5	143	121 518.23
	France	4	85	77 522.24
	Holland	1	24	25 344.00
	United Kingdom	1	25	19 286.00
	Sub-Total	**18**	**466**	**422 625.39**
2002	Germany	2	80	83 628.35
	Italia	8	172	156 886.36
	France	2	46	31 846.72
	United Kingdom	3	189	219 069.07
	Sub-Total	**15**	**487**	**491 430.50**

The European Commission aflatoxin regulation (EC) N° 1525/98 of 16 July 1998 and Decision 2003/493/EC have resulted in an important fall in Brazilian exports of Brazil nuts to Europe of about 87 % between 2000 and 2004 (Figure 1). Between 2003 and 2004, a fall of close to 92 % for the exports of Brazil nuts in shell was registered while there was an increase of fivefold for kernels ([15]).

[10] Commission Regulation (EC) N° 1525/98 of 16 July 1998 setting maximum levels for certain contaminants in foodstuffs. OJL 201 of 17 July 1998, p. 43-46.
[11] Commission Decision 2003/493/EC of 4 July 2003 imposing special conditions on the import of Brazil nuts in shell originating in or consigned from Brazil, OJL 168 of 5 July 2003, p. 33-38.
[12] Final report of a mission carried out in Brazil from 27th January to 7th February 2003 to assess the facilities and measures in place for the control of aflatoxin levels in Brazil nuts intended for export into the European Union. Food and Veterinary Office – FVO, 09/10/03 – 52200, 27 p.
[13] Commission Regulation (EC) N° 1525/98 of 16 July 1998 setting maximum levels for certain contaminants in foodstuffs. OJL 201 of 17 July 1998, p. 43-46.
[14] Commission Directive 98/53/EC of 16 July 1998 laying down sampling methods and the methods of analysis for the official control of the levels for certain contaminants in foodstuffs, OJL201 of 17 July 1998, p. 0093-0101.
[15] http://aliceweb.desenvolvimento.gov.br/alice.asp.

The reduction of Brazil nut exports has affected the economy of the producing regions and contributed to the clearing of larger areas of the Amazonian forest by local populations forced to look for other economic resources.
In addition, the rejected contaminated Brazil nut lots that meet the less restrictive aflatoxin requirements of Brazil or other importing countries, could be reintegrated in to the national or other export markets and constitute a risk for the health of the population through the consumption of the nuts themselves or nut based-products (flour, biscuits, bread etc.). Some of these nut based-products are frequently being used for the afternoon snack of school students in the Brazilian North region.

In the Brazil nut producing areas, there is the occurrence of fungi and aflatoxins all along the production chain, from collection of the nuts to the final exported lots. This occurrence may not only favoured by conditions typical of a tropical rain forest (high temperatures and air humidity levels, rain during the harvesting period, damage to the pods and nuts by wild animals, etc), but also by the low technological and organizational level of the productive chain and inappropriate product handling ([16]). Due to the extensive area of the Amazon region, transport distances can be very long and the rivers are the major and easiest way of transport for people and cargo because there are few paved and ground roads. The Brazil nuts are collected from thousands of spots and shipped from hundred of points to reach the processing plants in the main cities of the region. For these reasons and the different extractive practices, the time between collection and final drying for safe storage of the nuts is extremely variable and can be up to several weeks.

Aflatoxin control and monitoring in the Brazil nut production chain

In Brazil, the federal government through the Ministry of Agriculture (MAPA) and its executive services, the national agricultural research institute (EMBRAPA), universities and Non-Governmental Organizations among others initiated a series of actions to attend the international sanitary standards related to aflatoxin limits, in particular the stricter European regulations.

In 1998, MAPA implemented the first project on Brazil nut monitoring for assessing the frequency and levels of contamination by aflatoxins. Five hundred Brazil nut samples were collected in different sites of Brazil from 1998-2004 and analyzed for total aflatoxins and aflatoxin B1.

The actions also include research work carried out by EMBRAPA and universities since 2000 to identify the Critical Control Points(CCP) and factors for fungi growth and aflatoxin contamination from the Brazil nut collection to the processing units, as well as the publication of guidelines and technical regulations on good practices in the Brazil nut production chain and the development of post-harvest technologies (prototypes of warehouses, washing and drying equipment) for preventing and reducing aflatoxin contamination.

In 2004, the "Manual of safety and quality for Brazil nut production" was published by the Brazilian Program of Safe Foods (PAS) - Farm Sector ([17]) and the PAS for Brazil nut initiated. The PAS was created in 2002 with the support of EMBRAPA and other Brazilian institutes to guaranty the food safety and quality by using the principles of the HACCP (Hazard Analysis and Critical Control Points) system for preventing physical, chemical and biological hazards. The objective of the PAS-Farm Sector is to train producers, technicians and primary production industries in the adoption of Good Agricultural Practices (GAP).

Within the framework of its National Plan for Safety and Quality of Plant Products (PNSQV) initiated in 2003, MAPA published a technical regulation on basic hygiene and handling measures for the Brazil nut production chain (Normative Instruction N°13, 27 May 2004). This Normative Instruction also includes a technical regulation on the general requirements and basic criteria for Brazil nut sanitary certification and traceability in the processing stage. In addition, there is a technical regulation on the procedures for Brazil nut sampling and aflatoxin analyses.

[16] Manual de segurança e qualidade para a cultura da castanha-do-Brasil. CampoPAS, Série qualidade e segurança dos alimentos, Brasília, DF, 2004, 62 p.
[17] Manual de segurança e qualidade para a cultura da castanha-do-Brasil. CampoPAS, Série qualidade e segurança dos alimentos, Brasília, DF, 2004, 62 p.

In 2005, the 37th Session of the Codex Committee on Food Additives and Contaminants (CCFAC) agreed to establish a working group led by Brazil in order to prepare an Appendix to the Code of Practice for the Prevention and Reduction of Aflatoxin Contamination in Tree Nuts that includes additional specific measures for Brazil nuts. The elaboration of this Appendix which will be discussed in the next CCFAC session in 2006, was proposed by the Brazilian Ministry of Agriculture (MAPA) because it was considered that the collection and other particular features of the crop make it impossible to apply the same ordinary agricultural practices indicated for cultivated tree nuts. In order for these measures to be effective, it will be necessary for collectors, processors and other agents of the Brazil nut productive chain to consider the general principles established by the Code, while taking into account current practices associated with the Brazil nut collecting.

Training courses in Brazil nut sampling methods in accordance with the European Directive 98/53/EC were recently provided for federal inspectors. There were also training courses in good practices for extension agents and key stakeholders of the Brazil nut production chain.

According to the reports of two missions carried out in Brazil in 2003 and 2004 by the Food and Veterinary Office of the European Commission ([18],[19]) to assess the facilities and measures in place for the control of aflatoxin levels in Brazil nuts intended for export into the European Union, the following shortcomings were revealed:

- The current process of the Brazil nut collecting, transport, processing and export is poorly defined and varies significantly between producing regions.
- The crucial points of mould and aflatoxin contamination are not well known.
- There is currently inadequate research and fieldwork to identify at which stage in the process and in relation to variables such moisture content, time and temperature, *Aspergillus flavus*/*A. parasiticus* and aflatoxin contamination occurs. However, high moisture levels and mould activity were visually evident at all stages of the process.
- The actions undertaken to develop and disseminate GEP (Good Extractive Practices), GMP (Good Manufacturing Practices) and GHP (Good Hygiene Practices) guides are in their early stages and lacking the required scientific background. Up to now, the main investment has occurred in the processing plants, with very little to do with stages of collection, storage in the forest and transport.
- Currently, the control centres take a sample for aflatoxin analysis just prior to export instead of systematic quality control checks all along the production chain.
- The sampling procedure, which is crucial for aflatoxin analysis due to the very heterogeneous distribution of these mycotoxins in product consignments, is inadequate. This is because it does not provide a representative and dependable result and it is not in accordance with Directive 98/53/EC.
- The control over the treatment of the sample during the dispatch to the laboratory is inadequate e.g. the aflatoxin content may change during transport.
- There is currently no adequate traceability system in place in relation to the Brazil nuts, either during the production chain, or in relation to the export procedures and certification.
- There are no 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 the HACPP system.

Because of the short period since their identification, the shortcomings indicated above still need to be addressed.

[18] Final report of a mission carried out in Brazil from 27th January to 7th February 2003 to assess the facilities and measures in place for the control of aflatoxin levels in Brazil nuts intended for export into the European Union. Food and Veterinary Office, 09/10/03 – 52200, 27 p.

[19] Draft report of a mission carried out in Brazil from 11th March to 18th March 2004 to assess the facilities and measures in place for the control of aflatoxin levels in Brazil nuts intended for export into the European Union and to follow-up on recommendations made in mission report SANCO 9027/2003. Food and Veterinary Office, 27/05/04 – 38326, 20 p.

In that context, the project proposes :

- To better characterize the Brazil nut production chain and formulate organizational and incentive strategies for safety control,
- To test and validate the recommended good practices in the Brazil nut production chain for aflatoxin control, in particular the CCFAC Code of Practices, and update them according to the conclusions of research, including the development of appropriate post-harvest technologies and traceability system,
- To validate and implement a rapid aflatoxin surveillance system for use along the Brazil nut production chain.

By doing so, it is envisaged that the current negative environmental, socio-economic and health effects resulting from the current practices would be reversed and renewed market confidence in the Brazil nuts originated from Brazil.

Brazil nut aflatoxin surveillance systems

Conventional analysis of aflatoxins is done by laboratory methods such as High Performance Liquid Chromatography (HPLC). The HPLC method EN 14123, is validated for aflatoxin B_1 and the sum of aflatoxin B_1, B_2, G_1 and G_2 to give a measure of total aftlatoxins in Brazil nuts. This method uses immunoaffinity columns for cleanup of sample extracts and HPLC with fluorescence detection after post column derivatization for aflatoxin identification and quantification.
Whilst the method meet the legislative requirements, it is time consuming, expensive, requires very specialised equipment and uses large volumes of organic solvents.
The Brazil nut industry faces the real dilemma of access to inexpensive and robust methods for the implementation of a HACCP type control system and 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. In summary, they utilise highly specific and sensitive antibodies to detect the target analyte. The presence of the analyte is then visualised using an enzyme-substrate system and read on a 96 well plate reader.
Through the production of specific antibodies, R-Biopharm AG (RBAG) have developed an ELISA system for aflatoxins which overcomes many of the problems associated with HPLC but generally still needs to be carried out in a laboratory type situation. Very recently RBAG have developed a one step, on-site and robust Lateral Flow Devices (LFDs) that only take a few minutes to perform, for total aflatoxins (sensitivity down to 4ppb) which will be brought in to the project.
A competitive LFD relies upon the competition for binding sites on specific aflatoxin antibodies linked to gold or latex particles. This competition is between aflatoxin in the sample and that in the test line on a nitro-cellulose-based membrane. Next to the test line is also one that acts as a control to ensure that the test is working correctly. After the addition of a few drops of sample extract to the well and release pad, the competition reaction is started (see figure 3).The ELISA systems have previously been successfully used on Brazil nuts and further basic checks will be made on this. The LFDs are at the prototype stage and will need to be linked with suitable sample extraction regimes and undergo validation before use. However it is not envisaged that this will pose any specific problems and would provide a means for inexpensive (for just a few dollars) testing of Brazil nuts throughout the production chain.

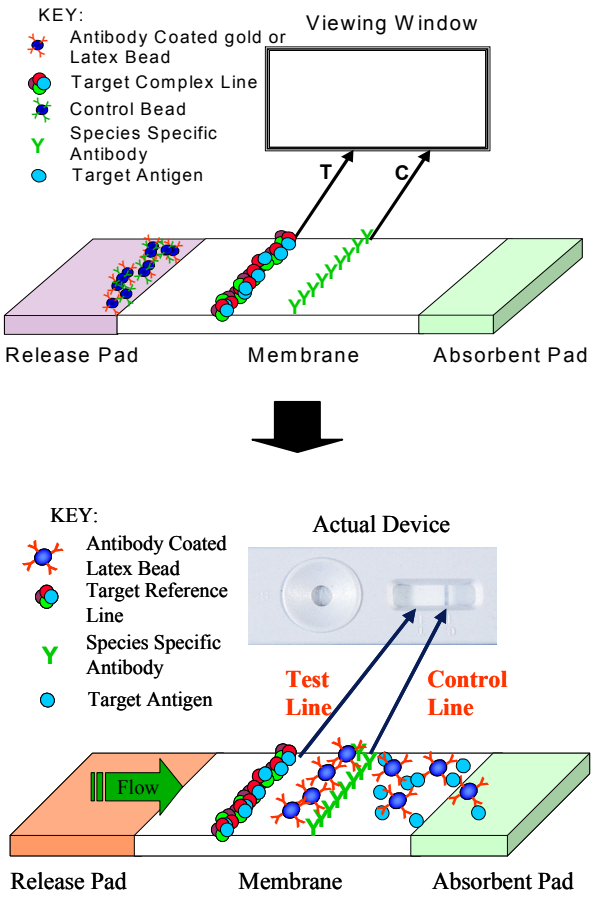


Figure 3: A schematic diagram of a competitive Lateral Flow Device for aflatoxins

APPENDIX 4
Detailed work plan, dissemination plan and evaluation plan

Detailed work plan

In order to comply with the objectives and the deliverables of the project, the work plan is organized into 3 main components (see diagram below).

The first component is dedicated to applied research aiming to answer the following scientific questions:

- What are the current conditions of Brazil nut production and commercialisation, their constraints and opportunities for aflatoxin control ? Based on this analysis, what would be the appropriate strategy in terms of production chain organization and incentives for improving the sustainability and the safety of the Brazil nut supply chain ? (**Specific objective 1**)
- Does the implementation of the already established recommendations of good practices in the Brazil nut production chain allow to reduce and control the occurrence of the aflatoxins to an acceptable level ? What improvements, including the development of appropriate low-cost and environmental-friendly post-harvest technologies and traceability system, could be brought in a scientific basis? (**Specific objective 2**)

This component also aims to set up a validated rapid aflatoxin surveillance system for use along the Brazil nut production chain to verify the effectiveness of the safety management system (**Specific objective 3**), rather than relying entirely on end-point aflatoxin analysis prior to export.

The research work is based on an integrated multidisciplinary approach, combining laboratory experiments to answer some basic scientific questions and in-field participatory studies involving the key stakeholders of the Brazil nut production chain. This approach intends to ensure the proposed aflatoxin management system will be compatible with the local socio-economic and environmental context.

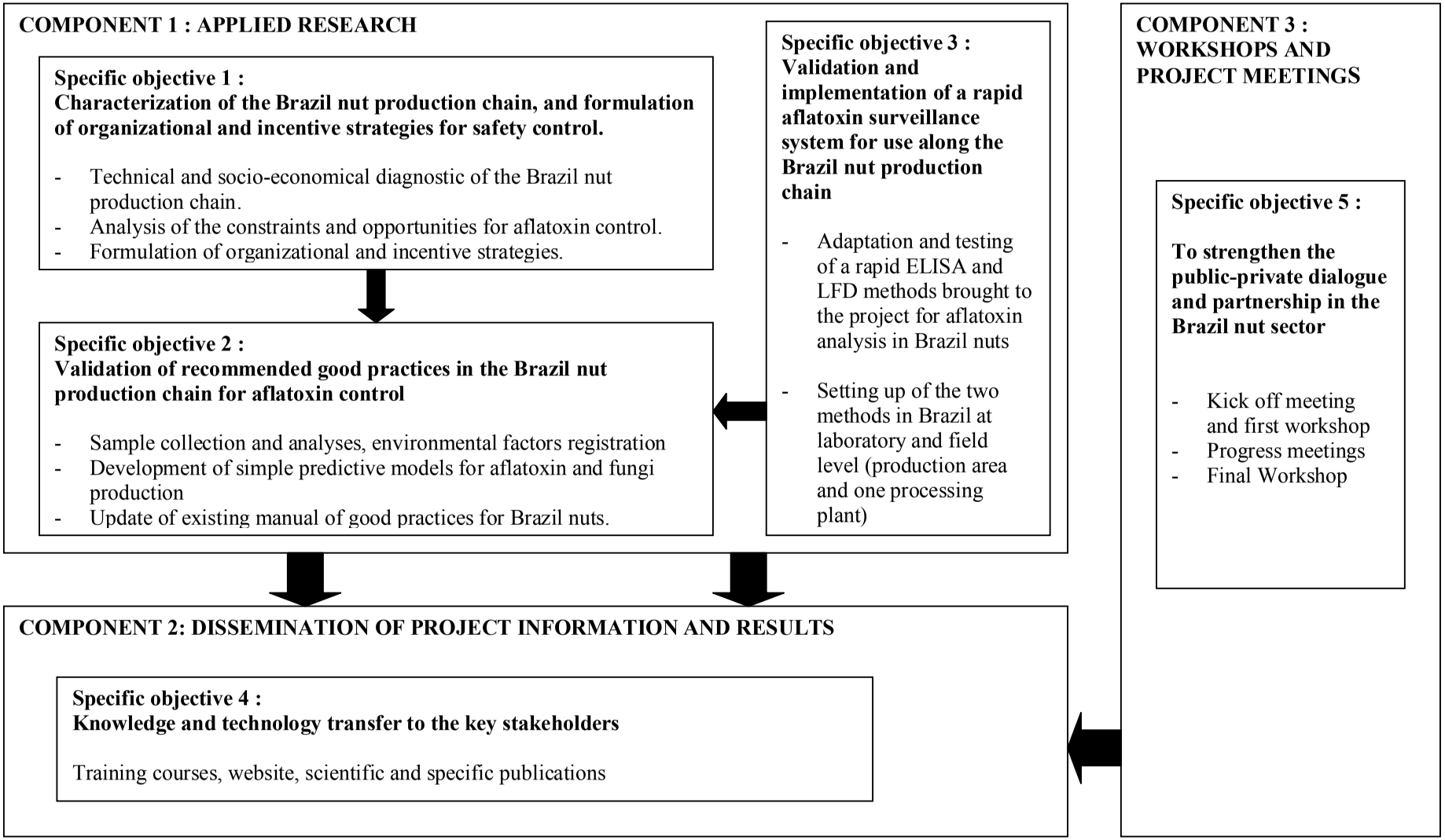
The first component aims two main project products:
1. Aflatoxin management tools (good practices and rapid analytical method) tested and validated for Brazil nuts on a scientific basis.
2. Appropriate organizational and incentive strategies formulated for the construction of a sustainable safety Brazil nut production chain.

The second component is dedicated to the dissemination of project information and results to the key stakeholders through the implementation of different information systems : training courses, website, scientific and specific publications (**Specific objective 4**).

The third component consists in the organization and implementation of project meetings and workshops in the Brazilian Amazon region throughout the duration of the project - a kick off meeting and a first workshop, a progress meeting and a final workshop - (**Specific objective 5**), in order to:
- Organize the implementation and execution of the project activities by the different partners,
- Synthesize and discuss the current knowledge in the Brazil nut area,
- Follow-up the progress of the activities to ensure the effective execution of the work plan (review of the scientific results and possible bottlenecks to be solved),
- Strengthen the public-private dialogue and partnership in the Brazil nut sector,
- Stimulate and facilitate network among the project participants,
- Contribute to the dissemination of the project results to the key stakeholders.

The Brazilian States of Para and Acre were selected for the execution of the project because they represent the largest Brazil nut producing and exporting States along with the State of Amazonas. Acre concentrates the exports to Bolivia, whereas Para is the major exporter to Europe and United States of America followed by Amazonas.

Schematic presentation of the project components showing their interdependencies



Specific objective 1: Characterization of the Brazil nut production chain, and formulation of organizational and incentive strategies for safety control.					
Activities / Partners	**CIRAD**	**NFA**	**CSL**	**MAPA**	**EMBRAPA**
A1.1: Technical and socio-economical diagnostic of the Brazil nut production chain in the Brazilian States of Acre and Para	**X**			**X**	**X**
A1.2: Analysis of the major constraints and opportunities in the Brazil nut production chain for aflatoxin control.	**X**	**X**		**X**	**X**
A1.3: Formulation of organizational and incentive strategies for a sustainable and safe Brazil nut production chain	**X**			**X**	**X**
Person-month per participant	**4**	**1**		**4**	**4**

Objectives:

1. Describe the current conditions of the Brazil nut production and commercialisation in the Brazilian States of Acre and Para.
2. Identify the major constraints and opportunities in the Brazil nut production chain, relevant to product safety management.
3. Formulate organizational strategies and efficient incentives aimed at improving the sustainability and safety of the Brazil nut supply chain

Description of work

A1.1: Technical and socio-economical diagnostic of the Brazil nut production chain in the Brazilian States of Acre and Para

- Inventory of the different regulations applied to Brazil nuts for commercialization.
- Determination of technical itinerates (steps, handling and processing parameters) from Brazil nut collection in the rainforest to final site for exportation.
- Identification of the actors, organization and mode of coordination, logistic, products and markets, pricing.

The data will be collected from published and unpublished (internal reports) literature, and complemented by field surveys if necessary.

A1.2: Analysis of the major constraints and opportunities in the Brazil nut production chain for aflatoxin control.

Based on the previous characterization, the technical and socio-economical constraints and opportunities affecting the implementation of aflatoxin control measures will be identified. Complementary qualitative surveys will be carried out as necessary.

A1.3: Formulation of organizational and incentive strategies for a sustainable and safe Brazil nut production chain

The formulation of appropriate organizational and incentive strategies will be based on the results obtained in A1.1 and A1.2. Working groups involving the private and public sector will be organized for promoting an interactive and fully inclusive analysis and dialogue between both sectors on the above topic. Incentive issues in the project will be based on the analysis of existing initiatives in the Brazil nut production chain, such as the Forestry Stewardship Council (FSC) certification.

Deliverables

- A report documenting the current conditions of Brazil nut production and commercialisation in the Brazilian States of Acre and Para **by month 7**.
- A report documenting the major constraints and opportunities for aflatoxin control **by month 13**.
- A report documenting the organizational and incentive strategies formulated for the construction of a sustainable and safe Brazil nut production chain **by month 20**.

Milestones and expected results

- Research methodology defined **by month 3.**
- Secondary data collected, and an Info Gap Matrix constructed **by month 4.**
- Primary data collected **by month 5.**
- Data processing, analysis and synthesis completed **by month 6.**
- Current conditions of Brazil nut production and commercialisation described **by month 7**.
- Major constraints and opportunities in the Brazil nut production chain identified **by month 13.**
- Organizational and incentive strategies formulated **by month 20.**

Specific objective 2: Validation of recommended good practices in the Brazil nut production chain for aflatoxin control					
Activities/Partners	**CIRAD**	**NFA**	**CSL**	**MAPA**	**EMBRAPA**
A2.1: Identify existing or set up Brazil nut production systems following recommended code of practices	**X**			**X**	**X**
A2.2: Collection of data (*A. flavus / A. parasiticus*, aflatoxins, water activity, moisture content, relative humidity, temperature, insect infestation)	**X**	**X**	**X**	**X**	**X**
A2.3: Development of a simple predictive model for aflatoxin and fungi production in the Brazil nut production chain.		**X**			
A2.4: Formulate recommendations to update the existing manual of safety and quality in Brazil nuts	**X**	**X**	**X**	**X**	**X**
Person-month per participant:	**4**	**6.5**	**0.2**	**4**	**4**

Objectives

1. Test the effectiveness of the already published code of practices for aflatoxin control in the Brazil nut production chain.
2. Determine the points in the production chain, where the infection of the causative fungi takes place (ecology of fungi) and where toxin production starts.
3. Analyze the effect of environmental factors (moisture content/water activity, relative humidity, temperature, and insect infestation) on the rate of infection and toxin production.
4. Develop a simple predictive model that describes the effect of moisture content/water activity, temperature and time on the rate of growth of the toxin producing fungi and aflatoxin production.
5. Update the existing manual of safety and quality in Brazil nuts using the data from the model and collected data.

Description of work

A2.1: Identify existing or set up Brazil nut production systems following recommended code of practices

- Constitution of a multi-disciplinary team with relevant competences (microbiology, chemistry, post-harvest technology, agronomy, food science, quality and safety management in food production chain) and the participation of key stakeholders for a participatory research.
- Selection of a Brazil nut production system in each of the States of Acre and Para, and set up as necessary the recommended good practices with emphasis on the Code of Practice of the Codex Committee on Food Additives and Contaminants (CCFAC).
- Construct and verify the Brazil nut Flow Diagrams (BnFD) for the selected production chains.

A2.2: Collection of data (*A. flavus/A. parasiticus*, aflatoxins, water activity, moisture content, relative humidity, temperature, insect infestation)

- Definition of sampling plans for the selected Brazil nut production chains.
- Collection of Brazil nut samples from the rainforest (tree) to the final site for exportation, using the European sampling procedure (Directive 98/53/EC).
- Sample screening for *A. flavus/A. parasiticus* using AFPA agar plate methodology.
  Sample screening for aflatoxins using ELISA technology (Rida Screen, R-Biopharm AG, Darmstadt, Germany).

- Water activity and moisture content in nut samples, air relative humidity and temperature measured through the production chain from tree to final site for exportation to investigate favourable conditions for fungal growth and aflatoxin production.
- Investigate the occurrence of insects, specifically *Ephestia cautella.*

A2.3: Development of a simple predictive model for aflatoxin and fungi production in the Brazil nut production chain.

Laboratory experiments will be performed to establish critical limits for safe storage and transport time at different moisture contents and temperatures.

- Published laboratory investigations of *A. flavus /A. parasiticus* growth and aflatoxin production do not consider the micro-conditions inside the nutshell. This part of the project will firstly investigate the relative humidity and the contents of O_2 and CO_2 inside the nutshell of fresh and unprocessed nuts, partially processed (semi-dried nuts) and processed nuts using sensor techniques.
- The knowledge obtained above will subsequently be used to perform studies of fungi growth and aflatoxin production on Brazil nuts using an incubator where temperature, relative humidity, O_2 and CO_2 content are controlled. This will be carried out with the aim of mimicking realistic events in the nut from the rainforest to the final processed stage. The nuts will be inoculated using fungal strains previously isolated from consignments of Brazil nuts containing high levels of aflatoxins. By using different combination of the parameters, data will be produced and used to model the probability of fungal growth and aflatoxin production.

One way to evaluate the relation between various environmental factors and biological events, such as toxin formation by microorganisms, is to use logistic regression. Based on the data collected in A2.2, a simple statistical model describing the probability of *A. flavus/ A. parasiticus* growth and toxin production will be developed. The model will be used to estimate the probability of aflatoxin production at different stages of the production chain.

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

- Identification of the Critical Control Points using the information in A2.1, A2.2 and the model developed in A2.3.
- Draft recommendations to update the existing manual of safety and quality in Brazil nuts, including the development of appropriate post-harvest technologies and traceability system, and circulation to the key stakeholders for comments on feasibility of suggested changes.
- Finalise the recommendations and the manual.

Deliverables

- A report documenting the verified BnFD of the selected production chains **by month 7**.
- A report documenting the effectiveness of the already published code of practices, and at which steps in the BnFD the mycotoxin hazard originates, or concentrations increase to unacceptable levels **by month 13**.
- A simple statistical model describing the probability of *A. flavus/A. parasiticus* growth and aflatoxin production **by month 17**.
- Updated manual of safety and quality in Brazil nuts **by month 21**.

Milestones and expected result

- Brazil nut production chains following recommended code of practices set up **by month 7.**
- Completion of verified BnFD **by month 7.**
- Sampling plans completed **by month 7.**
- Analytical methods for *A. flavus/A. parasiticus* and aflatoxins implemented in Brazilian laboratories close to the Brazil nut production areas (EMBRAPA Acre and LANAGRO-Para laboratories), and the staff trained (see Specific objectives 3 and 4 below) **by month 7.**
- Data collection completed **by month 13.**
- A laboratory model for establishing critical limits completed **by month 17.**

Specific objective 3: Validation and implementation of a rapid aflatoxin surveillance system for use along the Brazil nut production chain					
Activities/Partners	**CIRAD**	**NFA**	**CSL**	**MAPA**	**EMBRAPA**
A3.1: Adapt and validate existing rapid ELISA brought in to the project for aflatoxins in Brazil nuts.			**X**		
A3.2: Set up in Brazil a rapid ELISA for aflatoxins in the laboratory, the Brazil nut production area and one processing plant.			**X**	**X**	**X**
A3.3 Adapt and validate existing rapid, on-site Lateral Flow Device (LFD) brought in to the project for aflatoxins in Brazil nuts.			**X**		
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).			**X**	**X**	**X**
Person-month per participant:			**3.3**	**2**	**2**

Objectives
Adapt, validate and transfer to Brazilian laboratories and Brazil nut production chain (production area and processing plant) rapid methods brought in to the project (Enzyme Linked Immunosorbent Assay - ELISA and on-site Lateral Flow Device-LFD) for aflatoxins in Brazil nuts.

Description of work

A3.1: Adapt and validate existing rapid ELISA brought in to the project for aflatoxins in Brazil nuts.
Existing rapid ELISA systems for the detection of aflatoxins will be brought in to the project by R-Biopharm AG (RBAG). They will undergo a short fit for purpose evaluation on Brazil nuts through collaborative work in both the United Kingdom and Germany by CSL and RBAG. This will involve the comparison of data obtain from these assays with that from the already implemented and validated standard HPLC method, data obtained from R-Biopharm and also results in the literature on their use in Brazil nuts.

A3.2: Set up in Brazil a rapid ELISA for aflatoxins in the laboratory, the Brazil nut production area and one processing plant.
Rapid ELISA for aflatoxins would be set up in Brazil in the laboratory, the Brazil nut production area and one processing plant. This would be in conjunction with Specific objective 4 and the manufacturer (R-Biopharm) where there would be specific training in all the techniques associated with performing this assay. There will be on-going Quality Control checks against an already implemented and validated standard HPLC method.

A3.3 Adapt and validate existing rapid, on-site Lateral Flow Device (LFD) brought in to the project for aflatoxins in Brazil nuts
A rapid on-site Lateral Flow Device (LFD) for the detection of aflatoxins will be brought in to the project by R-Biopharm AG (RBAG). Because the LFD is a brand new assay, preliminary extraction protocols involving such aspects as milling, solvents (such as methanol) at various concentrations previously established will be confirmed for their applicability on Brazil nuts through colaborative work in both the United Kingdom and Germany by CSL and RBAG. As with the ELISA, this will involve the comparison of data obtain from these

assays with that from an already implemented and validated standard HPLC methods and that obtained from R-Biopharm.

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)

As for the ELISA, the LFD would be set up in Brazil in the laboratory, the Brazil nut production area and one processing plant. As the LFD is a very simple one step assay taking only a few minutes to perform, training would be very straightforward. This would be in conjunction with Specific objective 4 and the manufacturer (R-Biopharm) where all aspects associated with performing this assay would be covered. During the use of this assay in the project, as for the ELISA, there will be on-going Quality Control checks against already implemented and validated standard HPLC method.

Deliverables

- Report documenting the adapted and validated ELISA for aflatoxins in Brazil nuts detailing the standard operating procedures **by month 5**.
- Report documenting the adapted and validated rapid on-site LFD for aflatoxins in Brazil nuts detailing the standard operating procedures **by month 12**.
- The ELISA and LFD methods set up and validated in Brazil in the laboratory, the Brazil nut production area and one processing plant **by month 20**.

Milestones and expected results

- Rapid ELISA brought in to the project for aflatoxins in Brazil nuts adapted and validated **by month 5.**
- Rapid ELISA for aflatoxins in Brazil nuts set up in Brazil in the laboratory, the Brazil nut production area and one processing plant in conjunction with Specific objective 3 **by month 6.**
- Extraction procedure for existing rapid on-site LFD brought in to the project for aflatoxins in Brazil nuts developed and validated **by month 9**
- Testing and validation of rapid on-site LFD and comparison with HPLC and best other immunoassays (e.g. ELISA) completed **by month 12**
- Rapid on-site LFD for aflatoxins in Brazil nuts set up in Brazil in the laboratory, the Brazil nut production area and one processing plant in conjunction with Specific objective 3 **by month 14**
- Results obtained from the analysis of samples taken over the Brazil nut production chain in conjunction with Specific objective 2 evaluated **by month 20.**

Specific objective 4: Knowledge & technology transfer to the key stakeholders					
Activities/Partners	**CIRAD**	**NFA**	**CSL**	**MAPA**	**EMBRAPA**
A4.1: Training course in AFPA agar plate methodology for the identification of aflatoxin producing fungi in Brazil nuts	**X**	**X**		**X**	**X**
A4.2: Training courses in ELISA and Lateral Flow device (LFD) for aflatoxin analyses in Brazil nuts	**X**		**X**	**X**	**X**
A4.3: Training course in good practises in the Brazil nut production chain	**X**	**X**		**X**	**X**
A4.4: Development of a project specific website	**X**	**X**	**X**	**X**	**X**
A4.5: Scientific and specific sector publications	**X**	**X**	**X**	**X**	**X**
Person-month per participant:	**4**	**3**	**2**	**4**	**4**

Objectives

1. To spread all the scientific and technical results delivered by the project to the key stakeholders.
2. To disseminate project general information and other relevant information related to the topic to end-users.
3. To deliver appropriate information tools to promote promises innovations in the Brazil nut production chain.

Description of work

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

Training on the identification and quantification of *Aspergillus flavus/parasiticus* using AFPA agar plate methodology, which is a simple, low cost and validated standard methodology (Method No. 177, Nordic Committee on Food Analysis), will take place. This methodology is rapid (48 hrs) compared to other conventional agar plate methods. The training course will consider the following aspects:

- Introduction in food mycology with specific emphasis on aflatoxin producers,
- Description of the AFPA selective media including advantages, disadvantages, strengths, weakness, and interpretation of results,
- Practical laboratory work on mycological methodology using dilution and direct plating, identification, quantification and isolation of *Aspergillus flavus/ A. parasiticus,*
- Storage techniques for isolates,
- Safety considerations.

It will be offered in Brazil to the project participants in order to make it available at the beginning of the project for data collection of the Specific Objective 2.

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

For the technical transfer of the ELISA and LFD rapid methods that were adapted and validated within the Specific objective 3, in conjunction with the manufacturer (R-Biopharm) of these assays / kits, there would be full hands in training courses. This would involve :

- An introduction to the theory of immunoassays,
- A description of the advantages, disadvantages, strengths and weakness of each of the assays,
- Understanding the protocols / Standard Operating Procedures and training material,
- Hands on practical tuition of the use of all the equipment necessary to carry out the assays e.g. use of pipettes for small volumes, use of immunoassay readers, handling ELISA plates and LFDs etc.
- Full hands on tuition and experience of carrying out assays e.g. extraction of samples, making up reagents, coating plates, washing plates and all other aspects of performing ELISA and LFDs,

- Interpretation of results and data,
- Safety considerations.

Two training courses will be organized in Brazil: the first one for ELISA and the second one for LFD. The training course in ELISA will take place at the same period than the training course in AFPA agar plate methodology in order to make it also available at the beginning of the project for data collection of the Specific Objective 2, whereas the training course in LFD will be organized during the project progress meeting.
Both rapid methods will be offered not only to the project partners in order they can use them to carry out the project research activities, but also to all key stake holders of the Brazil nut productive chain as an aflatoxin management tool. It is indeed considered important that all people involved in the Brazil nut industry should have the opportunity to be trained in both methods. Those stakeholders that have basic laboratory facilities will have the choice of using both methods. The LFD for speed and on the spot semi quantitative decisions and the ELISA with a high throughput and a fully quantitative result.

The surveillance systems for both the causative fungi and the rapid methods for aflatoxin analysis provide the tools for the management of aflatoxins in Brazil nuts. These will be used at the crucial testing points identified during the course of the project. These points are likely, but not necessarily, to include the critical control points for the control of aflatoxins throughout Brazil Nut supply chain. Of particular relevance to the Brazil nut industry are the logistical considerations because of the very long and difficult transport of the nuts for distribution and export. It is envisaged that the rapid and inexpensive assays will enable real time local decisions (in conjunction wit the predictive model) to be made if it is worth transporting a consignment. For example if aflatoxin levels are less than 4 ppb then the decision is likely to be that it is worth the time, effort and cost for transport with a view to export. If levels are between 4 and 30ppb then it might be decided to divert a consignment intended for export to local markets or if the levels are higher to animal feed. Likewise all along the supply chain these sorts of “on the spot” decisions can be made and would lead to much greater efficiency in the use of resources, health of the local population, facilitate compliance with legislation and reduce the chances of possible ultimate rejection of a consignment after expensive export.
For enforcement, rapid methods would provide the means for a large number of samples to be screened for low or negative amounts of aflatoxins. Conformation of positive samples containing high amounts of aflatoxins would be by conventional standard methods.
The rapid and inexpensive methods for aflatoxin analysis would facilitate research and provide the information to evaluate control measures implemented after the EU missions in 2003 and 2004 and to update codes of practice and the “Manual of safety and quality for Brazil nut production”. However, regulatory decisions would need to be formulated by the individual state’s internal authorities and this project would be able to encourage and assist this process. However, recommendations concerning this will be formulated at the workshops.
At present conventional methods of aflatoxin analysis (e.g. by HPLC) although very accurate and extremely important as a method for reference and confirmation is prohibitively expensive and slow for the uses outline above. The rapid methods would overcome some of these barriers and aid informative on the spot decisions to be at the local level.

In relation to the rapid methods, there will be continued support after the completion of the project by the R-Biopharm local network in Brazil. Training manuals and other literature emanating from the project would be available to the whole of the Brazil nut industry to assist in the long term sustainability of the project outputs

A4.3: Training course in good practises in the Brazil nut production chain
On the basis of the results of the Specific objective 1 and 2, training courses in the updated and validated good practices in the Brazil nut production chain will be offered to the key stakeholders (collectors, processors, exporters) and extension agents of the local government executive agencies. This course will consider the following aspects:
- Introduction to aflatoxin producing fungi and aflatoxin contamination, and their risk for human heath,
- Aflatoxin contamination in the Brazil nut production chain and its socio-economic and environmental impact,
- Characterization of the Brazil nut production chain,

- Description of the Critical Control Points along the Brazil nut production chain,
- Description of the good practices updated and validated in the project,
- In-field demonstration sessions,
- Demonstration of the rapid assays (LFD and ELISA) updated and validated in the project for aflatoxin analyses to be used at the best testing points identified in the production chain. This will be in conjunction with the training course in A4.2 as there will be a lot of cross over between that objective and the current one.

The core group of agents of the Brazil nut production chain that was involved in the project validation and updating on the good practices will act as trainers/consultancies and serve as pilot examples/demonstration for the other stakeholders during and after the end of the project. The trained extension agents will ensure knowledge and technology transfer continuity after the end of the project.

A4.4: Development of a project specific website

A project specific website will be established and updated with inputs from all the participants following a procedure that will be implemented to check, validate and organize the information to be published.

This website will provide general information on the project and its results, as well as other relevant information related to the topic useful for all the participants and other users including beneficiaries of the Brazil nut production chain, policy makers, etc. It will also consist of the logos of all project partners and the supporting organisations within the STDF programme, and hyperlinks to their websites and other stakeholders for rapid dissemination of information/outputs.

The project specific website will be implemented through the website of a Brazilian project partner (EMBRAPA or MAPA) with the aim to maintain it after the project.

A4.5: Scientific and specific sector publications

The results of the project will be published in peer-reviewed scientific journals. They will also be reported orally and as posters, and published as abstracts or proceedings at scientific seminars and congresses organized at national, regional or international level.

The information and outputs generated by the projects will also be disseminated through specific sector publications in English, Portuguese and eventually Spanish.

Deliverables

- Training materials for the technical transfer of AFPA agar plate methodology, ELISA, LFD and good practices in Brazil nut production chain **by month 5, 5, 13 and 21 respectively.**
- Training courses in AFPA agar plate methodology, ELISA, LFD and good practices in Brazil nut production chain completed **by month 6, 6, 14 and 22 respectively**.
- Project specific website implemented and updated **by month 24**.
- Scientific and specific sector publications.

Milestones and expected result

- Project specific website established **by month 3**.
- Complete preparation of materials for training course in AFPA agar plate methodology, and ensure trainers are full prepared and have all the equipment necessary **by month 5**.
- Complete preparation of material for training course in ELISA, and ensure trainers are full prepared and have all the equipment necessary **by month 5.**
- Complete preparation of material for training course in LFD, and ensure trainers are full prepared and have all the equipment necessary **by month 13**.
- Complete preparation of material for training course in good practices in Brazil nut production chain, and ensure trainers are full prepared and have all the equipment necessary **by month 21**.

Specific objective 5: To strengthen the public-private dialogue and partnership in the Brazil nut sector					
Activities/Partners	**CIRAD**	**NFA**	**CSL**	**MAPA**	**EMBRAPA**
A5.1: Kick off meeting, and first workshop with the participation of Bolivian and Peruvian key partners	**X**	**X**	**X**	**X**	**X**
A5.2: Progress meeting	**X**	**X**	**X**	**X**	**X**
A5.3 Final workshop with the participation of Bolivian and Peruvian key partners	**X**	**X**	**X**	**X**	**X**
Person-month per participant:	**3**	**2.5**	**1.7**	**3**	**3**

Objectives
Organize and implement project meetings and workshops: a kick off meeting and first workshop, a progress meeting and a final workshop.

Description of work

A5.1: Kick off meeting, and first workshop with the participation of Bolivian and Peruvian key partners
A two days kick off meeting will be organized in the Amazon region at the beginning of the project with the participation of key representatives of the project partners. The objective of this meeting is to plan and organize the implementation and execution of the project activities by the different partners, including administrative, financial and scientific issues.
The Kick off meeting will be preceded by a one day workshop aiming to synthesize and discuss the current context of the Brazil nut sector, the results and conclusions of the main research work that has been carried out up to now on the topic in the Amazon region and the research and development projects in progress. This workshop that will involve the project partners and other key Brazilian, Peruvian and Bolivian entities of the public and private sector, will be the opportunity for the different teams to meet, get to know each other better and each others respective activities.

A5.2: Progress meeting
At the mid point of the project, a two day progress meeting will be organized in the Amazon region with the participation of the project partners, representatives of the governments of the States of Acre and Para and stakeholders of the Brazil nut production chain that have been involved in the project activities. This meeting is aimed to follow-up the progress of the activities and evaluate the effective execution of the work plan, to reinforce the knowledge of the Brazil nut situation between the participants and if necessary to agree adjustments to the direction of research. The scientific and technical results will be reviewed, analyzed and interpreted and solutions to the possible production chain bottlenecks discussed. Administrative and financial issues will also be addressed.

A5.3 Final workshop with the participation of Bolivian and Peruvian key partners
A three day final workshop will be organized at the end of the project with the participation of all the project partners, and other key Brazilian, Peruvian and Bolivian entities and stakeholders of the Brazil nut production chain. The objective of this workshop is aimed to disseminate and report all the results and conclusions generated by the project to the regional and international community. The participation of a European Commission expert and a representative of the STDF partner institutions is planned. Two days of demonstration sessions on the safety management tools (good practices, fungi and aflatoxin rapid analytical methods) tested and validated during the project will be organized.

All the meetings and workshops will stimulate networking between the project participants and contribute to strengthen the public-private dialogue and partnership in the Brazil nut sector.

Deliverables

- Kick off meeting and first workshop **by month 2.**
- Progress meeting **by month 13**.
- Final workshop **by month 23**.

Milestones and expected results

- Organization and final program of the kick off meeting and first workshop **by month 2.**
- Organization and final program of the progress meeting **by month 13.**
- Organization and final program of the final workshop **by month 23**

Dissemination plan

Appropriate dissemination of knowledge delivered by the project is a major issue for its success. For this reason, a project component (Specific objective 4) is dedicated only to this activity, and the Specific objective 5 will also contribute to it through the organisation of project meetings and workshops.

The project outputs will be disseminated to various target audiences :

- To all the participants of the project including "active" private and public co-participants,
- To the different key stakeholders of the Brazil nut production chain,
- To the other largest Brazil nut producing countries, Peru and Bolivia,
- To the scientific community including universities and research centers,
- To the Non-Governmental Organizations,
- To regulatory authorities,
- To official institutes as the national linking with European Union.

For that, different information systems will be used:

- An open website on the Internet, brief overview of the project, partner information, contact points, links to the various partners, a calendar of forthcoming workshops, seminars and conferences and the presentation of the main results and publications. The project web-side will provide the study results together with explanation in order to make it understandable to any audience. This web-side will also consist of the logos of the supporting organisations within the STDF programme and hyperlinks to their websites and other stakeholders.

- A leaflet describing the project will be produced and distributed. This publication will be formulated in layman's language and easily readable by non-specialists.

- There will be two meetings and two workshops within the project. In conjunction with the two meetings, training courses transferring technical information and materials (such as assays) will take place. The second will be in the second year of the project to update information from the first meeting and for the dissemination of current results. After the end of the project there will be a final dissemination workshop where all the activities and results of the project will be reported, with the participation not only of the core group of the project and the co-participants, but also of the regional and international community, including in particular Peruvian and Bolivian key partners.

- Dissemination of the project information and results through the EMBRAPA Information Technology Department, in particular, through the organization of interactive TV programmes via satellite for all the country (public questions asked by free phone calls, fax or emails and answered by specialists). Such a TV programme has been already used for the dissemination of Good Practices in the Brazil nut productive chain, with the support of the EMBRAPA-Acre.

- Scientific and technical results will be channelled through the NGO networks and newsletters, that act actively in the Amazon region.

- Contact will be made with the Brazilian Mycotoxin Network and other such industrial and academic networks as necessary.

- Every effort will be made to inform the general public, the Brazil Nut industry and national food agencies of the results and their implications.

- Progress will be reported orally and as posters at scientific seminars, conferences and symposia and published as abstracts or proceedings.

- The results will be published in prominent international scientific journals.

- Each publication, leaflets, books, etc. coming out of this project will provide full reference to the support from the STDF. A copy of each of these publications will be provided to the STDF together with the relevant annual and final reports and suitable information will be included on the project web-site.

A particular attention will be given to the dissemination of the project outputs to the key stakeholders of the other largest Brazil nut producing countries, Peru and Bolivia, for an effective regional impact. This will be ensured through:

- the participation of key Peruvian and Bolivian partners in the project workshops and progress meeting. In addition to the dissemination of the project outputs, this will facilitate and strengthen the exchange of scientific and technical information and experiences on Brazil nut issues between the different countries, as well as the dialogue and debate on research results (of the Brazil nut STDF project and other projects) and their application in the Brazil nut production chain and on further research needs. This will also promote the implementation of a stronger regional network in the area;

- the participation of key Peruvian and Bolivian partners in the training courses organized by the STDF Brazil nut project. These training courses which will concern Brazil nut good practises and methods for fungi identification and aflatoxin analysis, will be organized in conjunction with the project workshops and progress meeting. The objective is to provide performant tools that are necessary in the implementation of an efficient quality and safety management system along the Brazil nut production chain;

- the project web-site where all outputs and other relevant information on Brazil nut issues will be disseminated;

- the diffusion of documents published by the project such as validated code of good practices and standards operating procedures for fungi and aflatoxin surveillance system.

The project partners have already developed a partnership with Peruvian and Bolivian partners of both the private and public sector that are dedicated to the sustainable development of the Brazil nut extractive areas. The project will take advantage of this existing collaboration to ensure a regional impact, in particular in the framework of the regional Brazil nut network MAP (**M**adre de Dios, Peru ; **A**cre, Brazil ; **P**ando, Bolivia) in which the Embrapa Acre participates actively.

Evaluation plan

Achievement of project objectives
The delivery of original objectives and outputs as defined in the proposal will be evaluated.

Performance summary
The performance of the project will be assessed using the following criteria:

Baseline Start Date:	Actual Start Date:	Start Variance:
Baseline Finish Date:	Actual Finish Date:	Finish Variance:
Baseline Budget:	Actual Cost:	Cost Variance:
Baseline Work Days:	Actual Work Days:	Work Variance:

Any key variances will be identified

Achievement of expected results
Results, outputs and deliverables will be examined to determine if the expected benefits have been achieved. For example has the project outputs lead to the reformulation of the Manual of Safety and Quality in Brazil Nuts? If the answer is a negative, is it possible to reverse this result?

Impact of approved changes
If any changes to the proposal were made during the lifetime of the project, the impact (positive or negative) won the results, outputs and deliverables will be assessed.

Project management and planning
How the project has been managed will be reviewed. This will look specifically at the planning process, the frequency that plans were updated and their accuracy.
Also the effectiveness of the team and the degree of engagement with and by the other key stakeholders will be examined

Recommended actions
If there are any appropriate recommendations that can be made, these will be recorded.

Any such recommendations will tend to focus on positives rather than negatives with a view to assisting other STDF projects or their management,

Future priorities
Any future priorities identified from the project will be recorded. Consideration will be given as to if such future priorities can be achieved either within or after the project or if an additional funding programme would be necessary.

APPENDIX 5
Detailed budget

Total budget : 826 219 $ (USD)
75 % STDF contribution requested : 619 664 $ (USD)

CIRAD, France In $ (USD)	**Year 1**	**Year 2**	**Sum**
Person month	**7,5**	**7,5**	**15**
Personnel	82674	82674	165348
Travel International (France-Brazil) and local travel	13300	23300	36600
Consumables	5710	5330	11040
Other specific costs	0	0	0
Sub-contracts	0	0	0
Total			212988
75 % contribution			**159 741**

NFA, Sweden In $ (USD)	**Year 1**	**Year 2**	**Sum**
Person month	**9,5**	**3,5**	**13**
Personnel	95712	36312	132024
Travel International (Sweden-Brazil) travel	13901	7309	21210
Consumables	27654	0	27654
Other specific costs	1235	0	1235
Sub-contracts	0	0	0
Total			182123
75 % contribution			**136 592**

CSL, UK In $ (USD)	**Year 1**	**Year 2**	**Sum**
Person month	**3,6**	**3,6**	**7,2**
Personnel	43616	44924	88540
Travel International (UK-Brazil) travel	4933	4933	9866
Consumables	10962	10962	21924
Other specific costs	0	0	0
Sub-contracts (R-Biopharm AG)	10344	10534	20878
Total			141208
75 % contribution			**105 906**

R-Biopharm AG months estimated in kind contribution
Total = $ 26 527

MAPA, Brasil In $ (USD)	**Year 1**	**Year 2**	**Sum**
Person month	**10**	**7**	**17**
Personnel	42000	29400	71400
Travel Local travel + 2 journeys for two Peruvian partners	16000	23500	39500
Consumables	12550	9250	21800
Other specific costs Equipment for laboratory	10000		10000
Sub-contracts	0	0	0
Total			142700
75 % contribution			**107 025**

EMBRAPA, Brazil In $ (USD)	**Year 1**	**Year 2**	**Sum**
Person month	**10**	**7**	**17**
Personnel	42000	29400	71400
Travel Local travel + 2 journeys for two Bolivian partners	15000	21000	36000
Consumables	18550	11250	29800
Other specific costs Equipment for laboratory	10000		10000
Sub-contracts	0	0	0
Total			147200
75 % contribution			**110 400**

APPENDIX 6
Work planning

By considering that the project will start in May 2006, the work planning is the following:

Year	**2006**								**2007**												**2008**			
Month Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Activities / Months with the project starting in May (05) 2006	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**01**	**02**	**03**	**04**
Specific objective 1: Characterization of the Brazil nut production chain, and formulation of organizational and incentive strategies for safety control.																								
A1.1: Technical and socio-economical diagnostic of the Brazil nut production chain in the Brazilian States of Acre and Para			—	—	—	—	**D**																	
A1.2: Analysis of the major constraints and opportunities in the Brazil nut production chain for aflatoxin control.							—	—	—	—	—	—	**D**											
A1.3: Formulation of organizational and incentive strategies for a sustainable and safe Brazil nut production chain														—	—	—	—	—	—	**D**				
Specific objective 2: Validation of recommended good practices in the Brazil nut production chain for aflatoxin control																								
A2.1: Identify existing or set up Brazil nut production systems following recommended code of practices			—	—	—	—	**D**																	
A2.2: Collection of data (*A. flavus / A. parasiticus*, aflatoxins, water activity, moisture content, relative humidity, temperature, insect infestation)								—	—	—	—	—	**D**											
A2.3: Development of a simple predictive model for aflatoxin and fungi production in the Brazil nut production chain.									—	—	—	—		—	—	—	**D**							
A2.4: Formulate recommendations to update the existing manual of safety and quality in Brazil nuts																—	—	—	—	—	**D**			

Year	2006								2007												2008			
Month Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
Activities / Months with the project starting in May (05) 2006	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**01**	**02**	**03**	**04**
Specific objective 3: Validation and implementation of a rapid aflatoxin surveillance system for use along the Brazil nut production chain																								
A3.1: Adapt and validate existing rapid ELISA brought in to the project for aflatoxins in Brazil nuts.		—	—	—	**D**																			
A3.2: Set up in Brazil a rapid ELISA for aflatoxins in the laboratory, the Brazil nut production area and one processing plant.					—	**D**	—	—	—	—	—	—	—	—	—	—	—	—	—	**D**				
A3.3 Adapt and validate existing rapid, on-site Lateral Flow Device (LFD) brought in to the project for aflatoxins in Brazil nuts.							—	—	—	—	—	**D**												
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).													—	**D**	—	—	—	—	—	**D**				
Specific objective 4: Knowledge and technology transfer to the key stakeholders																								
A4.1: Training course in AFPA agar plate methodology for the identification of aflatoxin producing fungi in Brazil nuts					—	**D**																		
A4.2: Training courses in ELISA and Lateral Flow device (LFD) for aflatoxin analyses in Brazil nuts		ELISA			—	**D**					LFD		—	**D**										
A4.3: Training course in good practises in the Brazil nut production chain																					—	**D**		
A4.4: Development of a project specific website		—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	**D**
A4.5: Scientific and specific sector publications												—	—								—	—	—	**D**
Specific objective 5: To strengthen the public-private dialogue and partnership in the Brazil nut sector.																								
A5.1: Kick off meeting, and first workshop with the participation of Bolivian and Peruvian key partners	—	**D**																						
A5.2: Progress meeting													—	**D**										
A5.3 Final workshop with the participation of Bolivian and Peruvian key partners																						—	**D**	