Standards and Trade Development Facility

project SafeNut

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

Second Progress Report:

Reporting Period from 1 October 2006 to 31 January 2007

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GENERAL DATA ON THE PROJECT

Donor number: STDF 114

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

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

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

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

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

PROJECT STATUS

1. Describe briefly the major tasks or activities achieved during this reporting period (attach details on available training material, summary reports, etc...)

1.1. Management project activities

Signature of the contracts of technical and financial cooperation between CIRAD and the other Safenut partners

- Contract between CIRAD and CSL was signed in October 2006.
- Contracts between CIRAD and the Brazilian partners (EMBRAPA-FUNARBE and MAPA-FUNARBE) are still under validation before signature. Portuguese translation of these contracts and its annexes was required by EMBRAPA and MAPA.

Fund transfer to the Safenut partners

CIRAD has transferred to CSL 50 % of its total budget. Fund transfer to MAPA and EMBRAPA will be carried out as soon as the contracts of technical and financial cooperation have been signed with these partners. Meanwhile, CIRAD continues to assume directly the expenses of the Brazilian partners, as necessary, in order not to compromise the execution of the project activities. The advanced amounts will be invoiced to the Brazilian partners as supporting documents for their accounts, and deduced from the first fund transfer to these partners.

Organization and participation in Safenut meetings for organizing and follow up project activities:

- Meeting in the state of Pará, November 2006 – C. Brabet, CIRAD + E. Vargas, MAPA + MAPA local team – Organization of the field and laboratory activities in the state of Pará (project specific objective 2)
- Meeting in the state of Pará, December 2006 – C. Brabet, CIRAD + MAPA local team + agents (association of producers and industry) of the Brazil nut production chain – Organization of the field and laboratory activities in the state of Pará (project specific objective 2)

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

Contacts were made with the key stakeholders of the Brazil nut production chain (cooperatives and associations of Brazil nut producers/extractivists and processors/exporters) to involve them in the activities of the Safenut project, in particular those related to the project specific objective 2. The general coordinator supported the Brazilian partners for making these contacts and formalizing the collaboration.

Supervised students for Safenut activity support

- Olivier Devillers, French engineering student, ENSAR (Ecole Nationale Supérieure Agronomique de Rennes), December 2006 – May 2007: Execution of the activities A2.1 and A.2.2 in the state of Acre (project specific objective 2);
- Felicia Maria Nogueira Leite, Brazilian MSc student, UFAC (Universidade Federal do Acre) / SEAPROF (Secretaria de Extensão Agroflorestal e Produção Familiar), January 07-May 08: Execution of the activities A2.1, A.2.2 and A.2.4 in the state of Acre (project specific objective 2);
- Sarah Biruel, Brazilian student, Colégio Politécnico Bento Quirino: Safenut website design and hosting (project specific objective 4).

Reporting to the STDF Working Group

First progress report for the reporting period June-September 2006

1.2. Scientific & dissemination project activities

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

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

Deliverables expected during the reporting period:
A report documenting the current conditions of Brazil nut production and commercialisation in the Brazilian States of Acre and Pará by month 7 (December 06)

Milestones expected during the reporting period:
- Primary data collected by month 5 (October 06).
- Data processing, analysis and synthesis completed by month 6 (November 06).
- Current conditions of Brazil nut production and commercialisation described by month 7 (December 06).

Two reports were delivered during the reporting period:
1) A first synthetic report inventorying the international and Brazilian regulations applied to Brazil nuts for commercialization;
2) A first broad view of the state of art on the Brazil nut production chain in the states of Acre and Pará.

The second report, which objective is to characterize the different segments of the Brazil nut production chain and their interactions from a technical and socio-economical point of view, describes for both states of Acre and Pará:
- The evolution and location of Brazil nut production,
- The Brazil nut domestic and export markets,
- The Brazil nut flow diagram with a characterization of the different segments (input suppliers, producers/extractivists, associations and cooperatives, intermediaries, agro-industries and exporters),
- The institutional environment.

This report also includes a comparative analysis of the Brazil nut production chain between the states of Acre and Pará.

The collection of primary data (field surveys) for complementing the description of the Brazil nut production chain in the states of Acre and Pará has not been initiated yet. Consultants will be identified for supporting this work in both states.

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

Deliverables expected during the reporting period:
A report documenting the verified BnFD of the selected production chains by month 7 (December 06)

Milestones expected during the reporting period:
- Brazil nut production chains following recommended code of practices set up by month 7 (December 06).
- Completion of verified BnFD by month 7 (December 06).
- Sampling plans completed by month 7 (December 06).
- 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 (December 06).

In both states of Acre and Pará, the following activities were initiated:

Constitution of a multi-disciplinary team with relevant competences (Annex 1)
In Acre, the team involves staffs from EMBRAPA and students, whereas in Pará it involves staffs from MAPA and consultants. In both states, the teams benefit of a scientific and organizational support from the other Safenut partners.

Selection of a Brazil nut production chain in each of the states of Acre and Pará
The production chains concern the steps from the rainforest (tree) to the storage of the dried unshelled Brazil nuts in the processing plant.
Contacts with the private sector were made in both states of Acre and Pará in order to select the Brazil nut production chains:
- In Acre, with a cooperative of producers and one of its associations of producers;
- In Pará, with an association of communities of producers and one of its communities, and a processing industry.

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

Construction and verification of the Brazil nut Flow Diagrams (BnFD) of the selected production chains
- In Acre, the BnFD in the selected association of producers and processing plant;
- In Pará, the BnFD in the selected community of producers.

Other field visits and contacts with the private sector are planned to complement and confirm the selection of the Brazil nut production chains. Then, the BnFD and sampling plans will be completed.

Two training courses were organized in October 2006 (see project specific objective 4) with the aim to implement analytical methods for *Aspergillus flavus/parasiticus* (AFPA agar plate methodology) and aflatoxins (ELISA and LFD methods) in the Brazilian laboratories of EMBRAPA Acre and LANAGRO Pará, and get the staff trained.

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

Deliverables expected during the reporting period:
Report documenting the adapted and validated ELISA for aflatoxins in Brazil nuts, detailing the standard operating procedures by month 5 (October 06)

Milestones expected during the reporting period:
- Rapid ELISA brought in to the project for aflatoxins in Brazil nuts adapted and validated by month 5 (October 05).
- 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 (November 06).

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

Existing rapid ELISA (Enzyme Linked Immunosorbent Assay) and LFD (Lateral Flow Device) assays for the detection of aflatoxins were brought in to the project by R-Biopharm AG. The RIDASCREEN® ELISA is available in several different formats for the measurement of total aflatoxins (RIDASCREEN® FAST Aflatoxin multi-standards and RIDASCREEN® FAST Aflatoxin SC single control) and also aflatoxin B1 (RIDASCREEN® Aflatoxin B1 30/15), and the RIDA®QUICK Aflatoxin LFD for the measurement of total aflatoxins.

A first fit for purpose study on Brazil nuts spiked with aflatoxin B1 was carried out and a deliverable report on this work was written (Annex 2). Both the RIDASCREEN® Aflatoxin ELISA formats and the RIDA®QUICK Aflatoxin LFD resulted in values that met the target ones. It is thus concluded that any of these assays seems to be suitable to accurately measure the levels of aflatoxins in Brazil nuts at the required export/import control levels for the European Union.
An additional study on Brazil nuts naturally contaminated with aflatoxins will be carried out to add data to the first study and ensure the suitability of the ELISA and LFD assays for the Safenut project.

For technology transfer of these assays, as a first step a training course was organized in October 2006 (see project specific objective 4).

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

Deliverables expected during the reporting period:
- Training materials for the technical transfer of AFPA agar plate methodology and ELISA by month 5 (October 06).
- Training courses in AFPA agar plate methodology and ELISA completed by month 6 (November 06)

Milestones expected during the reporting period:
- 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 (October 06).
- Complete preparation of material for training course in ELISA, and ensure trainers are full prepared and have all the equipment necessary by month 5 (October 06).

Two training courses were organized at EMBRAPA Acre, Rio Branco, state of Acre, Brazil in October 2006:

Training course in AFPA agar plate methodology, 25-27 October 2006 for the identification and quantification of *Aspergillus flavus/parasiticus* by using a validated standard method (Method No. 177, Nordic Committee on Food Analysis) which is simple, low cost and rapid (48 h) compared to other conventional agar plate methods.

This course was organized by NFA with the local support of EMBRAPA Acre, CIRAD and MAPA, and offered to the Safenut partners in order to make the method available for data collection of the project specific objective 2, and to key stakeholders of the Brazil nut sector as a safety management tool. It was free of charge for the participants who had to pay only the travel expenses as necessary.
Thirteen participants joined the course: 11 from Brazil (EMBRAPA Acre, LACQSA-MG and LANAGRO-PA / MAPA, SEAPROF), 1 from the Brazil nut processing industry Tahuamanu SA, Bolivia and 1 from CIRAD (Annex 3).

The course was led by two members of NFA (Pernilla Johnsson and Ann Gidlund) and consisted of an introductory lecture and hands on practice in a laboratory.
The lecture, which was also available for participants joining the ELISA/LFD course (see below), dealt with the following aspects:
- Introduction to food mycology with specific emphasis on aflatoxin producing moulds,
- Description of the AFPA selective medium including advantages, disadvantages, strengths, weaknesses, and interpretation of results,
- Laboratory procedures in mycological methodology including dilution and direct plating, identification, quantification and isolation of *Aspergillus flavus/ A. parasiticus,*
- Storage techniques for isolates,
- Safety considerations.

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

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

Documents 2-6 were translated to Portuguese and distributed to all participants at the beginning of the course.

Training course in ELISA and LFD assays for aflatoxin analyses in Brazil nuts, 25-26 October 2006

This course was organized by CSL and R-Biopharm with the local support of EMBRAPA Acre, CIRAD and MAPA. As for the mycology course on AFPA agar plate methodology, it was offered to the Safenut partners and key stakeholders of the Brazil nut sector as a safety management tool, and was free of charge for the participants who had to pay only the travel expenses as necessary.

The attendance had to be limited to 8 participants: 7 came from Brazil (EMBRAPA Acre; CGAL-DF, LACQSA-MG, LANAGRO-MG and LANAGRO-PA / MAPA) and 1 from the Brazil nut processing industry Tahuamanu SA, Bolivia (Annex 4).

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

The first lecture by John Banks was a general overview and introduction to immunochemistry and the second by Dan Kaplan was on the specific assays being used on the course and the principles behind them. Participants were trained in different assays ranging from the traditional and generally laboratory based ELISA through to rapid systems that can be used on site in the field without specialist equipment: the LFD plus the Aflacard (a flow through device). The following R-Biopharm kits were used for the training:
- The RIDASCREEN®FAST Aflatoxin SC,
- The RIDA®QUICK Aflatoxin LFD,
- The Aflacard.

Other relevant project progress:

Safenut specific website

The website proposal for the Safenut project was approved by STDF and consequently put on the internet (http://www.stdf-safenutproject.com/).

Scientific and specific sector publications

Announcement of the Safenut training courses organized at EMBRAPA Acre in October 2006 in the rubric "Noticias" of the Embrapa Acre web site (http://www.cpafac.embrapa.br/) : “Pesquisadores testam métodos para analise e identificaçao de aflatoxina”, 23/11/2006.

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

Ring-tests for fungal analyses
These tests were organized and carried out by NFA and the team of EMBRAPA Acre in charge of the identification and quantification of *Aspergillus flavus/parasiticus* in collected Brazil nut samples (project specific objective 2). Such ring-tests consisted in analyzing a freeze dried fungal mixture produced by NFA by using the AFPA agar plate method. Since the quantitative content of the freeze dried samples of fungal mixture is statistically certified, the ring-tests enable the evaluation and comparison of laboratory skills. The tests will also be carried out by the team of LANAGRO-PA/MAPA.

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

No Deliverables expected during the reporting period.

2. Briefly list any issues/problems which impeded project implementation during this reporting period or which might affect project implementation in the future:

Fund transfer to EMBRAPA and MAPA of 50% of their total budget was delayed due to the delay in the signature of the contracts of technical and financial cooperation with these partners (Portuguese translation of the contracts and its annexes was required by EMBRAPA and MAPA). Nevertheless, as mentioned previously in 1.1, CIRAD continued to assume directly the expenses of the Brazilian partners, as necessary, in order not to compromise the execution of the project activities. The advanced amounts will be deduced from the first fund transfer to these partners.

3. Describe briefly any measurable project impacts in the reporting period (as distinct from project outputs)

Training of the Safenut partners and key stakeholders of the Brazil nut sector in rapid methods for aflatoxin and fungal analysis through the organization of training courses in AFPA agar plate methodology (13 participants), and rapid ELISA and LFD methods (8 participants).

Evaluation and improvement of laboratory skills of Safenut partners for fungal analysis through the organization of ring-tests by NFA by using the AFPA agar plate methodology and internal reference material.

Training of students
1 Brazilian MSc student and 1 French engineering student

ANNEXES

Annex 1:

Multi-disciplinary teams in both states of Acre and Pará

The multi-disciplinary team in the state of Acre

Team members	Institution	Fields of expertise	Responsibilities / Tasks
Cleísa Brasil da Cunha Cartaxo	EMBRAPA Acre	Food technology	Coordination of EMBRAPA activities. Support for contact with the Brazil nut stakeholders, CFD construction, sample collection and analysis
Joana Maria Leite de Souza	EMBRAPA Acre	Food Technology	Support for sample collection and analysis
Rivadalve Coelho Gonçalves	EMBRAPA Acre	Phyto-pathology	Fungal analysis
Support of two students at EMBRAPA Acre			
Felicia Maria Nogueira Leite	UFAC / SEAPROF	MSc in agronomy	Execution of the activities A2.1, A2.2 and A2.4
Olivier Devillers	ENSAR	Engineering student in agronomy	Execution of the activities A2.1 and A2.2.

The multi-disciplinary team in the state of Pará

Team members	Institution	Fields of expertise	Responsibilities / Tasks
Mauricio Quaresma de Araújo	LANAGRO-PA / MAPA	Chemistry	Brazil nut sample preparation Water activity and aflatoxin analysis
Nilce Limeira Medeiros	LANAGRO-PA / MAPA	Microbiology	Fungal analysis
Poliana Carla Góes de Souza	LANAGRO-PA / MAPA	Microbiology	Fungal analysis
Otavio César Durans de Oliveira	SFA-PA/MAPA	Brazil nut sampling and sanitary certification Good practices in Brazil nut production chain	Support for updating good practices
José Carlos Barroso Junior	SFA-PA/MAPA	Brazil nut sampling and sanitary certification Good practices in Brazil nut production chain	Support for contact with the Brazil nut stakeholders, CFD construction and sample collection Support for updating good practices
Gilson Pedrosa dos Santos	SFA-PA/MAPA	Brazil nut sampling	Brazil nut sample collection
+ Consultants to be identified for supporting Brazil nut sample collection, preparation and analysis			

Scientific and organizational support from the other Safenut partners

Team members	Institution	Fields of expertise	Responsibilities / Tasks
Catherine Brabet	CIRAD	Food science Quality and safety management in food production chain	Scientific and organizational support for CFD construction, sample collection and analysis
Monica Olsen	NFA	Mycology	Scientific support for sampling design, sample preparation & fungal analysis
Pernilla Johnson	NFA	Mycology	Scientific support for sampling design, sample preparation & fungal analysis
Eugenia Azevedo Vargas	LACQSA/LANAGRO-MG / MAPA	Chemistry, Food Science	Scientific support for sampling design, sample preparation & aflatoxin analyses
John Banks	CSL	Immunodiagnostics Mycology	Scientific support for aflatoxin analysis by ELISA/LFD kits
Patrick Taylor Guilherme Andrade, Dan Kaplan	R-Biopharm AG	Enzyme immunoassays	Scientific support for aflatoxin analysis by ELISA/LFD kits

Annex 2:

First fit for purpose study on Brazil nuts spiked with aflatoxin B1: Deliverable report

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

Feasibility and fit for purpose study for the ELISA and rapid on–site LFD for aflatoxins in Brazil nuts

Deliverable Report for
- 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**.

Introduction

Rapid ELISA and Lateral Flow Devices (LFD) were brought in to the project by R-Biopharm. The RIDASCREEN® ELISA is available in different formats for the measurement of total aflatoxins and also aflatoxin B1 and the RIDA®QUICK Lateral Flow Device for the measurement of total aflatoxins.

When the project proposal was written it was not sure how much validation the assays had been subjected to. However, during the development of these assays, there has been considerable internal validation on nuts, including Brazil Nuts, before general release of the assays on to the market.

At the kick off meeting of the project in July 2006, it was agreed that that an additional Fit for Purposes study would be carried out and is shown in this report along with plans for continued checks on the assays in the project.

Methods

ELISA Test

Samples were prepared according to the instruction leaflet of RIDASCREEN® Aflatoxin ELISA. In brief:
Brazil Nuts were milled and 5g amounts weighed into vials with 0 and 1 µg/ml aflatoxin B1 in methanol to yield 0 ("zero" samples) and 20ppb of aflatoxin. To each vial, 25ml of 70% methanol was added and mixed for 10 minutes with end of end rotation. The suspension was centrifuged at 3,000g for 12 minutes at room temperature and 0.5ml of the supernatant mixed with 0.5ml of distilled water. From this mixture, 50µl was added to the ELISA.

Lateral Device : LFD

Samples were prepared according to the instruction leaflet of RIDA®QUICK Aflatoxin LFD. In brief:
Brazil Nuts were milled and 5g amounts weighed into vials with 0 and 1 µg/ml aflatoxin B1 in methanol to yield 0 ("zero" samples) and 20ppb of aflatoxin. To each vial, 10ml of 70% methanol was added and mixed for 10 minutes with end of end rotation. The suspension was centrifuged at 3,000g for 12 minutes at room temperature and 50µl of the supernatant mixed with 100µl of mobile solvent and 100µl applied to the application window of the LFD.
Appearance of the control and test bands was observed as in the instructions over a period of 16 minutes.

Results and discussion

ELISA Test
Results of the different RIDASCREEN® ELISA formats are shown in the Table 1.
All the "zero" samples gave result of < the limit of detection (LOD) and for the 20 ppb sample, results were within 1ppb of the spike value for the Fast Aflatoxin and Aflatoxin B1 ELISAs and between 19.3 and 22.8 for the Fast Aflatoxin SC.

Table 1: RIDASCREEN® ELISA results in Parts per Billion (PPB) and the %B/BO of the different formats for total aflatoxins and aflatoxin B1 in Brazil nuts containing zero or 20ppb aflatoxin B1.

Sample	RIDASCREEN®					
	Fast Aflatoxin Lot 02266		Fast Aflatoxin SC Lot 02106		Aflatoxin B_1 Lot 05356	
	PPB	%B/BO	PPB	%B/BO	PPB	%B/BO
1239-1	<LOD	95.7	<LOD	97.3	<LOD	95.3
1239-1: 20ppb	21.0	32.6	19.3	47.2	19.1	26.9
1239-2	<LOD	82.6	<LOD	99.3	<LOD	96.4
1239-2: 20ppb	20.5	33.0	22.8	42.9	20.2	26.0
LOD or test	1.7ppb		2.0ppb		1ppb	
Range of test	1.7 – 45ppb		2.0 - 100ppb		1 – 50ppb	

Note: %B/BO = Standard or sample net absorbance (absorbance of sample minus absorbance of reagent blank) divided by the net absorbance of zero (absorbance of zero minus absorbance of reagent blank) times 100

Lateral Flow Device : LFD
Results of the RIDA®QUICK Lateral Flow Device are shown in the Table 2.
All the "zero" samples gave a negative result which was below the limit of detection of 4ppb. For the 20ppb samples, a band was present from 5 minutes which was just after the usual reading time of 4 minutes indicating that the analysed level was close to 20ppb.

Table 2. RIDA®QUICK Lateral Flow Device results indicating the appearance of the test band at different times after the start of the test and the assay evaluation result of brazil nut samples containing zero or 20ppb aflatoxin B1

RIDA®QUICK Lot 02136				
Sample	Test Band appearance			Evaluation
	4 min	8 min	16 min	
1239-1	-	-	-	<4ppb
1239-1: 20ppb	(+)*	+	+	Approx. 20ppb
1239-2	-	-	-	<4ppb
1239-2: 20ppb	(+)*	+	+	Approx. 20ppb

(+)* Test Band was clearly visible after 5 min.

Conclusion

Both the results from the RIDASCREEN® ELISA formats and the RIDA®QUICK Lateral Flow Device indicate that these methods can be used to accurately measure the levels of aflatoxins in Brazil Nuts.
The work presented in this report confirms earlier company development work for the assays. It is concluded that any of the above assays could usefully be employed in the SafeNut -STDF project for the analysis of Brazil Nut samples along the production chain. Within this analytical programme, it is anticipated that there will be further confirmation by standard samples of known aflatoxins content and HPLC checks.

Annex 3:

Training course in AFPA agar plate methodology: Participants and illustrating photos

Name	Institution
1. Catherine Brabet	CIRAD
2. Rivadalve Coelho	EMBRAPA Acre
3. Francisco Roberto Vieira Sampaio	EMBRAPA Acre
4. Josivaldo da Silva Saraiva	EMBRAPA Acre
5. Edwin Gustavo Cañazaca Choquehuanca	EMBRAPA Acre
6. Elizângela Barbosa de Lima Oliveira	EMBRAPA Acre
7. Fabiana Silva Reis	EMBRAPA Acre
8. Sônia Maria Lima Santos do Vale	EMBRAPA Acre
9. Felícia Maria Nogueira Leite	SEAPROF Acre
10. Cláudio Roberto Sosoranga Uchuari	Universidade Nacional de Loja
11. Julio César Garcia	LACQSA-MG / MAPA
12. Poliana Carla Góes de Souza	LANAGRO-PA / MAPA
13. Evangelina Jurado	Tahuamanu SA, Bolivia

Annex 4:

Training course in rapid ELISA and LFD methods: Participants and illustrating photos

Name	Institution
1. Joana Maria Leite de Souza	EMBRAPA Acre
2. John Lennon Mesquita Catão	EMBRAPA Acre
3. Alex da Silva Santos	EMBRAPA Acre
4. Giovana Aparecida Amaral Gonçalves	LANAGRO-MG / MAPA
5. Eliene Alves dos Santos	LACQSA-MG / MAPA
6. Maurício Quaresma de Araújo	LANAGRO-PA / MAPA
7. Adriana Claudia Chagas	CGAL-DF / MAPA
8. Justina Zurita	Tahuamanu SA, Bolivia