Project Progress Summary

Section 1: PROJECT IDENTIFICATION Information to be provided for project identification	**NOT CONFIDENTIAL**

Title of the project

Development of Quantitative and Qualitative Molecular Biological Methods to Identify Plant and Animal Species in Foods

Acronym of the project

MolSpec-ID

Type of contract		**Total project cost** (in euro)
Shared Cost RTD		3 109 579 €
Contract number	**Duration** (in months)	**EU contribution** (in euro)
QLK1-CT-2001-02373	36 Months	1 395 074 €

Commencement date	**Period covered by the progress report**
1 December 2001	1 December 2001 – 30 November 2002

PROJECT COORDINATOR

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Key words (5 maximum - Please include specific keywords that best describe the project.).

Species, Molecularbiology, Food, Identification, Quantification

World wide web address (the project's www address)

www.MolSpec.org

List of participants

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- Co-ordination of the project
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- Task-leader of work package 3
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Section 2: Project Progress Report **NOT CONFIDENTIAL**

Objectives:

Qualitative and quantitative detection methods for the identification of plant and animal species in food and feed playing a role in fraudulent replacement or as component of particular health risk will be developed and tested with processed materials. For this purpose materials with defined amounts of the respective animal or plant species will be produced. Apart from this, authentic samples will be made available by partners to cover non domestic animals. For the identification of the following plant species qualitative methods will be evaluated: *Leguminosae* (soybean, pea, peanut), at least two species of nuts (walnut and almond) and gluten-containing cereals (wheat, barley, oat, rye). Qualitative methods will be evaluated for the following animal species: beef, pork, lamb, horse, chicken, turkey, duck, crab meat and at least for the following non domestic animal species: deer, ostrich and kangaroo. Quantitative methods will be approached for the identification of the following species: beef, pig, lamb, turkey chicken, duck, horse, soybean. Methods will be based on DNA analysis. One method will be developed based on immunological techniques (antibody production). The subject of investigation in this case will be crab meat in surimi products. This investigation includes a systematic comparison with a PCR based approach. The method development and testing will be accompanied during the initial period of the project by investigations on DNA extraction and the development of appropriate amplification control protocols and systems that will be made available to all partners.

Four selected methods shall be validated in intercomparison studies as a prerequisite for further standardisation by international standardisation committees. The methods will be selected during the ongoing project by partners and co-ordination

1. **At least one quantitative method.**
2. **Two qualitative methods that can optionally be opened for quantitative analysis according to progress in the respective work packages.**
3. **One protein based approach (qualitative) which in parallel will be accompanied by a PCR-based approach.**

All qualitative approaches will include a sensitivity study.

Further objectives of the project are the enhancement of efficiency, throughput, which results in improved cost effective applications. For these purpose multiplex systems - allowing the identification of several species in one and the same approach - shall be developed and used for the establishment of micro array based techniques. In parallel PCR-ELISA system shall be settled using the input of multiplex system development.

Finally a data base will be developed to collect all information about species identification in foodstuffs including information about sequences suited for identification, primer and probes, guidelines for handling processed foodstuffs (DNA extraction), literature, contacts etc. The database shall guarantee a broad dissemination of results.

Results and Milestones:

Until November 2002 differently processed meat and fish products were provided with defined amounts of different species as well as authentic raw materials for qualitative and quantitative analysis. Using these and other materials the efficiency of extraction of nucleic acids (DNA) was evaluated using commercially available KITS or standard procedures. Critical steps as well as optimal isolation methods were identified. Internal standards were developed to monitor and compare DNA-isolation efficiencies, but results have been inconsistent so far.

A broad range of qualitative detection methods have been developed or are in an advanced state for the identification of sheep, goat, diverse bovide and cervide species, pig, chicken, turkey, duck, kangaroo, ostrich, gluten-containing cereals (barley, ray, wheat strains), soybean, walnut, almond and hazelnut. For the latter a special technique (SCAR) had to be used, because only very few sequence data are published, which are necessary for the development of PCR (polymerase chain reaction) based systems.

Methods for the relative quantification of cattle, pig, chicken, duck, turkey and horse in relation to total mammalian and bird DNA (reference system for meat) have been tackled and described.

Performance criteria like specificity, sensitivity and selectivity are available for most of the methods.

Protein based methods have been developed (antibodies against shellfish/crab) or commercially available kits were used and statistically evaluated to be compared with DNA based methods. Work on surimi (fish/crab-meat) started.

Multiplex assays aiming to identify several species in one assay were approached for a) cattle, goat, sheep, b) horse, ostrich, kangaroo, c) turkey, chicken and duck. d) pig and cattle. The systems need further optimisation and validation.

A first conceptual data base structure for the collection of detection methods was worked out. Input of scientific data about the developed methods has started.

Benefits and Beneficiaries:

a) new methods for the analysis of health risk posing species (potential allergens) and fraudulent replacements in food, b) information flow by scientific publication and project's website including public access, c) identification of "critical" samples/procedures and development of approaches to overcome those problems, d) starting point of a new data collection with broad practical applicability in future, e) the development of high throughput detection systems will improve cost effectiveness f) a standard validation protocol facilitating comparison of methods.

Future Actions (if applicable):

It will be considered, if validated methods under the topic "health posing" species could be presented to the CEN/TC 275/WG 12 "Food allergens" to be converted into EU standards.

First data base releases will be tested in autumn/winter 2003 (distribution of a MolSpec-database prototype will take place in October 2003).