**Danish Research Centre for Organic Farming**

# Midterm Status Report 2003 and Application for Continuation in 2004

For research projects financed by grants from
The Directorate for Food, Fisheries and Agro Business
under the Danish Ministry of Food, Agriculture and Fisheries

## 1. Research program

Research in organic farming 2000-2005 (DARCOF II)

## 2. Project title and number

Organic food and health – a multigeneration animal experiment. No. III.4.

## 3. Head of project

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## 6. Project period (month, year)

Start of project: 01.05.01
End of project: 31.12.04

# 7. Midterm description of the project, its results and progress, and application for continuation in 2004

## A. Project summary

*Introduction*
The project aims to determine if differences between organic and conventional cultivation systems can result in differences in the health of those who eat the products.
So the principle is to grow crops in models of organic and conventional cultivation systems, process them to food, and feed them to rats in a multigeneration experiment. Then the products and the rats are thoroughly investigated to determine if and what differences occur.

In the first call we were requested to establish a multigeneration experiment within a fixed budget of 6 mill. Dkr. Due to this limitation, we could only use plant material produced in one growth season. However, with only one growth season the results would only be applicable to this particular year. So in 2001 we applied for an extension of the project, with one more growth season and one more animal generation, using the offspring of the same rats, and received an additional grant of 1.83 mill. Dkr in 2002. The combined project will now provide independent data on the fertility of rats raised on feed from 2001 as well as on feed produced in 2002, and thus provide much better possibilities for a statistical evaluation of the results than what was originally planned.

In 2003 the central part of the project is taking place, the animal feeding experiments over 3 generations of rats. So this year, the practical work with detailed planning and execution of several interlinked tasks has been in focus. All activities have now commenced, and for some of them the experimental work has been completed, and the activities have entered the data analysis phase just prior to publication.

*Project overview:*
***Table A.1: Work package list (from application)***

| No. | Work package title | Participants* | Budget (1.000 DKr) | Start | End | Deliverable No(s): |
|---|---|---|---|---|---|---|
| 1 | Cultivation of feed plants | JPM, KTK, HLP | 753 | April 01 | Oct. 02 | 1.1, 1.2 |
| 2 | Characterisation of picture-developing properties of plant materials and feed mixtures | JOA | 485 | Nov. 01 | Dec. 02/ Dec. 03 | 2.1, 2.2 |
| 3 | Characterisation of secondary metabolites in plant material | RN, UK | 1124 | Dec. 01 | Aug. 03/ Aug. 04 | 3.1 |
| 4 | Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures | CL, HJ | 620 | May 01 | June 03/ June 04 | 4.1 |
| 5 | Characterisation of mineral content and pesticide residues of feed mixtures | SHB, LF, KB + subcontractors | 382 | Sept. 01 | Dec. 03/ Aug. 04 | 5.1, 5.2 |
| 6 | Management and recording of feeding and breeding | MR | 1113 | Sept. 01 | Feb. 04/ Oct. 04 | 6.1, 6.2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 7 | Digestion and utilisation of nutrients and effect on health status | CL, SHB, LF + sub-contrac-tors | 2206 | Nov. 01 | Aug. 04/ Dec. 04 | 7.1-7.3 |
| 8 | Data management and analysis | UH | 721 | Jan. 02 | Dec. 04 | 8.1-8.3 |
| 9 | Coordination, dissemination and evaluation | KB + all | 450 | April 01 | Dec. 04 | 9.1-9.7 |
| Total | | | 7.860 | | | |
| x | Screening of phytochemical changes in plant material | HR | (400) | Aug. 01 | April 02/ April 03 | x.1 |

* Responsible participants are underlined

Methods used in each workpackage (WP)

*WP1 Cultivation of feed plants*
The agricultural treatments used are the following:
1. A model of a distinct organic cultivation system, with low input of nutrients through animal manure and use of catch crops, and no pesticides.
2. A model of a distinct conventional cultivation system, with high input of nutrients through mineral fertiliser and use of as much pesticides as is allowed.
3. A combination of 1 and 2, with low input of nutrients, primarily through animal manure, and use of as much pesticides as is allowed.
This design provides 3 separate cultivation system models, 2 of which are similar to those actually used for food production. And it also allows an evaluation of the relative importance of nutrient supply compared with plant protection, for any effects on product composition or animal health.

The treatments were established on 2 sites, wheat, potatoes and oilseed rape were produced in Foulum, and carrots, kale and mature peas in Årslev.
Potato, peas and kale are cooked and freeze dried, oil produced from the rapeseed, and raw carrots and apples are shredded and freeze dried. The plan was to grind wheat and bake it to bisquits, but a fire destroyed the material, so the plans were changed to make instead a feed without wheat, with mature peas and potatoes as the major components.
The processing is intended to ensure that the feed mixture will contain only components that are also parts of human diets, this is the reason for avoiding eg. raw peas or raw wheat.

The cultivation has taken place in 2001 and 2002. Using two years provides material for a repetition of the analyses of the materials, and in the feeding trial.

*WP2 Characterisation of picture developing properties of plant materials and feed mixtures*
The picture-developing properties of fresh plant material are examined for carrot, potato, kale, apple, pea and spring wheat samples, as well as for processed feed mixtures by means of biocrystallization. The pictures are quantified by means of computerized image analysis techniques (texture analysis of grey-levels) and / or by means of quantitative visual scoring techniques.
Combined with the other WPs this work will allow a quantification of the reproducibility and precision of the biocrystallization method for detecting effects of cultivation conditions.

*WP3 Characterisation of secondary metabolites in plant material*
Characteristic secondary metabolites are measured in selected feed material from each treatment: Polyacetylenes, isocoumarins and volatile compounds in carrot, volatiles and phenolic compounds in apples, glucosinolates, volatiles and phenolics (including flavonoids) in kale, glucoalkaloids, phytin, coumarin and phenolic acids in potatoes, and phytins in peas. Selected phenolic or other compounds will be isolated and identified where standards are not available.
This WP will provide data on bioactive components suspected, but not known, to be important for the effects of food on health. It will also provide fingerprint-type data, which are known to be sensitive detectors of differences in the physiological status of plants (metabolomics).

*WP4 Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures*
The major nutrients in the feed plants of the cultivated treatments of WP1 are determined, and the biological value of major protein sources of feed plants will be assessed. Based on these results, feed mixtures are prepared based on defined weight percentages of each material from each treatment.
The proportions of each crop are chosen as far as possible to provide at least adequate amounts of each known nutrient in any of the diets. For a few nutrients, including methionine and vitamin E, any mixture of these crops will be inadequate to meet the tabulated demands of the reproducing animals. So adequate amounts of each of those nutrients will be added to the diets, the same amount to each treatment. Otherwise the health of the rats would be strongly influenced by nutrient deficiencies, which would be irrelevant for the purpose of the project of providing a model for human health, since no deficiencies of any of the nutrients in question are likely to occur in a European population. Based on this, the composition of the diet became: 30% potatoes, 13% carrots, 47.2% pea, 1% kale, 1% apple and 13% rapeseed oil. To this was added the amino acid methionine as well as selected vitamins and minerals, for which it was assessed that none of the treatments would be able to provide the recommended daily intake for rats. The content of rapeseed oil is higher than recommended, corresponding to the average level in Danish human diets. The intention with this choice is to be able to assess health aspects related to a superoptimal energy intake, which are particularly relevant for human health in developed countries.

*WP5 Characterisation of mineral content and pesticide residues of feed mixtures*
Concentrations of 16 elements are measured by ICP-OES (Ca, Cu, Fe, K, P, Mg, Mn, Na and Zn) or ICP-MS (Cd, Co, Cr, Mo, Pb, Te og V) on the plant material harvested in WP1, and a standard multi-analysis of 140 pesticides is carried out on each of the final diets.
The data on minerals will be used to determine which minerals will be further studied for bioavailability in WP7, and were also used to determine what extent of supplementation of single essential minerals was necessary to avoid deficiencies.

*WP6 Management and recording of feeding and breeding*
Young rats are fed with the 3 feed mixtures, and bred for 3 generations. It was originally planned that the first two generations would be raised on plant materials grown in 2001, while the third generation would receive feed from 2002-material. The plan was revised so now half of the rats (and their offspring) are raised on feed grown in the 2001 season throughout the experiment, while the other half receives feed grown in 2002 during the second and the third generation. The numbers and genotypes of rats was determined in pilot experiments with 5 different strains, and it was determined that the experiment was done with 2 strains for the first generation (Brown Norway and GK/mol), while one strain

(GK/mol) continued for the two subsequent generations. Reproductive characteristics and weight gains are recorded in each generation.

*WP7 Digestion and utilisation of nutrients and effect on health status*
Subgroups of the second generation of rats are selected for an intensive study in which uptake and excretion of energy and protein and selected micronutrients are determined. Respiration trials are performed to assess the energy metabolism, and simultaneous measurements of the activity levels of the rats are performed. A non-invasive method using analysis of isotope composition of exhaled air after ingestion of specific substrates will be used to evaluate the liver function and the gastric motility. In addition, blood and tissue samples of the rats will be obtained to study the effect of the dietary treatments on the immunological, antioxidant, and health status of the rats. A preference-test will be performed with rats of the same strain (GK/mol), from a group raised on standard rat feed, the test will take place immediately after weaning. Instead of the life expectancy study, a study will be made with older rats, using the female parents from the second generation after their young have been weaned. The animals will be assessed for the liver function and the gastric motility, and will then be killed at a specified age and autopsied, so the condition of key organs can be assessed and ranked under comparable conditions. This WP has been substantially revised compared with methods described in the application, to obtain more and better data within the allocated budget.

*WP8 Data management and analysis*
Data from the experiments are assembled and analysed using relevant models, and combined with the data on feed to determine which associations are significant, and to provide inputs for the final scientific papers. A model for a discrimination analysis of the biocrystallization pictures is developed, as well as the methods for the data analysis for WP6.

*WP9 Coordination, dissemination and evaluation*
Coordination and management of the project, primarily through periodic meetings and progress reports. Publications and other dissemination activities are planned and coordinated to maximise precision and extent of the impact of the results. In particular it is important that the more far-reaching results are subjected to a peer review process before they are released to the public, even though this means that most of the results will only be published after the project is finished. Since due to the substantial public interest in the issue, it would be a particularly serious problem if preliminary data were released, and later had to be corrected.

*WPx Screening of phytochemical changes in plant material*
TLC screening of fresh plant material from each crop and cultivation treatment for phytochemical changes to provide an estimate of similarity and facilitate the selection of analysis and isolation of secondary compounds. This WP is financed from external funding (co-financing). NERI's work is based on support from a company that expects to develop a test that can reveal whether a plant has been sprayed with pesticides. The patterns provide a quick screening to show which types of compound differ among treatments, and will therefore provide a valuable lead for optimising the work in WP3.

## B. Objectives and expected achievements

The overall objective is to determine if a well controlled animal feeding experiment comparing food products produced by conventional and organic methods shows differences in animal physiology, of a type and magnitude that indicates that such products can affect humans differently.

Partial objectives are the following:

1. To produce food products from strictly controlled, comparable fields, representing the versions of agricultural treatments described in the methods section below, to ensure that the field treatments are the only important variables related to the food, and that the diet made from the food products is both nutritionally adequate for the experimental animals and a relevant model for human diets.

2. To determine if the foods show differences in contents of macronutrients (protein amount and composition, energy content and distribution) of such a magnitude that this is likely to affect food consumption and/or growth of animals under nutrient-limited conditions.

3. To determine if the foods show significant differences in picture-developing characteristics of the fresh sample materials or processed feed mixtures, or differences in contents of any other compounds (secondary metabolites, vitamins or minerals) of such a magnitude that this is likely to affect consumption and/or growth of animals.

4. To determine if the 3 diets described above affect growth, fertility and level of activity of experimental animals for 3 generations.

5. To analyse biomarkers for relevant disease indicators, adaptations to biological value of the feed, bioavailability of selected minerals and secondary metabolites and other health related responses for differences among the 3 groups during the 2nd generation.

## C. Midterm results and progress

### C.1 Description (summary) of main results and conclusions

It was possible to obtain adequate yields of all the crops in all treatments. The materials from the 2001 and 2002 harvests were processed and all except rapeseed oil analysed for contents of: protein, ash, selected macrominerals (Ca, Cu, Fe, K, P, Mg, Mn, Na Zn) and phenolics, including flavonoids and phenolic acids. Relevant materials were analysed for carotenoids, volatile compounds, polyacetylenes, glycoalakaloids, 6-methoxymellein (isocoumarin) and other coumarins.

Until now the analyses of composition of the various foods that have been evaluated have shown differences of the expected magnitude (10-25 %). The differences may or may not be significant, which will be revealed when the data from the 2001 and 2002 harvests have been analysed statistically. For some types of compounds (e.g. content of volatile secondary metabolites) it appears that the difference between years of cultivation is greater than the effect of cultivation treatment, however, the effect of the cultivation treatment is still systematic across years for most crops (the ranking of the three treatments is the same in both years).

For example, a “crude summary” of the data for volatile compounds in apples:

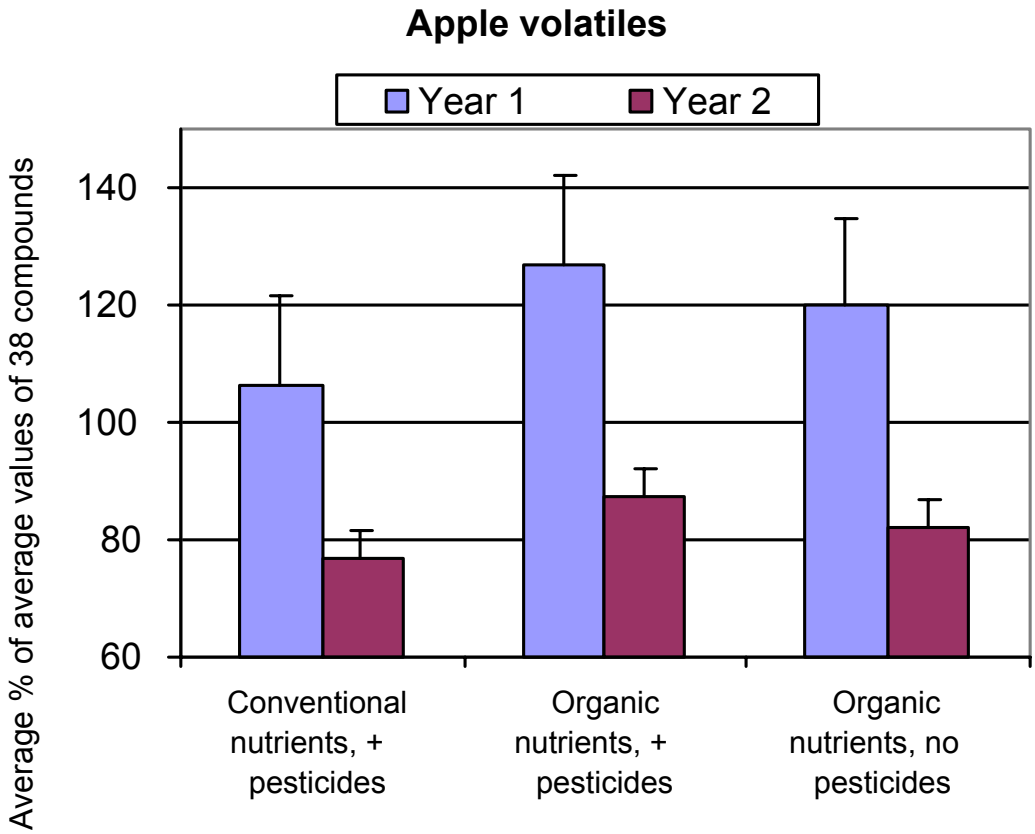


Figure 1: Effect of cultivation treatments on 38 volatile compounds in freeze-dried apples. For each compound the average content of that compound is set at 100%, and the value of each treatment is expressed as the percentage of the average value. In the figure is shown the average percentages obtained for a large number of compounds. This method for visualisation highlights trends that are common for many compounds and is not affected by the absolute amount of each compound (which varies by several factors of magnitude). The error bars represent standard error.

For the crops potato, pea, kale and carrot, as well as for the feed mixtures, biocrystallization pictures of sufficient quality (in terms of percentage of single-centered pictures) for comparisons within and among years were produced from material from the 2001 and 2002 harvests.

TLC biomarker patterns were produced from potato, pea, kale, apple and carrot from the 2001 harvest, showing an average of 4 compounds differing by at least 50 % in spot size between the two most different treatments.

Sensory evaluation of carrots harvested in 2001, performed by students at the Royal Veterinary and Agricultural University, showed significant differences in sweetness and bitter aftertaste between blanched carrots grown with the fully organic or the fully conventional treatments, while the third treatment was not different from any of the others. A test of the same carrots served as raw showed smaller differences, only significant for sweetness, but the treatment averages were ranked in the same sequence. From the 2002 harvest a similar test was made for green, blanched peas, and also here small, but significant differences were detected.
Based on these pilot experiments, a proposal was made to the “Foundation for Organic Agriculture” (Fonden for økologisk landbrug), a section of the Danish agricultural levy foundations, which allocated 129000 Dkr. for a separate project in 2004, where a trained panel at DIAS will carry out full scale sensory evaluation of the frozen material of carrots,

potato and kale from project III.4 for both the 2001 and 2002 harvests.

As far as has been analysed, data from the first generation rat study in WP 6 shows significant differences in some of the reproductive characteristics between treatments, however it is too early for any conclusions to be drawn.

These preliminary data are also in accordance with the expected differences based on earlier investigations comparing otherwise equivalent organically and conventionally cultivated crops.

### C.2 Fulfilment of deliverables and milestones

*The tables comprise deliverables and milestones from both the original project and the extension. Those belonging to the extension or changed due to it are marked by an **

| WP 1 Cultivation of feed plants | Time schedule according to application | Deviations, if any* |
|---|---|---|
| Task | | |
| | | |
| Deliverables | | |
| D1.1 The harvested materials. | Nov. 01 and Nov. 02 | Achieved in 2001 and 2002. |
| D1.2 The processed materials. | March 02 and March 03 | Most crops OK, the wheat was lost (burned!). |
| | | |
| Milestones | | |
| M1.1 The materials are harvested | Nov. 01 and Nov. 02 | Achieved in 2001 and 2002 (no wheat was grown in 2002). |
| M1.2 The materials are processed. | March 02 and March 03 | Achieved for all crops except wheat |

| WP 2 Characterisation of picture-developing properties of plant materials and feed mixtures | Time schedule according to application | Deviations, if any* |
|---|---|---|
| | | |
| Deliverables | | |
| D2.1 Scientific manuscript on picture-developing properties of the examined samples. | Aug. 03 * | Delayed by approximately 8 months |
| D2.2 Contribution to scientific paper(s) on the correlation between picture-forming characteristics and other data from the project. | Dec. 04 | On time |
| | | |
| Milestones | | |
| M2.1 The analyses are completed. | April 03 * | Delayed by approximately 4 months |

** Deviations are to be further discussed in D2*

| WP 3 Characterisation of secondary metabolites in plant material | Time schedule according to application | Deviations, if any* |
|---|---|---|
| | | |
| Deliverables | | |
| D3.1 At least one scientific manuscript on the contents of secondary metabolites in the materials. | Aug. 04 | On time |
| | | |
| Milestones | | |
| M3.1 The analyses are completed. | Aug. 03 | On time |

| WP 4 Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures | Time schedule according to application | Deviations, if any* |
|---|---|---|
| | | |
| Deliverables | | |
| D4.1 Contribution to scientific manuscript | Dec. 03 | Delayed by approximately 5 months |
| | | |
| Milestones | | |
| M4,1 Formulation and production of experimental diets for animal experiment. | Oct. 02 and June 03 [e] | Delayed by approximately 1 month |

** Deviations are to be further discussed in D4*

| WP 5 Characterisation of mineral content and pesticide residues of feed mixtures | Time schedule according to application | Deviations, if any* |
|---|---|---|
| | | |
| Deliverables | | |
| D5.1 (month 20): List of elements selected for the balance study of the second generation in WP7. | Dec. 02 * | Delayed by approximately 10 months |
| D5.2 (month 20): Data on contents of pesticides in each of the diets. | Dec. 02 and Aug. 03* | Delayed by approximately 2 months |
| | | |
| Milestones | | |
| M5.1 (Month 20): The analyses are completed. | Dec. 02 and Aug. 03 * | Delayed by approximately 2 months |

** Deviations are to be further discussed in D5*

| WP 6 Management and recording of feeding and breeding | Time schedule according to application | Deviations, if any* |
|---|---|---|
| | | |
| Deliverables | | |
| D6.1 Weaned rats of second generation for WP7 | June 03* | Delayed by approximately 1 month |
| D6.2 Scientific manuscript on reproductive effects. | April 04 | - |
| | | |
| Milestones | | |
| M6.1 In the first generation, a sufficient number of young is produced in order to make the planned recordings and experiments. | Dec. 02* | Delayed by approximately 1 month |
| M6.2 In the second generation, a sufficient number of young is produced in order to make the planned recordings and experiments. | April 03* | Delayed by approximately 1 month |
| M6.3 In the third generation, a sufficient number of young is produced in order to make the planned recordings and experiments. | Aug. 03* | Delayed by approximately 1 month |

** Deviations are to be further discussed in D6*
*- Too early to asses if deviations are going to occur*

| WP 7 Digestion and utilisation of nutrients and effect on health status | Time schedule according to application | Deviations, if any* |
|---|---|---|
| | | |
| Deliverables | | |
| D7.1 Scientific manuscript(s) on bioavailability of nutrients and secondary metabolites. | Dec. 04 | - |
| D7.2 Scientific manuscript(s) on behaviour, activity levels and food preference. | Dec. 04 | - |
| D7.3 Scientific manuscript(s) on immunological status, frequency of diseases and other aspects of health status in aged animals. | Dec. 04 | - (life expectancy study changed) |
| | | |
| Milestones | | |
| M7.1 The tests on the first (now third) generation rats are completed. | June 03* | Delayed by approximately 8 months |
| M7.2 The tests on the second generation rats are completed. | Oct. 03* | Delayed by approximately 4 months |
| M7.3 The occurrence of diseases and deficiencies in aged animals of the second generation rats are characterised. | Dec. 04 | On time |

** Deviations are to be further discussed in D7*
*- Too early to asses if deviations are going to occur*

| WP 8 Data management and analysis | Time schedule according to application | Deviations, if any* |
|---|---|---|
| | | |
| Deliverables | | |
| D8.1 Detailed plan for initial experiments and exchange of materials and data. | Feb. 02 | On time (changed to WP 9) |
| D8.2 Optimised plan for the animal experiments. | Oct. 02* | Delayed by approximately 6 months |
| D8.3 Report or inputs to papers, with the relevant statistical analyses. | Aug. 04 | - |
| | | |
| Milestones | | |
| M8.1 Detailed plan for experiments and exchange of materials and data is prepared. | Feb. 02 | On time (changed to WP 9) |
| M8.2 Optimised plan for the animal experiments is prepared. | Oct. 02* | Delayed by approximately 6 months |

** Deviations are to be further discussed at in D8*
*- Too early to asses if deviations are going to occur*

| WP 9 Coordination, dissemination and evaluation | Time schedule according to application | Deviations, if any* |
|---|---|---|
| | | |
| Deliverables | | |
| D9.1 Startup meeting M1 | April 01 | Achieved |
| D9.2 Progress meeting M2 | March 02 | Achieved |
| D9.3 Progress meeting M3. At this meeting those details of the research plans for WPs 6 and 7, which depend on WPs 2-5, and, if necessary, relevant redistributions of resources, are determined for the remains of the project | Oct. 02 | Delayed by approximately 1 month |
| D9.4 Progress meeting M4 | April 03* | Delayed by approximately 2 months |
| D9.5 Progress meeting M5 | Feb. 04 | On time |
| D9.6 Progress meeting M6 | Oct. 04 | - |
| D9.7 Final report, including implementation and further progress. | Dec. 04 | - |
| | | |
| Milestones | | |
| M9.1 Determination of detailed research plans for the animal studies. | Oct. 02* | Delayed by approximately 6 months |
| M9.2 Publication plan is prepared. | April 03* | Delayed by approximately 8 months |
| M9.3 Final report on implementation and further progress is prepared. | Dec. 04 | - |

** Deviations are to be further discussed in D9*
*- Too early to asses if deviations are going to occur*

| WP x Screening of phytochemical changes in plant material | Time schedule according to application | Deviations, if any* |
|---|---|---|
| | | |
| Deliverables | | |
| Dx.1 Participation in publications where the results are used to identify secondary compounds. | Dec. 03 | On time/reduced |
| | | |
| Milestones | | |
| Mx.1 The analyses are completed. | Dec. 02 | On time/reduced |

## D. Description of deviations and subsequent adjustments of plans

*Only comprises deviations that are not included in the 2002 report.*
*D1, deviations in WP1.*
This WP is completed, no further deviations since 2002

*D2, deviations in WP2.*
The work was delayed during 2003 due to other tasks which could not be postponed, both for the picture preparation, which was completed in August 03, and for the statistical analysis of the results, which is not yet completed. However, the extent and content of the work has not changed.

*D3, deviations in WP3.*
None.

*D4, deviations in WP4.*
A few analyses of amino acids in the feed material has not yet been performed, and this has delayed the work with the manuscript accordingly, even though all other tasks were done approximately on time.
Due to delayed delivery of crops from the second harvest, diet preparation was delayed by approximately one month.

*D5, deviations in WP5.*
The analyses of feed material are completed, but the evaluation of the data is delayed due to paternity leave for a researcher, having taken into consideration that the information will not be needed until the end of 2003 anyway, due to the delays caused by the changed plans in WP7. This means that the work on bioavailability has been postponed to 2004 (with corresponding revisions of the budget for Risø).
The pesticide analyses were delayed due to minor logistic issues, they are expected to be finished within October 2003.

*D6, deviations in WP6.*
The start of the experiment in late 2002 experienced a minor delay due to delays in provision of the feed. The progress since then has been according to schedule.

*D7, deviations in WP7.*
The plans were changed slightly to make them fit better with other activities and provide better data than in the original plan. The investigations planned for animals from the first generation was changed to the third generation, and the health assessment and life expectancy studies were combined to a study using the parents from the second generation rather than young rats. This has made it necessary to transfer some funds from 2003 to 2004, as reflected in the budgets for DIAS and KVL.

*D8, deviations in WP8.*
The plans for the experiments were extensively changed as described above, which took longer than expected, but the delay was without consequences for the budget or the experimental work.

*D9, deviations in WP9.*
The plans were delayed as described above. The publication plan was postponed from the meeting in June 2003 until the next meeting, which will be held as soon as there is an overview of the trends in the results from WP6. This meeting is tentatively planned for January 2004.

*Dx, deviations in WPx.*
This WP is completed, no further deviations since 2002

## E. Project publications and other products

1. Articles in international, scientific journals with review procedures

Brandt, K. & Kidmose, U. 2002, ***Nutritional Consequences of Using Organic Agricultural Methods in Developing Countries.** In: Impacts of Agriculture on Human Health and Nutrition, edited by Cakmak I., Graham R.D., and Welch R.M., in Encyclopedia of Life Support Systems (EOLSS), Developed under the Auspices of the UNESCO, Eolss Publishers, Oxford, UK, [http://www.eolss.net]. Provided for the Organic Eprints website as doc-file.

Brandt, K. & Mølgaard, J.P., 2001. ****Organic agriculture: does it enhance or reduce the nutritional value of plant foods**? J. Sci. Food Agric. 81, 924-931. Provided for the Organic Eprints website as pdf-file.

2. Papers presented at congresses, symposiums etc.

Jensen, M.N, Halekoh, U., Brandt, K., Jegstrup, I.M., Ritskes-Hoitinga, M. 2003. **The effects of organically and conventionally cultivated plant feed on fertility and health in two inbred rat strains**. Oral presentation at Scandlas Meetings, Lahti in Finland 2003. Presentation provided for the Organic Eprints website as Powerpoint –file.

Brandt, K., Bügel, S.H., Ritskes-Hoitinga, M., Frøsig, L. et al. 2003. **Organic food and health – a multigeneration animal experiment.** Poster presented at the EFFoST conference "New Functional Ingredients and Foods - Safety, Health and Convenience" April 9-11 2003 in Copenhagen. Provided for the Organic Eprints website as Powerpoint-file.

Brandt, K., Ejlersen, A., Nørbæk, R. & Lindhard Petersen, H. 2003. ***Effects of Cultivation Conditions for Apples on Growth Rates of Fruit Fly Larvae and Contents of Phenolics.** Poster presented at the EFFoST conference "New Functional Ingredients and Foods - Safety, Health and Convenience" April 9-11 2003 in Copenhagen. Provided for the Organic Eprints website as Powerpoint -file.

Brandt K. 2002. **Organic food and human health**. Oral presentation at the LMC "Food Congress", 17-18 Jan. 2002. Abstract and presentation provided for the Organic Eprints website as doc and Powerpoint –files, respectively.

Christensen, T.F., Diedrichsen, B., Adsersen, A., Ravn, H.W., & Andersen, J.B. 2002. **Plant Biomarker Pattern, Screening Programme for Phytochemical Differences in Plants Exposed to Stress**. Poster presented at Natur og Miljøforskningskonferencen (The Conference on Research on Nature and the Environment), H.C. Ørsted Institutet, Copenhagen, 22-23- August 2002. Provided for the Organic Eprints website as pdf-file.

Brandt K., Nygaard Larsen H., Andersen J.-O., Mølgaard J.P., Lauridsen C., Jørgensen H., Gundersen V., Larsen E., Badsberg J.H. and Thorup-Kristensen K.. 2001. **Organic Food and Health: A new project to study the effects of plant cultivation methods (organic and conventional) on nutritional value, health and reproduction in an animal experiment.** Poster presented at "FOOD and NUTRITION for BETTER HEALTH" (HEALFO Conference), 13-15 June 2001, Lanciano, Italy. Provided for the Organic Eprints website as Powerpoint-file.

3. Reports, articles in agricultural journals, etc.

Brandt, K. and Kristensen, E.S. 2003. **Investigations of organic food and health.** Innovations in Food Technology 20, 68-69. Provided for the Organic Eprints website as pdf-file.

Slot, J., Andersen, T.S. and Kristensen, H.T. 2003. ***Sensorisk bedømmelse af ærter** (Sensory evaluation of peas). Report from a 3-week course in sensory evaluation at The Royal Veterinary and Agricultural University, taught by prof. Magni Martens, using material provided from the project. This report is not available in electronic form.

Nøddekær, T., Sandberg, C, Kreutzmann, S., and Albrechtsen, H.O. 2002. ***Sensorisk bedømmelse af gulerødder** (Sensory evaluation of carrots). Report from a 3-week course in sensory evaluation at The Royal Veterinary and Agricultural University, taught by prof. Magni Martens, using material provided from the project. This report is not available in electronic form.

Ravn, H.W., Christensen, T.F., Diedrichsen, B. 2002. **A new Phytochemical Screening Programme used for Organic and Conventional Crops**. M.Sc. thesis report from NERI and The Royal Danish School of Pharmacy, Dept. of Medicinal Chemistry, Copenhagen. Published on the Organic Eprints website as pdf-file.

4. Oral presentations, public meetings, field days, etc.

The project was presented at the “FØJO open house” event in Årslev on Aug. 7, 2003.

The part of the project that comprises cultivation of vegetables in 2002 was presented at the “FØJO open house” event in Årslev on Aug. 8, 2002.

The part of the project that comprises biocrystallisation analyses were presented at a visit of the Special Committee for Organic Agriculture and local organic committees, 65 persons in total, at Biodynamic Research Association, Herskind, on May 30, 2002

The part of the project that comprises cultivation of vegetables in 2001 was presented to representatives of the Danish Association of Vegetable Growers, including organic growers and consultants, during a field excursion on Aug. 16, 2001.

## F. Scientific education

The only Ph.D.-student presently involved in the project is Ulla Kidmose, who works on the project for approximately one month, analysing carotenoids, which is also the subject of her thesis work.

The project group comprises 4 post doctoral scientists (RN, IMJ, LF and JOA).

The M. Sc. student Marianne Nygaard Hansen, University of Southern Denmark, is affiliated with the project. Her thesis work regards male rat fertility evaluated from quantitative and qualitative measures of semen along with testicular histology. She makes use of the male rats after breeding is completed in each generation.

M. Sc. students Trine F. Christensen and Brigitte Diedrichsen, The Royal Danish School of Pharmacy, Dept. of Medicinal Chemistry and NERI, were affiliated with the project for their thesis on biomarkers visualised by TLC.

Apple material was used by B.Sc. student Astrid Ejlersen in experiments with the growth

rate of fruit flies reared on material made using different cultivation strategies. And her results were published together with some of the analyses of secondary metabolites in apples.

Material from the project (raw and processed carrots) was used in the experimental course “Sensory evaluation”, the Royal Veterinary and Agricultural University, June 2002, and peas from the project were used in the same course in June 2003 (see section E3).

## G. National and international cooperation

Together with project no. III.8, the project forms part of the basis for an EU-project for support for a concerted action on “Recommendations for improved procedures for securing consumer oriented food safety and quality of certified organic foods from plough to plate (Organic HACCP)”. This project, which is coordinated by Kirsten Brandt, started in February 2003 and will finish in January 2005. The project website is http://www.organichaccp.org.

Another related EU-project, the Integrated Project “Improving quality and safety and reduction of cost in the European organic and “low input” food supply chains” (QualityLowInputFood) is currently under contract negotiations, it is coordinated by the University of Newcastle upon Tyne in UK. In this project one of 7 subprojects is on investigations of impact of production systems on nutrient content, safety and health, which is coordinated by Kirsten Brandt, and part of this work incorporates concepts from the presently reported DARCOF project, as well as several other DARCOF activities.

DARCOF has become member of the International Organic FQH Research Association (http://www.organicfqhresearch.org/) with Kirsten Brandt as contact person.

For some of the harvested crops, and the animals from the experiments, some surplus is available for the possible use in other, related projects. Projects that may be established in parallel with the present one, or collaborators, including students, that are interested to carry out additional tests in a project with well-defined material. Five examples of such use are listed in section F, and a new project specifically intended to provide additional data on the material is described in section C1.

## Critical reflection on the project

Regarding the work and collaboration within the project:
This project is extremely multidisciplinary, comprising participants from 9 departments in 5 different institutes, with very different traditions and modes of work, and an interest in organic agriculture and health as the only common feature. In addition, it attempts to address a question that is notoriously difficult to answer, and where there is a high risk that the results will be used by different interest groups to support their different views, no matter what the results actually show. Still, and despite several significant problems in the first years of the project, we have managed to use the need for changes to develop the planning further in a positive way, so the project is now substantially better designed than when it started.

Regarding the perspectives of the work for society and science:

Even though the work is difficult, it is clearly very important that the question of the project is addressed, in a good scientific manner, because it is a field where well controlled data are very seriously needed. Without a project like this, the press and various interest groups will be completely unrestrained in their statements. If we succeed fully as planned, the project will provide a very significant contribution to the development of interdisciplinary research relevant for organic (and conventional) agriculture. And the overall design is now so robust that we can be certain that some of the results will be useful in their own right, even if some parts of the project may yield results that are difficult to interpret.

During the course of the project until now, the discussions at the meetings, and in particular the processes of preparing and receiving evaluations of the two applications that provided its funding, we have also become very aware of the limitations of the project, and how important it is not to extrapolate its data too much. Once it is finished, no matter what the results show, they will primarily be valuable as basis for further research, which should then be designed to dissect and quantify the correlations or differences we find in the project. We can provide an upper limit for an estimate of how much the differences among model cultivation systems could affect the health of rats. This will not tell us how much real cultivation systems affect the health of humans, only define which types and magnitudes of effects should be concentrated on. But due to its inclusion of both tropic levels, the results of this project will still be much better for this purpose than many other studies, which attempt to address this question by working only on part of the production chain, analysing only the outcome of primary production or that of market surveys.

# 8. Budget

## A. Account for any change in budgets

Since the submission of status report for 2002 the following budget changes have occurred:

1. Some funds at KVL, Institute of Human Nutrition, moved from 2003 to 2004 due to the postponement of 5 person-months of work. See details in D7

2. Some funds at DIAS, Department of Animal Nutrition and Physiology were moved from 2003 to 2004 due to the postponement of 3.7 person-months of work. See details in D7

3. Some funds at Risø were moved from 2003 to 2004 due to the postponement of 2.2 person-months of work. See details in D5

Since these changes are due to relatively minor delays, rather than actual changes in plans, and all revisions of plans have been within the WPs affected, these changes have no effect on the amount of funding for each workpackage, nor on the load or content of the work.

## B. Budget for the whole project (1.000 DKK)

Total consumption of funds from DARCOF and expected consumption this year and coming years

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 41 | 23.1 | 20.8 | 0 | 84.9 |
| Technical personnel | 28.3 | 31.9 | 10.7 | 0 | 70.9 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 1550 | 948 | 851 | 0 | 3349 |
| Technical personnel | 653 | 733 | 255 | 0 | 1641 |
| Other operational costs | 689 | 437 | 310 | 0 | 1436 |
| Equipment | 10 | 115 | 0 | 0 | 125 |
| Others (please specify) | 0 | 0 | 0 | 0 | 0 |
| Direct costs | 2903 | 2233 | 1416 | 0 | 6552 |
| Indirect costs (20% of direct costs) | 580 | 446 | 283 | 0 | 1310 |
| Total | 3483 | 2679 | 1699 | 0 | 7862 |

**Comments:**

# 9. Signatures and stamps

| Name | Institute | Date | Signature |
|---|---|---|---|
| Head of project | | | |

# Appendix I. Detailed budget

## A. Budget for each participating institute (1.000 DKr)

Name of Institute: Danish Institute of Agricultural Sciences

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 25.3 | 14.8 | 18.1 | 0 | 58.2 |
| Technical personnel | 21.3 | 18.5 | 4 | 0 | 43.8 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 988 | 621 | 742 | 0 | 2351 |
| Technical personnel | 494 | 388 | 79 | 0 | 961 |
| Other operational costs | 545 | 357 | 271 | 0 | 1173 |
| Equipment | 0 | 20 | 0 | 0 | 20 |
| Others (please specify) | 0 | 0 | 0 | 0 | 0 |
| Direct costs | 2028 | 1386 | 1092 | 0 | 4506 |
| Indirect costs (20% of direct costs) | 405 | 277 | 219 | 0 | 901 |
| Total | 2433 | 1663 | 1311 | 0 | 5407 |

**Comments:**

Name of Institute: The Royal Veterinary and Agricultural University

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 8.7 | 1 | 1.3 | 0 | 11 |
| Technical personnel | 0 | 4 | 5 | 0 | 9 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 322 | 39 | 50 | 0 | 411 |
| Technical personnel | 0 | 113 | 121 | 0 | 234 |
| Other operational costs | 38 | 20 | 34 | 0 | 92 |
| Equipment | 0 | 0 | 0 | 0 | 0 |
| Others (please specify) | 0 | 0 | 0 | 0 | 0 |
| Direct costs | 360 | 172 | 205 | 0 | 737 |
| Indirect costs (20% of direct costs) | 72 | 35 | 41 | 0 | 148 |
| Total | 432 | 207 | 246 | 0 | 885 |

**Comments:**

Name of Institute: Risø National Laboratory

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 1 | 1 | 0.5 | | 2.5 |
| Technical personnel | 1 | 2 | 1.7 | | 4.7 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 42 | 44 | 22 | | 108 |
| Technical personnel | 33 | 65 | 55 | | 153 |
| Other operational costs | 0 | 14 | 0 | | 14 |
| Equipment | 10 | 95 | 0 | | 105 |
| Others (please specify) | 0 | 0 | 0 | | 0 |
| Direct costs | 85 | 218 | 77 | | 380 |
| Indirect costs (20% of direct costs) | 17 | 44 | 15 | | 76 |
| Total | 102 | 262 | 92 | | 456 |

**Comments:**

Name of Institute: University of Southern Denmark

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 6 | 6.3 | 0.9 | | 13.2 |
| Technical personnel | 6 | 7.4 | 0 | | 13.4 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 198 | 244 | 37 | | 479 |
| Technical personnel | 126 | 167 | 0 | | 293 |
| Other operational costs | 106 | 46 | 5 | | 157 |
| Equipment | 0 | 0 | 0 | | 0 |
| Others (please specify) | 0 | 0 | 0 | | 0 |
| Direct costs | 430 | 457 | 42 | | 929 |
| Indirect costs (20% of direct costs) | 86 | 91 | 8 | | 185 |
| Total | 516 | 548 | 50 | | 1114 |

**Comments:**

## B. Budget for each participating department (1.000 DKK)

Name of Institute and department: Danish Institute of Agricultural Sciences, Department of Food Science

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 15.4 | 4.4 | 1.6 | | 21.4 |
| Technical personnel | 10.6 | 2.3 | 0 | | 12.9 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 597 | 196 | 86 | | 879 |
| Technical personnel | 250 | 59 | 0 | | 309 |
| Other operational costs | 360 | 117 | 7 | | 484 |
| Equipment | 0 | 0 | 0 | | 0 |
| Others (please specify) | 0 | 0 | 0 | | 0 |
| Direct costs | 1208 | 372 | 93 | | 1673 |
| Indirect costs (20% of direct costs) | 242 | 74 | 19 | | 335 |
| Total | 1449 | 446 | 112 | | 2007 |

**Comments:**

Name of Institute and department: Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 4 | 7 | 11.1 | | 22.1 |
| Technical personnel | 4 | 16.2 | 4 | | 24.2 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 142 | 262 | 384 | | 788 |
| Technical personnel | 85 | 329 | 79 | | 493 |
| Other operational costs | 60 | 230 | 255 | | 545 |
| Equipment | 0 | 20 | | | 20 |
| Others (please specify) | 0 | 0 | 0 | | 0 |
| Direct costs | 287 | 841 | 718 | | 1846 |
| Indirect costs (20% of direct costs) | 57 | 168 | 144 | | 369 |
| Total | 344 | 1009 | 862 | | 2215 |

**Comments:**

Name of Institute and department: Danish Institute of Agricultural Sciences, Department of Agricultural Systems

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 2.9 | 0 | 0 | | 2.9 |
| Technical personnel | 6.7 | 0 | 0 | | 6.7 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 110 | 0 | 0 | | 110 |
| Technical personnel | 159 | 0 | 0 | | 159 |
| Other operational costs | 117 | 0 | 0 | | 117 |
| Equipment | 0 | 0 | 0 | | 0 |
| Others (please specify) | 0 | 0 | 0 | | 0 |
| Direct costs | 386 | 0 | 0 | | 386 |
| Indirect costs (20% of direct costs) | 77 | 0 | 0 | | 77 |
| Total | 463 | 0 | 0 | | 463 |

**Comments:**

Name of Institute and department: Danish Institute of Agricultural Sciences, Department of Animal Breeding and Genetics

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 3 | 3.4 | 5.4 | | 11.8 |
| Technical personnel | 0 | 0 | 0 | | 0 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 139 | 163 | 272 | | 574 |
| Technical personnel | 0 | 0 | 0 | | 0 |
| Other operational costs | 8 | 10 | 9 | | 27 |
| Equipment | 0 | 0 | 0 | | 0 |
| Others (please specify) | 0 | 0 | 0 | | 0 |
| Direct costs | 147 | 173 | 281 | | 601 |
| Indirect costs (20% of direct costs) | 29 | 35 | 56 | | 120 |
| Total | 177 | 208 | 337 | | 722 |

**Comments:**

Name of Institute and department: The Royal Veterinary and Agricultural University, Research Department of Human Nutrition

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 0 | 0 | 1.3 | | 1.3 |
| Technical personnel | 0 | 4.0 | 5.0 | | 9.0 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 0 | 0 | 50 | | 50 |
| Technical personnel | 0 | 113 | 121 | | 234 |
| Other operational costs | 0 | 15 | 34 | | 49 |
| Equipment | 0 | 0 | 0 | | 0 |
| Others (please specify) | 0 | 0 | 0 | | 0 |
| Direct costs | 0 | 128 | 205 | | 333 |
| Indirect costs (20% of direct costs) | 0 | 26 | 41 | | 67 |
| Total | 0 | 154 | 246 | | 400 |

**Comments:**

Name of Institute and department: The Royal Veterinary and Agricultural University, Organic Farming Unit

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 8.7 | 1 | 0 | | 9.7 |
| Technical personnel | 0 | 0 | 0 | | 0 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 322 | 39 | 0 | | 361 |
| Technical personnel | 0 | 0 | 0 | | 0 |
| Other operational costs | 38 | 5 | 0 | | 43 |
| Equipment | 0 | 0 | 0 | | 0 |
| Others (please specify) | 0 | 0 | 0 | | 0 |
| Direct costs | 360 | 44 | 0 | | 404 |
| Indirect costs (20% of direct costs) | 72 | 9 | 0 | | 81 |
| Total | 432 | 53 | 0 | | 485 |

**Comments:**

## C. Budget for co-financing from each participating institute (1.000 DKK)

Name of Institute: National Environmental Research Institute

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Man-months | | | | | |
| Scientific personnel | 2 | 0 | 0 | 0 | 2 |
| Technical personnel | 3 | 0 | 0 | 0 | 3 |

| Year: | Consumption before 2003 | Expected consumption 2003 | 2004 | 2005 | Total |
|---|---|---|---|---|---|
| Salaries | | | | | |
| Scientific personnel | 90 | 0 | 0 | | 90 |
| Technical personnel | 75 | 0 | 0 | | 75 |
| Other operational costs | 50 | 0 | 0 | | 50 |
| Equipment | 0 | 0 | 0 | | 0 |
| Others (please specify) | 0 | 0 | 0 | | 0 |
| Direct costs | 215 | 0 | 0 | | 215 |
| Indirect costs (20% of direct costs) | 43 | 0 | 0 | | 43 |
| Total | 258 | 0 | 0 | | 258 |

**Comments: This was partly financed from NERI basal funds, since an anticipated external funding source was reduced, after the work was started, and the first set of results produced. The value of the work of 2 students is not included.**