Danish Research Centre for Organic Farming

Midterm Status Report 2002 and Application for Continuation in 2003

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The Directorate for Food, Fisheries and Agro Business
under the Danish Ministry of Food, Agriculture and Fisheries

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1. Research program

Research in organic farming 2000-2005 (DARCOF II)

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2. Project title and number

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

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3. Head of project

Kirsten Brandt (KB), Ph.D., senior scientist
Department of Horticulture, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, 5792 Årslev.
Tlf.: 6390 4343, fax.: 6390 4395, e-mail: kirsten.brandt@agrsci.dk

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4. Participating institutes

Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, 5792 Årslev
Tlf.: 63 90 43 43, fax.: 63 90 43 95

The Royal Veterinary and Agricultural University, Rolighedsvej 30, 1958 Frederiksberg C, Tlf.: 35 28 24 90, fax.: 35 28 24 83/24 69

Risø National Laboratory, Building 201, Postboks 49, 4000 Roskilde
Tel: 46 77 53 40, Fax: 46 77 41 93

University of Southern Denmark, Winsløwparken 23, 5000 Odense C
Tel.: 65 50 37 27, Fax: 65 90 68 21

National Environmental Research Institute
Vejlsøvej 25, P.O. Box 314, DK-8600 Silkeborg,
Tel.: 89 20 17 53, Fax: 89 20 14 13

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5. Other project staff

Danish Institute of Agricultural Sciences,
Department of Horticulture, Kirstinebjergvej 10, 5792 Årslev: Rikke Nørbæk (RN), Ph.D., scientist, Hanne Lindhard Petersen (HLP), Ph.D., senior scientist, Ulla Kidmose (UK), scientist and Kristian Thorup-Kristensen (KTK), Ph.D., senior scientist.

Department of Crop Physiology and Soil Science, P.O. Box 50, 8830 Tjele: Jens Peter Mølgaard (JPM), Ph.D., senior scientist and Uffe Jørgensen (UJ), Ph.D., senior scientist.

Department of Agricultural Systems, P.O. Box 50, 8830 Tjele: Ulrich Halekoh (UH), Ph.D., scientist.

Department of Animal Nutrition and Physiology, P.O. Box 50, 8830 Tjele: Charlotte Lauridsen (CL), Ph.D., senior scientist and Henry Jørgensen (HJ), Ph.D., senior scientist.

The Royal Veterinary and Agricultural University,
Research Department of Human Nutrition, Rolighedsvej 30, 1958 Frederiksberg C: Susanne Højbjerg Bügel (SHB), Ph.D., associate professor

Organic Farming Unit, Agrovej 10, 2630 Tåstrup:
Jens-Otto Andersen (JOA), Ph.D., research assistant.

University of Southern Denmark,
Biomedical Laboratory, Winsløwparken 23, 5000 Odense C: Merel Ritskes-Hoitinga (MR), professor, Inger Marie Jegstrup (IMJ), Ph.D., research assistant.

Risø National Laboratory,
Radiation Research Department, Building 201, P.O. Box 49, DK-4000 Roskilde: Lars Frøsig (LF), Ph.D., post doc., and Lis Vinther Kristensen (LVK), chief laboratory technician.

National Environmental Research Institute,
Department of Terrestrial Ecology, Vejlsøvej 25, P.O. Box 314, DK-8600 Silkeborg: Helle Weber Ravn (HR), Ph.D., senior scientist.

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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 2003

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 material produced in one growth season. However, with only one growth season the results would only be applicable to this particular year. So 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. The combined project will now allow a statistical comparison of the fertility in the 2nd generation of rats, raised on feed from 2001, with that of the 3rd generation, raised on feed produced in 2002.

A major task in 2002 has been to use statistical procedures to refine, and where relevant, revise, the detailed plans for the animal experiment, that will start up in the last month of the year. In particular for parts where reviewers had pointed out the need for this.

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, wheat was planned to be ground and baked to bisquits, oil produced from the rapeseed, and raw carrots and apples are shredded and freeze dried.
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 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.

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 150 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 also to what extent supplementation of single essential minerals will be necessary to avoid deficiencies.

WP6 Management and recording of feeding and breeding
Newly weaned rats are fed with the 3 feed mixtures, and bred for 3 generations. The first two generations will be raised on plant materials grown in 2001, while the third generation will receive feed from 2002-material. The number and genotypes of rats will depend on ongoing pilot experiments, where a few rats from each of 5 commonly used strains are bred under similar conditions, to estimate the levels and variability for litter size and chance for pregnancy. The ZDF-rat, which develops obesity and diabetes at a rate comparable to some human populations, could be particularly suitable for this project. Negotiations are in progress with the supplier in an attempt to obtain permission to breed this rat strain, which is normally not released for breeding. 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. 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.

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. Presently models for a discrimination analysis of the biocrystallization pictures is being developed, as well as the structure for the database for WP7

WP9 Coordination, dissemination and evaluation
Coordination and management of the project, primarily through periodic meetings and progress reports. Publications and other dissemination activites are planned and coordinated to maximise precision and extent of the impact of the results.

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 genera-

tion.

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 harvest 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, polyacetylenes, 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 corresponding data from the 2002 harvest become available.

For the crops potato, pea, kale, wheat and carrot, 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 harvest.

TLC biomarker patterns were produced from potato, pea, kale, apple and carrot, 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 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.

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 [e]

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 [e]	Achieved in 2001, on time for 2002.
D1.2 The processed materials.	March 02 and March 03 [e]	Most crops OK, the wheat was lost (burned!).
Milestones		
M1.1 The materials are harvested	Nov. 01 and Nov. 02 [e]	Achieved in 2001, on time for 2002.
M1.2 The materials are processed.	March 02 and March 03 [e]	On time or somewhat delayed for most crops, except wheat

** Deviations are to be further discussed in D1*

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 [e]	On time
D2.2 Contribution to scientific paper(s) on the correlation between picture-forming characteristics and other data from the project.	Dec. 04	-
Milestones		
M2.1 The analyses are completed.	April 03 [e]	On time

** Deviations are to be further discussed in D2*
- Too early to asses if deviations are going to occur

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	-
Milestones		
M3.1 The analyses are completed.	Aug. 03 [e]	On time

** Deviations are to be further discussed in D3*
- Too early to asses if deviations are going to occur

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	-
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*
- Too early to asses if deviations are going to occur

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 WP8.	Dec. 02	On time
D5.2 (month 20): Data on contents of pesticides in each of the diets.	Dec. 02 and Aug. 03 [e]	On time
Milestones		
M5.1 (Month 20): The analyses are completed.	Dec. 02 and Aug. 03 [e]	On time

** 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 [e]	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.	Dec. 04	- (life expectancy study changed)
Milestones		
M7.1 The tests on the first generation rats are completed.	June 03	Delayed by approx-imately 1 month
M7.2 The tests on the second generation rats are com-pleted.	Oct. 03	Delayed by approx-imately 1 month
M7.3 The life expectancy and occurrence of diseases of the second generation rats are characterised.	Dec. 04	-

** 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	On time/delayed, de-pending on availability of data
D8.3 Report or inputs to papers, with the relevant statisti-cal analyses.	Aug. 04	-
Milestones		
M8.1 Detailed plan for experiments and exchange of ma-terials and data is prepared.	Feb. 02	On time (changed to WP 9)
M8.2 Optimised plan for the animal experiments is pre-pared.	Oct. 02	On time/delayed, de-pending on availability of data

** Deviations are to be further discussed at in D*
- 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	-
D9.5 Progress meeting M5	Feb. 04	-
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 1 month
M9.2 Publication plan is prepared.	April 03	-
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 [e]	On time/reduced
Milestones		
Mx.1 The analyses are completed.	Dec. 02 [e]	On time/reduced

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

D. Description of deviations and subsequent adjustments of plans

D1, deviations in WP1.
For processing, the wheat from all 3 treatments was milled, and the flour was taken to a biscuit factory for the preparation and baking of biscuits from it. However, a technical error occurred, which caused the oven to overheat and catch fire, and all material from one of the treatments was lost. We considered possible actions and decided to continue the project plans based on the use of only the crops that were successfully processed (potatoes, cabbage, carrots, apples, peas).

In October – November 2001 problems appeared for Hanne Nygaard Larsens position at The Royal Veterinary and Agricultural University, Research Department of Human Nutrition. Due to a new strategy the Department requested that this part of the project be moved to another institute, and during this process Hanne Nygaard Larsen decided to leave the project for another job.
In relation to WP1 this delayed the calculation of the amount of material that was due to be processed, and therefore most of the processings in 2001-2002 were delayed by approx. 2 months.

Another problem concerned the second year of crop production, where it turned out to be impossible to arrange the relevant cultivation conditions for oilseed rape, so we could not grow a second crop of rape. However, after the recalculation mentioned above, it turned out that the amount of rapeseed oil produced in the first year was adequate to meet the needs of the entire project. So part of this oil will be stored and used for the second batch of material. Due to this we will not be able to test for differences between years in effects that are solely due to the rapeseed oil. We consider this a minor and reasonable risk, since rapeseed is the only crop where only part of the material is used, and this already prevents us from detecting any consequences of most relevant effects of growing conditions, such as differences in contents of oil or antinutritional glucosinolates.

All in all the production of enough material to meet the needs of the project was achieved with a delay of a few months. These problems resulted in some changes in composition of the experimental diets, which are unlikely to affect the conclusions of the entire project.

D2, deviations in WP2.
While most of the pictures made from carrots, kale, potatoes and peas were single-centered, the sample preparation procedure for the 2001 apple samples resulted in multi-centered pictures, which are known not to be suitable for visual nor instrumental evaluation. Thus it would not be possible to obtain useful comparisons, neither within the 2001 data nor with data from the 2002 harvest, so in 2002 apples were deleted from the analysis programme, as well as the wheat analyses originally planned. Instead additional analyses of the other crops will be carried out in 2002, including a study of the repeatability of the extraction procedure used, and one of the effects of freeze-drying on carrot pictures.

D3, deviations in WP3.
Since the measurements are made on the processed material, the start of the analyses was delayed due to the delayed processing. And when the material was ready for analysis, the responsible scientist, Erik Larsen, resigned from his job. However, the tasks have been taken over by two other scientists in the same research group, Rikke Nørbæk and Ulla Kidmose, and several minor tasks will be done by other scientists, so the work

is now progressing quickly, and we expect the results to become available within the period originally foreseen.

D4, deviations in WP4.
Despite the delays in processing, the materials were delivered to this WP almost at the planned time. However, then the supplier of the rat strain used for this experiment experienced disease problems, and was therefore unable to deliver as frequent as usual. Combined with a structural reorganisation of the department (Department for Analytical Chemistry) that analyses the samples for assessment of biological value and nutritional content of the cultivated crops, a delay of approximately 1 month is expected to have accumulated when the work is finished in November 2002.

D5, deviations in WP5.
According to the original plan, the analyses of minerals and pesticides should not be done before the feed has been mixed, after the completion of WP4. However, in order to optimise the planning and work of WP4 and WP7, and prevent further delays, we have decided to measure minerals in each crop, rather than just in the mixtures, and for this reason some work has already started in this WP, actually ahead of schedule.

It should be mentioned that the scientist originally responsible for this WP, Vagn Gundersen, has had to leave his working area and thus the project, due to a changed strategy at Risoe. His scientific responsibilities are taken over by Susanne Højbjerg Bügel. The laboratory work will still be made by Vagns assistant Lis Vinther Kristensen, as originally planned, together with post doc. Lars Frøsig. This has caused some changes in the distribution of funds between Risø and The Royal Veterinary and Agricultural University, see the section on budget changes.

D6, deviations in WP6.
This WP was supposed to have been the central part of Hanne Nygaard Larsens work. When she left the project, and the department did not want to provide another scientist for the work, several other possibilities were considered, and the choice fell on the Biomedical Laboratory (BL) of University of Southern Denmark. BL tends laboratory animals for all parts of the University, and carries out research in relation to the use of animals as experimental models for human physiology and diseases, so it has all relevant facilities and expertise for this task. This was also confirmed by external reviewers in relation to the application for supplementary funding, where BL was named as responsible.
The work suffers minor delay due to the delayed processing as well as most of the other WPs, but some preliminary work on selection of the optimal strain or strains of rats to use is now in progress, and the work will start up as soon as the feed is ready later this year.

D7, deviations in WP7.
The parts of this WP that Hanne Nygaard Larsen should have taken care of has been transferred to DIAS, where it will be carried out by Charlotte Lauridsen and Henry Jørgensen, as described in the section on budget changes. The work will be initiated when weaned rats of second generation from WP6 are available.
Regarding the planned life expectancy study, due to the delay and limitation in feed resources, as well as the question of obtaining enough experimental units (animals) per treatment, the setup will be changed, as it was also recommended by reviewers. The original plan was to feed relatively few rats (10) for a maximum of 2 years on each of the respective diets. The recordings consisted of registration after death of abnormalities and signs of diseases, including signs of cancer and vascular diseases. In order to fulfil the purpose of this work optimally, we will make use of the rats that will be raised for the

other studies. We plan to keep a selected group of rats for a longer period than originally planned for the other (non-invasive) experiments, the length of the period depending on availability of experimental diets as well as rat strain (preferring rats that quickly develop age related ailments). By using non-invasive methods, such as ^{13}C-infrared isotope analyser for monitoring function of vital organs and intestinal flora, as well as analysis of nutritional status and biomarkers indicative of health status in blood samples, we will provide measures of the onset of physiological disorders without killing the animals. This comprises postponing the macroscopic analysis of the organs and analysis of biomarkers etc. in of tissue samples until the non-destructive data are accumulated, and any differences in general health are likely to become increasingly defined as the animals age. This revised study design will answer the question regarding the influence of the respective diets on the listed aspects of health status and general well-being of the animals at different age using the same animal material, and thus more useful data in a shorter time and without added costs.

D8, deviations in WP8.
Due to other obligations Jens Henrik Badsberg, who was originally responsible for this WP, first delayed his engagement (as described in the status report from 2001) and later completely withdrew from the project. His colleague Ulrich Halekoh has taken over the work, and is working with the data as they turn up. However, this is also affected by the delays in other parts of the project, so it is requested that the person-months that were originally planned for 2001, and then transferred to 2002, are transferred further to 2004, which is when they will most likely be needed. This will ensure the placing of the most intensive effort during the period when the data on the results are being processed and published.

D9, deviations in WP9.
This WP is progressing on time, the planned meetings are being held, and at times a lot of work done to ensure the functioning of the project, in spite of changes in personnel and other problems that require coordination beyond the standard administration of a project.

Dx, deviations in WPx.
The participation of NERI was planned to be financied by a company Biotech Line AS, according to an agreement between this company and NERI. However, the budget was reduced, so the work presented in the workplan was reduced to consist of a screening fresh plant material of apples, potatoes, kale, peas and carrots from the 2001 harvest, while screening of fresh plant material obtained in 2002 can not be performed. Still, NERI has completed the work that was in progress, even before time, to a large extent based on the work of two students.

E. Project publications and other products

1. Articles in international, scientific journals with review procedures

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 DARCOF website as pdf-file.

2. Papers presented at congresses, symposiums etc.

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. Abstract available at:

http://www.cmns.mnegri.it/en/congres/healfo/selected/files11.htm

Brandt K. 2002. **Organic food and human health**. Oral presentation at the LMC "Food Congress", 17-18 Jan. 2002. Abstract available at:
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Ravn, H.W., Christensen, T.F., Diedrichsen, B., Kristensen, C.V., Jensen, A.K., Husted, C.L. Lindhard Petersen, H., Brandt, K. & Andersen, J.B. 2002. **Plant Biomarker Pattern, Apples grown with various availability of organic nitrogen and with or without the use of pesticides**. Poster, to be presented in relevant contexts. Provided for the DARCOF website as ppt-file.

Brandt, K., Bügel, S.H., Ritskes-Hoitinga, M., Frøsig, L. et al. 2003. **Organic food and health – a multigeneration animal experiment.** Poster abstract submitted for the EFFoST conference "New Functional Ingredients and Foods - Safety, Health and Convenience" to be held in March 2003 in Copenhagen. Provided for the DARCOF website as doc-file if accepted.

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 abstract submitted for the EFFoST conference "New Functional Ingredients and Foods - Safety, Health and Convenience" to be held in March 2003 in Copenhagen. Provided for the DARCOF website as doc-file if accepted.

3. Reports, articles in agricultural journals, etc.

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 The Royal Veterinary and Agricultural University, taught by prof. Magni Martens, using material provided from the project.

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. Provided for the DARCOF website as pdf-file.

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

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.

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

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 Sabine Jensen, University of Southern Denmark and DIAS, is affiliated with the project. Her thesis work regards the measurement of degradation products of glucosinolates in cruciferous vegetables, and she will analyse the kale material produced in the project for those compounds.

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.

Material from the project (raw and processed carrots) was used in the experimental course "Sensory evaluation", the Royal Veterinary and Agricultural University, June 2002. It is expected that this will be repeated in 2003.

Apple material is 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 will be published together with some of the analyses of secondary metabolites in apples.

G. National and international cooperation

The project forms part of the basis for an EU-project currently under contract negotiation with the European Commission, 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 is expected to start in December 2002 or January 2003.
A Nordic workshop on quality in organic agriculture was arranged to take place in Stange, Norway 22.-24.10.2001, with additional support from NKJ. However, due to an insufficient number of participants it was cancelled, although there is still a possibility that

it will be held at a later date.

For some of the harvested crops, some surplus plant material is saved 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. Three examples of such use are listed in section F.

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 mode of work, and an interest in organic agriculture 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 they actually show. And on top of this, the project seems to exert some strange attraction of accidents and unfortunate coincidences, which make it even more complicated than it had to be. Until now we have been able to use the buffers and partial redundancies of the design, which were included for this purpose, to minimise the impact of the various difficulties on the overall project, and have also experienced a few cases of good fortune. However, there is not much more buffer to use, so from now on major changes in personnel or losses of materials will hopefully be avoided.

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. So we simply have to solve the problems as they appear, and stay on the track. 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 hopefully robust enough to ensure that also partial results will be useful in their own right, even if some other part of the project is not completely successful.

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 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 it will still be much better for this than the many 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

The following budget changes have ocurred:

1. Vagn Gundersens departure from the project caused a transfer of funds to SHB (for bioavailability work) and KB (for subcontracting of pesticide analyses). Details in section D5.

2. Hanne Nygaard Larsens departure from the project caused a transfer of funds to MR (for WP6) and CL+HJ (for WP7). See details in sections D1, D6 and D7.

3. Delays in data inputs caused a postponement of 2 months of work in WP8. Details in section D8

Since most changes are due to redistributions of responsibilities for work, 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.

4. NERI's contribution is reduced (affecting only self-funded activities). Details in section Dx.

B. Budget for the whole project (1.000 DKK)

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

Year:	Consump-tion before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	10.0	31.0	28.5	15.3		84.9
Technical personnel	7.0	21.3	37.3	5.3		71.0

Year:	Consump-tion before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	386	1165	1145	653		3349
Technical personnel	161	491	872	116		1640
Other operational costs	163	526	591	156		1436
Equipment	0	10	115	0		125
Others (please specify)	0	0	0	0		0
Direct costs	710	2193	2723	925		6550
Indirect costs (20% of direct costs)	142	439	545	185		1310
Total	852	2631	3267	1110		7860

Comments: The budget is the sum of two separate contracts, one on 6.000.000 dkr. and one on 1.860.000 dkr.

9. Signatures and stamps

Name	Institute	Date	Signature
Head of project Kirsten Brandt	Danish Institute of Agri-cultural Sciences		

Appendix I. Detailed budget

A. Budget for each participating institute (1.000 DKr)

Name of Institute: Danish Institute of Agricultural Sciences

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	7.0	18.3	18.5	14.4		58.1
Technical personnel	7.0	14.3	18.5	4.0		43.8

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	273	715	746	617		2351
Technical personnel	161	333	387	79		961
Other operational costs	148	397	481	147		1173
Equipment	0	0	20	0		20
Others (please specify)	0	0	0	0		0
Direct costs	582	1446	1634	843		4505
Indirect costs (20% of direct costs)	116	289	327	169		901
Total	698	1735	1961	1011		5406

Name of Institute: The Royal Veterinary and Agricultural University

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	3.0	5.7	2.3	0.0		11.0
Technical personnel	0.0	0.0	7.7	1.3		9.0

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	113	209	89	0		412
Technical personnel	0	0	197	37		234
Other operational costs	15	23	50	4		92
Equipment	0	0	0	0		0
Others (please specify)	0	0	0	0		0
Direct costs	128	232	336	41		737
Indirect costs (20% of direct costs)	26	46	67	8		147
Total	153	279	404	49		885

Name of Institute: Risø National Laboratory

Year:	Con-sumption before 2002	Expected consump-tion 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	0.0	1.0	1.5	0.0		2.5
Technical personnel	0.0	1.0	3.7	0.0		4.7

Year:	Con-sumption before 2002	Expected consump-tion 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	0	42	66	0		108
Technical personnel	0	33	120	0		153
Other operational costs	0	0	14	0		14
Equipment	0	10	95	0		105
Others (please spec-ify)	0	0	0	0		0
Direct costs	0	85	296	0		380
Indirect costs (20% of direct costs)	0	17	59	0		76
Total	0	102	355	0		457

Name of Institute: University of Southern Denmark

Year:	Con-sumption before 2002	Expected consump-tion 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	0	6.0	6.3	0.9		13.2
Technical personnel	0	6.0	7.4	0.0		13.4

Year:	Con-sumption before 2002	Expected consump-tion 2002	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 spec-ify)		0	0	0		0
Direct costs		430	457	42		928
Indirect costs (20% of direct costs)		86	91	8		186
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 Horticulture

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	7.0	8.4	4.4	1.6		21.3
Technical personnel	4.0	6.6	2.3	0.0		12.9

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	273	324	196	86		880
Technical personnel	92	158	59	0		309
Other operational costs	133	227	117	7		484
Equipment	0	0	0	0		0
Others (please specify)	0	0	0	0		0
Direct costs	498	710	372	93		1672
Indirect costs (20% of direct costs)	100	142	74	19		334
Total	598	851	446	112		2007

Name of Institute and department: Danish Institute of Agricultural Sciences, Department of Crop Physiology and Soil Science

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	0.0	4.0	10.7	7.4		22.1
Technical personnel	0.0	4.0	16.2	4.0		24.2

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	0	142	387	259		788
Technical personnel	0	85	329	79		493
Other operational costs	0	60	354	131		545
Equipment	0	0	20	0		20
Others (please specify)	0	0	0	0		0
Direct costs	0	287	1090	469		1846
Indirect costs (20% of direct costs)	0	57	218	94		369
Total	0	345	1307	563		2215

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

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	0.0	2.9	0.0	0.0		2.9
Technical personnel	3.0	3.7	0.0	0.0		6.7

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	0	110	0	0		110
Technical personnel	69	90	0	0		159
Other operational costs	15	102	0	0		117
Equipment	0	0	0	0		0
Others (please specify)	0	0	0	0		0
Direct costs	84	302	0	0		386
Indirect costs (20% of direct costs)	17	60	0	0		77
Total	101	362	0	0		463

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

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	0.0	3.0	3.4	5.4		11.8
Technical personnel	0.0	0.0	0.0	0.0		0.0

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	0	139	163	272		574
Technical personnel	0	0	0	0		0
Other operational costs	0	8	10	9		27
Equipment	0	0	0	0		0
Others (please specify)	0	0	0	0		0
Direct costs	0	147	173	281		601
Indirect costs (20% of direct costs)	0	29	35	56		120
Total	0	177	208	337		721

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

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	0	0.0	1.3	0.0		1.3
Technical personnel	0	0.0	7.7	1.3		9.0

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	0	0	50	0		50
Technical personnel	0	0	197	37		234
Other operational costs	0	0	45	4		49
Equipment	0	0	0	0		0
Others (please specify)	0	0	0	0		0
Direct costs	0	0	292	41		333
Indirect costs (20% of direct costs)	0	0	58	8		67
Total	0	0	351	49		400

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

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	3.0	5.7	1.0	0.0		9.7
Technical personnel	0.0	0.0	0.0	0.0		0.0

Year:	Consumption before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	113	209	39	0		361
Technical personnel	0	0	0	0		0
Other operational costs	15	23	5	0		43
Equipment	0	0	0	0		0
Others (please specify)	0	0	0	0		0
Direct costs	128	232	44	0		404
Indirect costs (20% of direct costs)	26	46	9	0		81
Total	153	279	53	0		485

Comments:

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

Name of Institute: National Environmental Research Institute

Year:	Consump-tion before 2002	Expected consumption 2002	2003	2004	2005	Total
Man-months						
Scientific personnel	0	2	0	0	0	2
Technical personnel	0	3	0	0	0	3

Year:	Consump-tion before 2002	Expected consumption 2002	2003	2004	2005	Total
Salaries						
Scientific personnel	0	90.000	0	0		90.000
Technical personnel	0	75.000	0	0		75.000
Other operational costs	0	50.000	0	0		50.000
Equipment	0	0	0	0		0
Others (please specify)	0	0	0	0		0
Direct costs	0	215.000	0	0		215.000
Indirect costs (20% of direct costs)	0	43.000	0	0		43.000
Total	0	258.000	0	0		258.000

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.