

Application for funding – FØJO II supplementary, Project VII.10 – November 2001

Title: Extension of the project “Organic food and health – a multigeneration animal experiment” with an additional cultivation season and an additional generation.

Acronym: Extension of “Organic health”

Date: Nov. 26th 2001

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Summary in Danish:*Problemformulering og baggrund*

Ved udarbejdelsen af ansøgningen til det igangværende projekt III.4, "Økologisk kost og sundhed - et flergenerations dyreforsøg" (se projektbeskrivelse vedlagt som appendix 3) har vi fra starten været opmærksom på det uheldige i, at det ikke var lykkedes at få plads til en gentagelse af dyrkningsforsøget. Derfor er der ikke mulighed for at afgøre, om de opnåede resultater vil være udtryk for generelle tendenser eller blot for tilfældige forskelle mellem afgrøderne i det ene dyrkningsår. Tilfældigt opståede forskelle mellem fodertyperne kan ikke opvejes af, at der under selve dyreforsøget er gentagelser i form af et passende stort antal rotter. På den anden side blev det bedømt som en alt for stor omkostning at medtage en sådan gentagelse, da det under den aktuelle budgetramme ville have betydet, at der stort set ikke havde været mulighed for registreringer af dyrenes tilstand og sundhed.

Formål

Formålet med projektet er at gentage dyrkningen af foderafgrøder i et senere dyrkningsår, og karakterisere disse afgrøder i et sådant omfang, at det vil være muligt at benytte resultaterne til at bedømme reproducerbarheden af resultaterne af det samlede forsøg. Herunder relevante analyser af materialet og relevante fodringsforsøg med rotter.

Metode

Dyrkning og foderfremstilling: Som i projekt III.4, WP 1, blot på mindre arealer. D.v.s. en model af et økologisk dyrkningssystem (organisk gødning i begrænsede mængder, ingen pesticider), af et konventionelt (mineralsk gødning i anbefalede mængder, med pesticider), og en faktoriel kombination (organisk gødning i begrænsede mængder, med pesticider).

Karakterisering: Som udgangspunkt udføres samme analyser som i III.4, WP 2-5, omfattende biokrystallisation, indhold og biologisk værdi af næringsstoffer, type og indhold af mineraler, pesticider og sekundære metabolitter. Dog vil der ske reduktion i antallet af analyser i det omfang resultaterne fra første dyrkningsår viser at det er fagligt forsvarligt.

Fodringsforsøg: Det frembragte foder vil blive benyttet til at forlænge reproduktionsforsøget med yderligere en generation, i sidste halvdel af 2003, og de samme registreringer vil blive udført som laves på rotterne i hver af de andre generationer, f.eks. frugtbarhed og tilvækst.

*Tidsplan**År 1 (2002):*

Dyrkning af afgrøder: vårhvede, kartofler, vinterraps, gulerødder, grønkål, ærter til modenhed og æbler. Stabilisering i form af frysning, tørring etc. Analyse af biokrystallisation, andre analyser påbegyndes.

År 2 (2003):

Analyser afsluttes og foderblandinger med veldefineret nærings sammensætning fremstilles. Fodringsforsøg og registreringer af rotternes reproduktion påbegyndes med nyligt fravænnede rotter fra 2. generation i det eksisterende projekt.

År 3 (2004):

Fodringsforsøg afsluttes og resultaterne publiceres.

I år 3 indgår resultaterne i de samme publikationer som er planlagt i det eksisterende projekt, og da dette først forventes afsluttet med udgangen af 2004, forventes det ikke at det supplerende projekt vil forsinke den endelige afslutning af det kombinerede projekt.

1. Summary

Background

At the preparation of the application for the project III.4, "Organic food and health – a multigeneration animal experiment" (see project description, enclosed as appendix 3) we have from the start been aware of the problem that it did not become possible to accommodate a repetition of the cultivation experiment. Due to this it is not possible to determine whether the results obtained will be caused by general tendencies or just to chance differences between the crops in the single year of cultivation. Randomly occurring differences in the feed can not be compensated for by the repetitions of the feeding study in terms of suitable numbers of animals and generations. On the other hand, it was considered too large a cost to repeat the entire experiment, since under the economic constraints that were given, it would have had the consequence that there would hardly have been funding left for any recordings of the state and health of the animals.

Objective

The overall objective is to repeat the cultivation of feed crops for one more year, and characterise these crops to such an extent, that that it will be possible to use the results to evaluate the reproducibility of the results of the original experiment. Comprising analyses of the material and relevant feeding experiments with rats.

Method

Cultivation and feed preparation: The same as in project III.4, WP1, just on smaller areas. Comprising: 1. a model of a distinct organic cultivation system, with low input of organic plant nutrients and no use of pesticides; 2. a model of a distinct conventional cultivation system, with high input of mineral nutrients and intensive use of pesticides, and 3. a combination of model 1 and 2, with low input of nutrients and intensive use of pesticides.

Characterisation: As a starting point the same analyses will be made as in III.4, comprising biocrystallisation, content and biological value of nutrients, type and content of minerals, pesticides and secondary metabolites. However, the number of analyses will be reduced as far as the results from the first year of cultivation show it is scientifically justified.

Feeding trials: The feed produced will be used to extend the reproduction experiment by one more generation, during the last half of 2003. The same recordings will be made as are done on the rats in each of the other generations, eg. fertility and weight gain.

Time schedule

Year 1 (2002):

Cultivation of crops: wheat, potatoes, oilseed rape carrots, kale, apples and mature peas. Stabilisation in terms of freezing, drying etc. Analysis of biocrystallisation, other analyses are started.

Year 2 (2003):

Analyses are finished and feed mixtures with well defined nutrient composition are prepared. Feeding experiments and recordings of the reproduction are started with newly weaned rats from the 2. generation of the existing project.

Year 3 (2004):

Feeding experiments are finished and the results will be published.

In year 3 the results will be included in the same publications as are planned in the existing project. Since this one is planned to last until the end of 2004, the supplementary project is not expected to delay the final completion of the combined project.

2. Research group

Danish Institute of Agricultural Sciences:

Department of Horticulture: Kirsten Brandt (KB), Ph.D., senior scientist, project coordinator; Erik Larsen (EL), Ph.D., senior scientist, Anette Sørensen (AS), pomology technician and Kristian Thorup-Kristensen (KTK), Ph.D., senior scientist.

Department of Crop Physiology and Soil Science: Jens Peter Mølgaard (JPM), Ph.D., senior scientist.

Department of Agricultural Systems: Ulrich Halekoh, Ph.D., scientist.

Department of Animal Nutrition and Physiology: Charlotte Lauridsen (CL), Ph.D., scientist and Henry Jørgensen (HJ) Ph.D., senior scientist.

The Royal Veterinary and Agricultural University:

Research Department of Human Nutrition: Susanne Højbjerg Bügel (SB), Ph.D., assistant professor

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

Hanne Nygaard Larsen (HNL), Ph.D.

Risø National Laboratory:

Lis Vinther Kristensen (LVK), chief laboratory technician.

National Environmental Research Institute:

Helle Weber Ravn (HER), Ph.D., senior scientist.

3. Introduction

Early in 2001 the project III.4, “Organic food and health – a multigeneration animal experiment” was established (see project description, enclosed as appendix 3). The overall objective of this project is to determine if a well controlled animal feeding experiment comparing conventional and organic food products shows differences in animal physiology, of a type and magnitude that indicates that such products will affect humans differently. Basically, the project consists of a cultivation experiment, where 7 crops are produced with 3 different experimental treatments, processed and extensively analysed, and a subsequent animal experiment, where rats are fed with feed mixtures made from the crops from the 3 treatments, and fertility is measured for 2 breeding cycles for rats fed on each mixture, as well as several physiological characteristics with relevance for human health, such as the use of energy and proteins, bioavailability of minerals etc., blood lipid composition, feed preference, immune response and longevity.

At an early stage in the preparation of the application for project III.4 a sum of 6 mill. Dkr. was allocated for the project. So from the start we have been aware of the problem that with this size of the project it would not be possible to accommodate a repetition of the cultivation experiment.

However, due to this, in the present project it cannot actually be determined whether the results obtained will be caused by general tendencies or could just be due to chance differences between the crops in the single year of cultivation. Randomly occurring differences in the feed can not be compensated for by the repetitions of the feeding study in terms of suitable numbers of animals and generations. On the other hand, it was considered too large a cost to repeat the entire experiment, since under the economic constraints that were given, it would have had the consequence that there would hardly have been funding left for any recordings of the state and health of the animals. So already during the process of evaluation in the spring of 2002, DARCOF decided to make an additional sum of 2 mill. Dkr available for this purpose, and the group was informed that it would be come possible to apply for supplementary funding at a later stage.

4. State of the art

State of the art for the question of differences in effect on health of organic and conventional foods was described in detail in the project description for project III.4 (appendix 3). Since then a few

more papers and reviews have appeared with relevance for the present proposal. A review prepared by the Soil Association in UK (Heaton 2001) declares that the available evidence points in the direction of organic food having a more positive effect on health than corresponding conventional foods. However, it is clear that the volume of good quality studies is much too low to make a solid scientifically based conclusion regarding this question, and it is the intention of the project group of the present proposal to try to increase this volume, without believing that any one study can be conclusive by itself. A very important aspect in this respect is how to accommodate the variability found among different organic farms and similarly among conventional farms. The investigator must choose to either investigate foods from a few, well controlled cultivation studies, or to collect foods from a broad and representative sample of both types of farms, to do both will generally be prohibitively expensive. If significant effects occur, the first type of investigation can be used to determine whether a difference is likely to occur between cultivation systems, but it can not show if it actually does occur in real life. Complementarily the second type will show if a difference occurs at the time and place of the survey, but not if it will disappear after the next adjustment of the practices in organic or in conventional farming. One way of contributing to solving this problem is to design a “narrow” study in a way where it will be possible to estimate which characteristics of food that are related to any differential effects found on health, and thus lead towards a better understanding of the cause of the differences, including means to ensure that the best health effects are preserved in future developments. Understanding those links is a prerequisite for meaningful broader surveys, where the food is only analysed for certain important characteristics. Until now many surveys have been done, but most of them have been based on (rather unfounded) assumptions that this was already known, eg. that any significantly higher content of vitamins, fibres, antioxidants and/or minerals would indicate a better effect on health. But in fact the real differences may be due to other factors in the food, that have not generally been determined in surveys, such as bioactive secondary metabolites (Brandt & Mølgaard 2001), in particular those that respond strongly to external stresses (Ravn & Løkke 2001). It is also still an untested hypothesis whether those unknown characteristics that are important, could be visualised using biocrystallisation, provided it is measured objectively by modern image analysis tools. (Andersen et al. 2001).

5. Objectives and expected achievements

The main objective of the proposed project is to improve the quality of the existing project “Organic food and health – a multigeneration animal experiment”, by supplementing it with additional experiments in those fields where we consider an extension most beneficial. Primarily to improve the reliability of the results, by providing an independent set of data comparable with ones to come from the already planned work.

The expected achievements are in the first place feed for an additional generation of rats, data on its composition and biological value, and data on the response of health markers of the rats to the differences in the feed mixtures. At the next level we will achieve an improved explanatory value of all the data of the already planned work, by comparing it with the new set of data, to identify which differences are consistently present between certain cultivation treatments in both years.

A minor objective, supported by funding external to the present programme, is to use a novel TLC-system to screen for stress-related differences in plant composition, and thereby possibly improve the efficiency of the identification of relevant plant secondary metabolites as well as provide additional insight in the physiological response of plants to cultivation treatments.

6. Description of workpackages including methods

The supplementary project described in the present proposal mainly repeats the same methods as the previous one, and for simplicity we have numbered the workpackages with the same numbers. Except WP 7, which does not comprise any work that is repeated in the proposed project. It is the intention that the workpackages of the new project will be integrated closely with the corresponding WPs of the present one, to provide maximal synergy and rationalisation of the work. Due to this, no specific resources are defined for WP 9, Coordination, dissemination and evaluation, since the ex-

tension does not require additional resources for these tasks, beyond what is already provided in project III.4.

However one method will be used that is not included in the present project, and which we have chosen to organise in the “vacant” WP 7. This is a TLC screening method intended to visualise the compositional changes that occur in plant material which is subjected to specific types of stress, in particular application of pesticides. The test is intended to be used for authenticity control, in particular to detect plants that have been illegally sprayed with pesticides, and the scientist and a company involved are very interested to use the material produced in the project for assessment of the performance of this method. Due to this, the entire cost of this work will be funded from other sources than the FØJO (DARCOF) grant which is presently being applied for (see appendix 2). However, the results could provide valuable leads for the work in WP 3 and it will also be interesting to see if the results correlate with those of WP 2, so we consider it an advantage that the work is done as an integrated workpackage of the proposed project, even though there is not the possibility of getting two years’ results, as for the other tasks.

In brief, the repeated workpackages comprise the following contents and methods:

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 factorial combination of 1 and 2, with low input of nutrients, primarily through animal manure, and use of as much pesticides as is allowed.

The treatments will be established on 2 sites, wheat, potatoes and oilseed rape will be produced in Foulum, and carrots, kale, apples and mature peas in Årslev.

Potato, peas and kale will be cooked and freeze dried, wheat is ground and baked to bisquits, oil produced from the rapeseed, and raw carrots and apples are shredded and freeze dried.

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.

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 wheat and peas.

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, either based on defined weight percentages of each material from each treatment, or, if substantial differences are found between material from the cultivation treatments, one feed mixture of each cultivation treatment is designed in such way that that the feed mixtures are adjusted to provide the same availability of protein and energy.

WP5 Characterisation of mineral content and pesticide residues of feed mixtures

Concentrations of approx. 20 elements and 150 pesticides are estimated by analysis of the final diets produced in WP4 from the plant material harvested in WP1.

WP6 Management and recording of feeding and breeding

Rats are fed with the 3 prescribed feed mixtures for 1 generation, succeeding the 2nd generation of project III.4, with 25 females per treatment and generation. Reproductive characteristics and weight gain are recorded.

WP7 (new) 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.

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.

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.

Table 1: Workpackage list

WP No	WP title	Responsible participant	Budget (1000 Dkr.)	Start	End	Deliverable, No
1	Cultivation of feed plants	JPM	320.7	2	11	1.1, 1.2
2	Characterisation of picture-developing properties of plant materials and feed mixtures	JOA	137.4	7	12/24	2.1, 2.2
3	Characterisation of secondary metabolites in plant material	EL	366.5	8	20/32	3.1
4	Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures	CL	274.9	11	18/30	4.1
5	Characterisation of mineral content and pesticide residues of feed mixtures	SB	210.7	18	20/32	5.1
6	Management and recording of feeding and breeding	HNL	366.5	18	26/34	6.1
7 (new)	Screening of phytochemical changes in plant material	HR	(400)	4	12/24	7.1
8	Data management and analysis	JHB	183.3	12	34	8.1
9	Coordination, dissemination and evaluation	KB	0 ¹	1	36	9.1-9.6
Total			2260 ²			

¹ Provided from the presently funded project III.4.

² 1860 applied from DARCOF, 400 (WP7) provided from other funds

Table 2: Description of workpackages

WP1: Cultivation of feed plants
<p>Workpackage number: 1</p> <p>Start date or starting event: When the soil is ready to sow or plant the crops (month 2)</p> <p>Responsible person: JPM</p> <p>Contributing persons: KTK, AS</p> <p>Person-months: 6</p>
<p>Objectives:</p> <p>To produce plant material for the feed.</p>
<p>Description of work:</p> <p>1. Production of plant material of spring wheat, rape, potato, carrot, kale, apples and dry peas, in 3 different cultivation treatments:</p> <p>(1) Organic: No pesticides and low input of plant nutrients derived from organic matter.</p> <p>(2) Conventional: With pesticides and high input of plant nutrients, provided as minerals.</p> <p>(3) Intermediate: With pesticides and low input of plant nutrients, derived from organic matter.</p> <p>Spring wheat of the cultivar ‘Vinjett’ and ‘Sava’ potatoes will be grown on the same field in Foulum, which has been cultivated organically for 5 years, and without pesticides for several years before that. The potato cultivation is integrated with project II.6 on refinement of organic potato cultivation. Treatments 1 and 3 will be fertilised with pig slurry, treatment 2 with standard mineral fertilisers. Winter rape ‘Bruno’ will be grown on separate, but nearby fields, which are already established as organic (since 1996) or conventional.</p> <p>Carrots ‘Bolero’, peas ‘Ambassadeur’ and apples ‘Otava’ are grown in the organic field experimental unit in Årslev (organic since 96), treatment 2 is made on adjacent conventional areas. Apple experiments are integrated with project I.2. Kale ‘Bona’ is grown on the additional organic experimental areas established in Årslev in 1997.</p> <p>As far as possible we will try to place treatments with and without pesticides in the same or immediately adjacent fields, to ensure comparable basic soil conditions. In all such cases the pesticide treated plots will be placed with concern for the risk of contamination, an appropriate separation will be defined between the harvested plots, and the spraying will be done with appropriately screened equipment, only under suitable weather conditions.</p> <p>2. Processing the materials to become storable: Rape is pressed to produce oil, the residue is discarded. Potato, kale and split peas are cooked and freeze-dried. Carrot and apple are shredded, frozen and freeze-dried. Wheat is milled and the whole grain flour is made into biscuits together with an appropriate amount of oil.</p>
<p>Deliverables:</p> <p>D1.1 (month 7): The harvested materials.</p> <p>D1.2 (month 11): The processed materials.</p>
<p>Milestones:</p> <p>M1.1 (month 7): The materials are harvested</p> <p>M1.2 (month 11): The materials are processed.</p>

WP2: Characterisation of picture-forming properties of plant materials and feed mixtures

Workpackage number:	2
Start date or starting event:	When fresh plant material is available (month 7)
Responsible person:	JOA
Contributing persons:	
Person-months:	3

Objectives:

To characterise the picture-forming properties of plant materials produced in WP1 and the feed mixtures made in WP4.

Description of work:

Production of biocrystallisation pictures on the basis of watery extracts of plant samples and feed mixtures, using the methods already developed in project III.4 and other projects. Examination of the field plot replicates of the treatments separately or pooled. Computerised image analysis and / or visual scoring of the crystallisation textures. Organisation of data with respect to the subsequent statistical analyses.

Deliverables:

D2.1 (month 24): Scientific manuscript on picture-forming properties of the examined samples.
D2.2 (month 34): Contribution to scientific paper(s) on the correlation between picture-forming characteristics and other data from the project.

Milestones:

M2.1 (month 16): The analyses are completed.

WP3: Characterisation of secondary metabolites in plant material

Workpackage number:	3
Start date or starting event:	When some processed plant material is available (month 8)
Responsible person:	EL
Contributing persons:	
Person-months:	8.5

Objectives:

To characterise secondary metabolites in plant material.

To assist in determining if feed made from the 3 treatments in WP1 are sufficiently different to warrant the inclusion of all of them in the animal experiment.

Description of work:

Characteristic secondary metabolites are measured in selected feed material from each treatment, based on the methods developed in project III.4 and using the isolated and identified compounds as standards.

1. Phenolic profile of all vegetable products used will be determined by HPLC and categorised as free and bound compounds, this comprises phenolic acids, coumarins and flavonoids where relevant.
2. Volatile compounds are measured in carrot, kale, apple and rapeseed oil by dynamic headspace sampling followed by GC and GC-MS, and in the same materials carotenoids are measured by HPLC.
3. Phytins are measured in peas, wheat and potatoes by HPLC.
4. Glucosinolates in kale are measured by HPLC, glycoalkaloids in potatoes by LC-MS, and polyacetylenes in carrots by GC-MS.

Deliverables:

D3.1 (month 32): At least one scientific manuscript on the contents of secondary metabolites in the materials.

Milestones:

M3.1 (month 20): The analyses are completed.

WP4: Characterisation of biological value of protein and energy content in feed material and preparation of feed mixtures

Workpackage number:	4
Start date or starting event:	When all processed plant material is available (month 11)
Responsible person:	CL
Contributing persons:	HJ
Person-months:	6.5

Objectives:

To determine the biological value of protein and the energy content in the feed material from the 3 treatments in WP1.

To assist in determining if feeds made from the cultivation treatments are sufficiently different to warrant the inclusion of all 3 of them in the animal experiment.

To design and produce batches of feed from each cultivation treatment, if necessary adjusted in composition to obtain similar protein and energy content.

Description of work:

1. Characterisation of the plant ingredients produced in WP1 with respect to their nutrient content and value. The nutrient analysis will include dry matter, crude fat, crude protein, ash, crude fibre and, in selected ingredients, the composition of amino acids and fibres. Biological value (BV) of protein in the major protein contributing plant ingredients is assessed in a balance experiment with rats (5 per treatment).
2. It is determined if the plant ingredients differ in their energy content and BV of protein to such an extent, that according to previous experiences it is likely to influence growth and health of the rats. If this is the case, the feed mixtures of the plant material of the cultivation treatments will be designed in such way that the energy and protein contents become similar. This will be done by adjusting the content of energy and protein contributing ingredients (e.g. wheat flour and pea meal). Depending on the results of this WP and of WP's 2, 3 and 5, such adjusted feed(s) will either be used to replace treatment 3 with an adjusted version of treatment 1, or both treatments 1 and 3 will be used only in adjusted form.

Deliverables:

D4.1 (month 32): Contribution to scientific manuscript with data on differences in macronutrient content and BV of protein in plant material produced in cultivation treatment, and the work involved in the design of the experimental diets.

Milestones:

M4,1 (month 18): Formulation and production of experimental diets for animal experiment.

WP5: Characterisation of mineral content and pesticide residues of feed mixtures

Workpackage number:	5
Start date or starting event:	When feed mixtures are available (month 18)
Responsible person:	SB
Contributing persons:	KB and Fødevareregion København, Danish Food Control Agency or other subcontractor for pesticide analyses
Person-months:	2.7

Objectives:

To measure a large number of elements and pesticide residues in the 3 feed mixtures.
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Description of work:

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| <ol style="list-style-type: none"> 1. Estimation (measurement) of the concentrations of selected numbers of essential, toxic and potentially interesting elements in the final diets produced from the plant material harvested in WP1. The elements may be Ca, Cr, Cu, Fe, K, Mg, Mn, Mo, Na, P, S, Se, Zn, Ba, Cd, Pb, Sr, Ce, Cs, Rb, and V. The final selection of elements to be measured in feed will be executed when a running human metabolic balance study is finished. Of these, elements that differ significantly among treatments, or where there is an indication that bioavailability can differ depending on other food components, will be selected for measurement in WP7. 2. Estimation (measurement) of the occurrence and concentration levels of pesticides in the final diets, using the cheapest standard analysis scheme that comprises the compounds used on the crops. |
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Deliverables:

D5.1 (month 20): Data on contents of elements and pesticides in each of the diets.
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Milestones:

M5.1 (Month 20): The analyses are completed.
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WP6: Management and recording of feeding and breeding
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Workpackage number:	6
Start date or starting event:	When feed mixtures are available (month 18)
Responsible person:	HNL
Contributing persons:	Subcontractor (Møllegården)
Person-months:	4

Objectives:

To manage the animals through 1 generation and record reproductive characteristics, weight gain and disease frequency (if relevant).
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Description of work:

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| <ol style="list-style-type: none"> 1. Separate sets of individually identifiable rats, selected from the corresponding feed treatments from the 2nd generation in project III.4 are fed with the 3 prescribed feed mixtures, and 25 pairs per diet group are allowed to breed, one litter per female. For each female, reproductive characteristics are recorded, including numbers of attempts before pregnancy, litter size, female/male ratio, stillborn/live born pups, litter size at weaning and possibly age of sexual maturity and anugenital distance. For all rats, weight gain is recorded weekly (for pups starting at the age of 4 weeks) and any signs of the presence of disease are recorded. |
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Deliverables:

D6.1 (month 34): Scientific manuscript on reproductive effects in rats fed organically versus conventionally produced feed over 3 generations.
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Milestones:

M6.1 (month 20): A sufficient number of young is produced in order to make the planned recordings and experiments.
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WP7 (new): Screening of phytochemical changes in plant material
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Workpackage number:	7
Start date or starting event:	When fresh plant material is available (month 4)
Responsible person:	HER
Contributing persons:	
Person-months:	9
Financial sources	Biotech Line A/S and National Environmental Research Institute

Objectives:

Screening of plant material for phytochemical changes to facilitate the selection of analysis and isolation of secondary compounds.

Description of work:

Fresh frozen plant material is pressed and the plant extract is screened using thin layer chromatography (TLC). Several series of different TLC-systems is developed to emphasising phytochemical changes. UV and/or fluorescence -spectra of each compound, corresponding to the phytochemical changes, with their chemical reagent can be performed using a Camag TLC-Scanner equipment (supported by SJVF). After isolation and identification or structure elucidation in WP2 of project III.4, the method can be used to verify the phytochemical changes as identified compounds. The phytochemical changes, presented as a pattern of compounds, will be used to develop a kit to identify organic plant food products from conventional plant products. This product development is financed by Biotech Line A/S and is performed as an associated project.

Deliverables:

D7.1 (month 24): Participation in publications where the results are used to identify secondary compounds.
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Milestones:

M 7.1 (month 12): The analyses are completed.

WP8: Data management and analysis

Workpackage number: 8
Start date or starting event: Month 12
Responsible person: UH
Contributing persons:
Person-months: 3.5

Objectives:

To establish optimal experimental designs and methods, in order to ensure compatibility and utilisation of all results.

Description of work:

1. Detailed planning of interaction among experiments and exchange of materials and data.
2. Data from the animal experiments (WP 6-7) 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.

Deliverables:

D8.1 (month 34): Report or inputs to papers, with the relevant statistical analyses.

Milestones:

M8.1 (month 18): Optimised plan for the animal experiments is prepared.

WP9: Coordination, dissemination and evaluation

Workpackage number:	9
Start date or starting event:	Month 1
Responsible person:	KB
Contributing persons:	All other participants
Person-months:	0 (resources are provided in project III.4)

Objectives:

To coordinate the work and ensure that all subprojects support each other and the overall objective.
 To evaluate the scientific progress in relation to the pre-set aims and to concurrent advances in other projects.

To disseminate the results of the project to relevant forums.

Description of work:

1. Coordination and management of the project, primarily through periodic meetings and progress reports. At each meeting, progress is evaluated and reported in relation to the pre-set aims and to concurrent advances in other projects, and relevant action is decided and implemented if deviations occur.
2. The data which are produced in WP's 1-7 and analysed in WP8 are assembled into scientific and popular papers and conference presentations, which are submitted to the most influential journals or events to reach the target audiences in the relevant fields, nationally and internationally. The target groups are nutrition scientists, agronomy scientists, farmers, consumers and politicians. Due to the high probability that the results will be somewhat controversial (almost irrespective of what they are), strict discipline will be required of the project members to avoid giving contradictory statements to the press, primarily in the pre-publication phases. A detailed plan will be prepared, describing which information will be disseminated where and under which conditions. E.g. some popular or conference publications must await acceptance of a particular paper by a journal before they can be released.

Deliverables:

D9.1 (month 1): Startup meeting M1

D9.2 (month 8): Progress meeting M2. 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 remainder of the project.

D9.3 (month 14): Progress meeting M3

D9.4 (month 24): Progress meeting M4

D9.5 (month 34): Progress meeting M5

D9.6 (month 36): Final report, including implementation and further progress.

Milestones:

M9.1 (month 8): Determination of detailed research plans for the animal studies.

M9.2 (month 14): Publication plan is prepared.

M9.3 (month 36): Final report on implementation and further progress is prepared.

7. Implementation and time schedule

Table 3: Deliverables list

Deliverable, No	Deliverable title	Delivery date	Meeting³	Nature
9.1	Startup meeting	1	M1	Oral
1.1	The harvested materials.	7	M2	O
1.2	The processed materials.	11	M2	O
9.2	Progress meeting M2	8	M2	Oral
9.3	Progress meeting M3	14	M3	Oral
5.1	Data on contents of pesticides in each of the diets.	20	M4	Re
2.1	Scientific manuscript on picture-forming properties of the examined samples.	24	M4	Pu
9.4	Progress meeting M4	24	M4	Oral
3.1, 4.1, 7.1	At least one scientific manuscript on the contents of nutrients and one on secondary metabolites in the plant materials.	32	M5	Pu
2.2, 6.1,	Several scientific manuscripts on the relationships between diet characterisation and animal performance.	34	M5	Pu
8.1	Report or inputs to papers, with the relevant statistical analyses.	34	M5	Re
9.5	Progress meeting M5	34	M5	Oral
9.6	Final report, including implementation and further progress.	36	M6	Re

³ Number of the meeting where the deliverable will be evaluated. Normally the first meeting planned after the date of the deliverable.

Table 4: Timetable			2002*				2003*				2004*				2005*			
Tasks	Co-ordination	Quarter	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
M1	WP1: Cultivation of feed plants					1.1	1.2											
1																		
2																		
M2	WP2: Characterisation of picture-developing properties of plant materials and feed mixtures						2.1											
1																		
M3	WP3: Characterisation of secondary metabolites in plant material						3.1											
1																		
2																		
3																		
4																		
M4	WP4: Characterisation of biological value in feed material and preparation of feed mixtures						4.1											
1																		
2																		
M5	WP5: Characterisation of mineral content and pesticide residues of feed mixtures								5.1									
1																		
2																		
M6	WP6: Management and recording of feeding and breeding								6.1									
1																		
M7	WP7 (new): Screening of phytochemical changes in plant material					7.1												
1																		
M8	WP8: Data management and analysis						8.1											
1																		
2																		
M9	WP9: Coordination, dissemination and evaluation: Meetings M1-M5					9.1	9.2				9.3							
1						1		11		18		24						36
2																		

8. Collaborative partners

Since the number and extent of analyses and recordings in both projects are limited by the level of funding allocated to the project, material from the project will be made available to be used by external collaborators according to specific agreements. All applicants encourage this type of collaboration, which will enhance the value of all that is done inside the project. Of course on the condition that the external work will be of good scientific quality, and this is the main condition that must be met when others want to collaborate in this way. There have been several enquiries about collaboration from scientists in other countries, all of which have been told that we are willing to recommend that their national funding bodies provide support for their efforts in a collaboration. One of the potential collaborators, Alberta Velimirov from the Ludwig Boltzmann Institute in Vienna, Austria, has applied for national funding for her part of a collaboration, but not yet received a decision.

Additionally an EU application is under preparation, with participation of several EU countries, Switzerland and one or more Eastern European countries, with the preliminary title “Safety and quality of organic food: Ensuring and improving safety, taste and health benefits”. The applied project will provide part of the Danish contribution to the work that will be done in this project, if the applications are successful.

9. References

Andersen, J.-O., Kaack, K., Nielsen, M. & Thorup-Kristensen, K., 2001. Comparative study between biocrystallization and chemical analyses of carrots (*Daucus carota* L.) grown organically using different levels of green manure. *Bio. Agric. Hort.* 19, 29-48.

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.

Heaton, S. 2001. *Organic Farming, Food Quality and Human Health*, p. 1-88. Published by the Soil Association, UK.

Ravn, H. & Løkke, H. (2001): Biomarker in plants exposed to glyphosate. (submit. *Chemosphere*).

10. List of appendices

Appendix 1: CV's of central persons, and description of role, qualifications, capacity and experience of each participant including maximum 5 relevant papers (max. 5 pages) (p 24-29).

Appendix 2: Letter of support from Hans Løkke, NERI, confirming the intention to provide funding for WP 7 (p 30).

Appendix 3 (in a separate file): The application with project description of the ongoing project “Organic food and health – a multigeneration animal experiment” (enclosed as a separate file).

Appendix 1: CV's of central persons, and description of role, qualifications, capacity and experience of each participant including maximum 5 relevant papers (max. 5 pages).

CURRICULUM VITAE for Kirsten Brandt

Danish Institute of Agricultural Sciences (DIAS)

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e-mail: kirsten.brandt@agrsci.dk

Tel. (+45) 63 90 42 44

Fax (+45) 63 90 43 95

Born: March 11, 1960

Employments:

1998-present Head of Research Group for Food Science and Natural Products Chemistry, Department of Horticulture, DIAS.

1992-1998 Senior scientist and head of Research Group for Plant Breeding and Propagation, Dept. Ornamentals, DIAS.

1990-1992 Scientist, Department of Plant Breeding and Propagation, DIAS.

1987-1990 Graduate scholarship, the Royal Veterinary and Agricultural University (RVAU) and DIAS.

Education:

1990 Ph.D., Plant breeding and physiology of secondary metabolites, RVAU.

1987 M.Sc. Odense University, Experimental Cell Biology.

Role in the project: Coordinator. Responsible for WP9. Manages the project and links it to relevant national and international initiatives.

Experience and qualifications:

Previous research on physiology and genetics of plant secondary metabolites. Secondary metabolites affecting quality of plant products, in particular effects on human health. Investigations of interactions between genetics and environment of plants, with main emphasis on secondary metabolites. Coordinator of the newly initiated FØTEK 3 research network "Health promoting substances in vegetable foods" and of two FØJO I multi-disciplinary and multi-institutional research projects on plant health and quality in organic agriculture. Participant in the EU Concerted Action NEODIET, Nutritional Enhancement of Plant Foods in Europe. 15 papers in international refereed journals, 20 papers or abstracts in proceedings etc., 18 reports and popular papers.

Five relevant publications:

K. Brandt and U. Kidmose 200x. Nutritional consequences of using organic agricultural methods in developing countries. Encyclopedia of Life Support Systems (EOLSS), paper no. 5.21.5.5 (in press).

Brandt, K. and Mølgaard, J.P. 2001. Organic agriculture: Does it enhance or reduce the nutritional value of plant foods? J. Sci. Food Agric. 81, 924-931.

Brandt, K. & Christensen, L.P., 2000. Vegetables as Nutraceuticals - Falcarinol in carrot and other root crops. In: Dietary Anticarcinogens and Antimutagens. Chemical and Biological Aspects, Ed. by I. T. Johnson and G. R. Fenwick, Norwich, UK., p.386-391.

Brandt, K., Christensen, L.P., Hansen, S.L., Young, J., Stagsted, J., Purup, S. & Knudsen, K.E.B., 2001. Functions of bioactive plant compounds and effect on humans. Innov. Food Tech. 10, 60-62.

Nørbæk, R., Brandt, K. & Kondo, T. 2000. Identification of flavone C-glycosides including a new flavonoid chromophore from barley leaves (*Hordeum vulgare* L.) by improved NMR techniques. Journal of Agricultural and Food Chemistry 48: 1703-1707.

Curriculum vitae**Name:** Hanne Nygaard Larsen**Date of birth:** 21.04.67**Education:**

2000: Ph.D. from The Royal Veterinary and Agricultural University (RVAU), Copenhagen (Ph.D. thesis in clinical nutrition).

1997: M.Sc. Food Science and Technology, RVAU, Copenhagen (Master thesis in nutrition)

Employment record:

1999-2001: Assistant professor, Research Department of Human Nutrition, RVAU.

1995-1999: Ph.D. student, Research Department of Human Nutrition, RVAU and Department of Medicine and Endocrinology, Aarhus University Hospital.

1997: Research assistant, Institute of Experimental Clinical Research, Aarhus University

1997: Research assistant, Research Department of Human Nutrition, RVAU.

Role: Responsible for WP6 and WP7. Takes care of research on reproductive characteristics, food preference and immunology.**Research experience:** My research for the last year has focused on a large scale literature review of health aspects of organic foods. My previous research has focused on the digestion and digestibility of carbohydrates and the glycaemic index concept. This has included human studies, acute and long term animal experimental dietary studies, in vitro studies as well as analytical work.**Relevant publications:**

Larsen H.N., Parvin S., Pathan F., Ali L., Thilsted S.H. and Hermansen K. Glycemic response to rice is affected by parboiling method: comparative studies in Bangladeshi and Danish NIDDM subjects. Submitted.

Larsen H.N., Rasmussen O.W., Rasmussen P.H., Alstrup K.K., Biswas S.K., Tetens I., Thilsted S.H. and Hermansen K. Glycaemic index to parboiled rice depends on the severity of processing: study in type 2 diabetic subjects. *European Journal of Clinical Nutrition*, 2000;54:380-385.Larsen H.N., Christensen C., Rasmussen O., Tetens I.H., Choudhury N.H., Thilsted S.H. and Hermansen K. Influence of parboiling and physico-chemical characteristics of rice on glycaemic index (GI) in non-insulin-dependent diabetic (NIDDM) subjects. *European Journal of Clinical Nutrition* 1996;50:22-27.**Curriculum Vitae***Scientist, Ph.D. Charlotte Lauridsen**Born 26th January 1969.*

M.Sc. in Agricultural Science 1994, Ph.D. in 1998 in Food Chemistry at the Royal Veterinary and Agricultural University, Copenhagen, Denmark.

1994-1995: Research Training Fellow, Department of Nutrition, University College Cork, Ireland.

1995-1998: Ph.D.-student, Department of Product Quality, Danish Institute of Agricultural Sciences, Denmark.

1998: Research Assistant, Department of Animal Nutrition and Physiology, Danish Institute of Agricultural Sciences, Denmark.

1998: Scientist, Department of Animal Nutrition and Physiology, Danish Institute of Agricultural Sciences, Denmark.

1999: Leave of absence. Research associate at the Linus Pauling Institute, Oregon State University, Corvallis, Oregon, USA.

Role in project: To evaluate the macro-nutrient content of the cultivated plant material and to design the experimental diets for the rat studies. In addition, to provide information on the effects of the experimental diets on the digestibility and energy metabolism of the rats.

Research experience: Basic and applied research concerning nutrition and physiology of monogastrics with major focus on fats, fatty acids, and selected vitamins, minerals, and antioxidants. The effects of fatty acids and antioxidants on oxidative stability of muscle and muscle food.

Key references of relevance for this project:

Lauridsen, C., Højsgaard, S. and Sørensen, M.T. 1999: Influence of dietary rapeseed oil, vitamin E and copper on performance and antioxidative and oxidative status of pigs. *Journal of Animal Science*, 77, 906-916.

Lauridsen, C., Nielsen, J.H., Henckel, P. and Sørensen, M.T. 1999: Antioxidative and oxidative status in muscles of pigs fed rapeseed oil, vitamin E and copper. *Journal of Animal Science*, 77, 105-115.

Lauridsen, C., Andersen, G., Andersen, M., Andersson, M., Danielsen, V., Engberg, R. and Jakobsen, K. 1999. Effect of dietary fish oil supplied to pigs from weaning to 60 kg liveweight on performance, tissue fatty acid composition and palatability of pork when slaughtered at 100 kg live weight. *Journal of Animal and Feed Sciences*, 8, 441-456.

Lauridsen, C., Jensen, S.K., Skibsted, L.H. and Bertelsen, G. 1999. Influence of supranutritional vitamin E and copper on α -tocopherol deposition and susceptibility to lipid oxidation of porcine membranal fractions of *M. Psoas Major* and *M. Longissimus Dorsi*. *Meat Science*, 54 (4), 377-384.

Sandström, B., Bügel, S., Lauridsen, C., Nielsen, F., Jensen, C., and Skibsted, L.H. 2000. Cholesterol-lowering potential in human subjects of fat from pigs fed rapeseed oil. *British Journal of Nutrition*, 84, 143-150.

Curriculum vitae

Name	Jens-Otto Andersen
Date of birth	August 15, 1949
Citizenship	Danish
Title	Research assistant
Education	Ph.D. and M.Sc. Agronomy, Royal Veterinary and Agricultural University, Denmark
Address	Royal Veterinary and Agricultural University, Denmark Department of Agricultural Sciences / Organic Farming Unit Agrovej 10, 2630 Taastrup, Denmark Phone +45 35 28 35 20, E-mail joa@kvl.dk Fax +45 35 28 21 75

Research and professional experience

1998- Coordinator of minor research projects funded by the Danish Ministry of Food, Agriculture and Fisheries.

2000- Coordinator of research at the private association Biodynamic Research Group, Heriskind, 8464 Galten, Denmark.

Research activities

Development and application of the biocrystallization method, including development of experimental techniques and image analysis software. Investigations concerning the effects of biodynamic preparations on the yield and quality of vegetable crops.

Membership of scientific committees

Danish Research Centre of Organic Farming (Darcovf), User Board.

Publications in international papers (as first author)

Andersen, J-O., Laursen, J. & Kølster, P. (1998). *A refined biocrystallization method applied in a*

- pictomorphological investigation of a polymer*. Elemente der Naturwissenschaft, **68**, 1-20.
- Andersen, J-O., Henriksen, C.B., Laursen, J. & Nielsen, A.A. (1999). *Computerized image analysis of biocrystallograms*. Computers and Electronics in Agriculture, **22**, 51-69.
- Andersen, J-O., Kaack, K., Nielsen, M. & Labouriau, R. (2001). *Comparative study between biocrystallization and chemical analyses of carrots (Daucus carota L.) grown organically using different levels of green manure*. Biological Agriculture & Horticulture, **19**, 29-48.

CURRICULUM VITAE for Ulrich Halekoh

Ph.D. (rer. nat), scientist

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Tel.: + 45 89 99 18 25, Fax: + 45 89 99 18 19; e-mail: ulrich.halekoh@agrsci.dk

Born: March 30, 1961

Employments:

- 1999 - Scientist at the Biometry Research Group, Danish Institute of Agricultural Sciences, Foulum
- 1995 - 1999 Scientist at the Centre for Data Analysis and Modelling, Freiburg, Germany
- 1989 - 1995 Research Assistant, Department of Epidemiology, Institute of Environmental Hygiene, Düsseldorf, Germany

Education:

- 1989 Diplom in Statistics
- 1996 Ph.D. (rer. nat)

Role in the project: To establish optimal experimental designs and methods, in order to ensure compatibility and utilisation of all results.

Experience and qualifications: Involved in projects of the analysis on health related data (epidemiological research, cancer mapping), development of new statistical methodology (Markov Chain Monte Carlo).

Consultancy for medical and agricultural researchers. Seminary talks in Germany (5), France (1), Denmark (1).

Five related publications:

- Halekoh, U. and Degens, P.O. (1991). "*Analysis of Data Measured on a Lattice*," in: Classification, Data Analysis, and Knowledge Organization}, eds. Bock, H.-H. and Ihm, P., Heidelberg: Springer Verlag, 91-98.
- Halekoh, U. and Degens, P.O. (1994). "*Least Squares Smoothers and Additive Decomposition*," in: New Approaches in Classification and Data Analysis, eds. Diday, E., Lechevallier, Y., Schader, M., Bertrand, P., Burtschy, B., Heidelberg: Springer Verlag 1994, 549-555.
- Pesch, B., Halekoh, U., Ranft, U., Richter, M., Pott, F. (1994). "*Atlas zur Krebssterblichkeit in Nordrhein-Westfalen*", Ministerium für Arbeit, Gesundheit und Soziales des Landes Nordrhein-Westfalen.
- Halekoh, U., Schweizer, K. (1999). *Analysis of the Stability of Clusters of Variables via Bootstrap* in: Classification in the Information Age (Eds.) Gaul, W., Locarek-Junge, H. Proceedings of the 22nd Annual Conference of the German Classification Society, March 4-6, 1998, Dresden. p. 171-178.

Halekoh, U. and Vach, W. (1999) *Bayesian Seriation as a Tool in Archaeology* Proceedings of the Conference on Computer Applications and Quantitative Methods in Archaeology, Birmingham, 1997, CD BAR S750, Oxford: Archaeopress.

Curriculum Vitae

Erik Larsen

Department of Horticulture

Danish Institute of Agricultural Sciences (DIAS)

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Phone: +45 63 90 43 11; Email: erik.larsen@agrsci.dk

Born: April 26, 1964, Denmark.

Education

1995 Ph. D. in chemistry at Odense University.

1989 M. Sc. in organic chemistry at Aarhus University.

1987 B. Sc. in physics at Aarhus University.

Research Experience

April 99- Senior scientist, Department of Horticulture, DIAS.

July 96-May 98 Post doc, Department of Chemistry and Biochemistry, University of Colorado, USA.

Sept. 95-Dec. 95 Assistant professor (temporarily). Department of Chemistry, Odense University.

Jan. 95-June 95 Research associate, Department of Chemistry, University of Iowa, U.S.A.

Jan. 93-Dec. 94 Ph. D. student, Department of Chemistry, Odense University.

Jan. 92-June 92 Researcher, Department of Chemistry, Odense University.

Role in the project: Responsible for workpackage 3, characterization of secondary metabolites in plant material.

Qualifications: Extensive experience in isolation, identification and synthesis of chemical compounds with effects on health, including many different biologically active secondary metabolites from plants.

Five Relevant Publications (out of 19 international and several national publications)

- 1) **Larsen, E.;** Andreasen, M. F.; Christensen, L. P. Regioselective Dimerization of Ferulic Acid in a Micellar Solution. *J. Agric. Food Chem.* In Press.
- 2) **Larsen, E.;** Christensen, L. P. A Simple Method for Large Scale Isolation of the Cyclic Arylhydroxamic Acid DIMBOA from Maize (*Zea mays* L.). *J. Agric. Food Chem.* **2000**, *48*, 2556-2558.
- 3) Christensen, L. P.; **Larsen, E.** Direct Emission of the Allergen Primin from Intact *Primula obconica* Plants. *Contact Dermatitis* **2000**, *42*, 149-153.
- 4) Danel, K.; **Larsen, E.;** Pedersen, E. B.; Vestergaard, B. F.; Nielsen, C. Synthesis and Potent Anti-HIV-1 Activity of Novel 6-Benzyluracil Analogues of 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine. *J. Med. Chem.* **1996**, *39*, 2427-2431.
- 5) **Larsen, E.;** Kofoed, T.; Pedersen, E. B. Synthesis of 2,3'-Anhydro-2'-deoxynucleosides and 2',3'-Didehydro-2',3'-dideoxyuridines Using Polymer Supported Flouride. *Synthesis* **1995**, 1121-1125.

CURRICULUM VITAE for Helle Weber Ravn,

Ph.D. (Lic. Pharm), Senior scientist

National Environmental Research Institute, Vejlsovej 25, P.O. Box 314, DK-8600 Silkeborg,

Tel.: + 45 89 20 17 53, Fax: + 45 89 20 14 13; e-mail: her@dmu.dk

Born: April 28, 1957

Employments:

- 1996 - Senior scientist and Head of Research Group for Plant Biomarkers, National Environmental Research Institute Dept. of Terrestrial Ecology, Silkeborg
- 1993 - 1996 International coordinator on Harmful Algal Bloom (Ass. Expert), Intergovernmental Oceanographic Commission, UNESCO, Paris
- 1989 - 1993 Researcher and Head of Group for Toxic Phytoplankton, National Environmental Research Institute Dept. of Marine Ecology and Microbiology, Roskilde
- 1989 Research Assistant, PharmaBiotec Research Center, The Royal Danish School of Pharmacy
- 1988 Assistant professor, Dept. of Pharmacognocny, The Royal Danish School of Pharmacy
- 1984 - 1985 Provisor, Åbenrå Løveapotek and Haderslev Hjorteapotek

External activities:

- 1998 Rapporteur, Université de Montpellier I, France (opponent in French Ph.D. project)
- 1998 - External examiner, The Royal Danish School of Pharmacy, Copenhagen

Education:

- 1984 Cand.pharm
- 1988 Ph.D. (Lic.pharm)
- Participating in 15 superimposed courses in Danmark, Japan, Italy and Germany, co-organizer of 4 Internationale courses.

Role in the project: Screening of plant material for phytochemical changes (plant biomarkers) to facilitate the selection of analysis and isolation of secondary compounds.

Experience and qualifications: Senior scientist in natural compounds in plants, animals, algae and fungi. Field of main research: Biomarkers in plants exposed to stress, especially herbicides. Research stays in France, Japan and U.S.A. Presented invited lectures in Japan (4), Denmark (7), Belgium (1), Norway (1), Germany (1), Finland (1), Italy (2), France (2). Supervisor of 17 student projects in or from Denmark (16), Japan (1), Iran (1), France (1) Active participating and/or co-organiser in/of International meetings: in Belgium (3), USA (1), Spain (2), France (7), Malta (1), Germany (1), Japan (1), Italy (1). Presented posters at Internationale conferences in USA (2), Danmark (3), Tyskland (1), Frankrig (6), Schweiz (1), Portugal (1).

International PCT-patentansøgning: *An assay method and kit for testing biological material for exposure to stress using biomarkers*; applied 30 may 2000 (1).

Five related publications:

- Per Mølgaard & Helle Ravn (1988): *Evolutionary aspects of caffeoyl esters in dicotyledons. A review*, *Phytochemistry*, 27, 8, 2411-2421.
- Helle Ravn & Leon Brimer (1988): *Structure and antibacterial activity of Plantamajoside, a caffeic acid sugar ester from Plantago major ssp. major*, *Phytochemistry*, 27, 11, 3433-3437.
- Helle Ravn, Claude Andary, Georg Kovacs & Per Mølgaard (1989): *Caffeic acid esters as in vitro inhibitors of plant pathogenic bacteria and fungi*, *Biochem. Syst. and Ecology*, Vol 17, No 3, 175-184.
- Helle Ravn, Sansei Nishibe, Michiko Sasahara & Li Xuebo (1990): *Phenolic compounds from Plantago asiatica*, *Phytochemistry*, Vol.29, No. 11, pp 3627 - 3631.
- Helle Ravn & Hans Løkke (2001): *Biomarker in plants exposed to glyphosate*. (submit. *Chemosphere*).

Appendix 2: Letter of support from Hans Løkke, NERI, confirming the intention to provide funding for WP 7.

Dr. Kirsten Brandt, Ph.D.
 Head of Research Unit
 Danish Institute of Agricultural Sciences
 Department of Horticulture
 Kirstinebjergvej 10
 DK-5792 Aarslev
 Denmark

MINISTRY OF ENVIRONMENT
 AND ENERGY

NATIONAL ENVIRONMENTAL
 RESEARCH INSTITUTE

Department of Terrestrial Ecology

File no. 151/201-0003
 Ref. HLO/her

Subject: Participation in the application project to FØJO with WP 7: Screening of phytochemical changes in plant material. November 23, 2001

Dear Kirsten Brandt,

The Department of Terrestrial Ecology, National Environmental Research Institute, Silkeborg has a collaboration agreement with the Danish private Company Biotech Line A/S, Slangerup. A part of the agreement is to develop a simple method kit to separate organic from conventional cultivated plant products. It is a fact that the plant material cultivated in the FØJO-project: "Organic food and health" is very useful to our project.

A part of our project includes a screening of phytochemical changes in fresh plant material to identify the best plant material for kit development. This part of our project could be a part of your project as suggested in the new WP 7 of the project. WP7 can support the new FØJO project with valuable information to focus on the most interesting groups of natural compounds for analysis, isolation, purification and identification.

The Department and Biotech Line A/S has agreed to offer 400.000 kr as presented in WP7 as own funding to the project, and hereby be a part of the new FØJO-project.

We are looking forward to collaborate with you.
 Yours sincerely,

Hans Løkke
 Head of Department

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