



## Progress Report 2008 and Application for Continuation in 2009

for research funding under the research programme:

**Research in Organic Food and Farming**  
International Research Co-operation and Organic Integrity  
(DARCOF III 2005-2010)

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1. **Project title and acronym:** Content, Bioavailability and Health Effects of Trace Elements and Bioactive Components of Food Products Cultivated in Organic Agricultural Systems (OrgTrace)

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2. **Project journal number** 3304-FOJO-05-45-01

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3. **Project period (month, year)**

**Start of project:** 01-01-2007

**End of project:** 31-12-2010

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4. **Head of project:** Associate Professor, Ph.D. Søren Husted, LIFE, KU

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### 7. Midterm description of the project, its results and progress, and application for continuation in 2009

#### A. Project summary

WP No.	WP title	Responsible scientist	Budget DKK	Start	End	Deliverable No.
1	Multielemental analysis of plant and soil, multivariate data analysis and project coordination	Søren Husted	2301	01-2007	12-2010	D1.1-D1.5
2	Identification of major bioactive plant constituents	Pia Knuthsen	1969	01-2007	12-2009	D2.1-D2.8
3	Analysis of crops	Erik H. Larsen	2380	06-2007	12-2009	D3.1-D3.6
4	Health and immunity elucidated by a rat model	Charlotte Lauridsen	4000	08-2007	12-2010	D4.1-D4.4
5	Human bioavailability	Susanne Bügel	3737	05-2007	12-2010	D5.1-D5.5
<b>Total</b>			<b>14.387</b>			

#### Objectives and expected achievements:

The main objective of OrgTrace is to study the impact of relevant organic and conventional agricultural practises on the ability of plants to assimilate trace elements from the soil and to synthesise bioactive secondary metabolites and antioxidant vitamins with health promoting effects. Moreover, the possible improved bioactivity and health in humans and animal models from complete diets will be studied.

The specific main objectives are:

1. To characterise and optimise the content of trace elements and bioactive compounds in crop plants harvested from a well-defined organic cultivation system including realistic combinations of plant species, soil type, crop rotations and fertilizers.
2. To assess the bioavailability of the bioactive compounds in human intervention studies employing prepared diets based on the crops produced within the systems.
3. To characterise how biomarkers for health and well-being are affected by the dietary treatments using a rat-model and to study the development of the immune defence system according to the dietary treatments.

The overall scope of the proposal is to improve the fundamental knowledge of organically grown foods in order to further document their quality in relation to agricultural practice. This will be achieved in the present project by investigating the influence of a variety of rigidly controlled cultivation methods on the content of bioactive compounds of importance in human nutrition as well as for promotion of human health.

The underlying hypothesis is that a careful choice of crop rotation and cultivation practice will allow for an organic crop production system characterised by crops of superior nutritional quality with respect to trace elements, bioactive metabolites and vitamins. Phytate, being a potential counteracting substance will also be analysed. Such an optimised cultivation system will respect and ensure the integrity and efficiency of the organic cultivation practice in all links of the chain from the consumer to the primary production.

## Midterm results and progress:

The midterm results achieved in OrgTrace from the periods 01-2007 to 08-2007 (*italics*) and 09-2007 to 08-2008 are broken down by the individual WP's and listed below:

### ***WP1 Multielemental analysis of plant and soil (Søren Husted):***

#### ***01-2007 to 08-2007:***

*From all geographical locations (Årslev, Flakkebjerg, Jyndevad and Foulum) soil and plant samples have been collected at two occasions during the spring 07. The sampling methodology has been optimised to minimize variation. Harvest of cereals and potatoes has been concluded whereas the remaining plant products are expected during the following two months. The sample logistics within the WP's have been detailed and planned.*

*An ICP-MS based semi-quantitative (Semi-Q) analytical method has been developed which enables simultaneous determination of 60 elements in soil and plant samples. Data from the Semi-Q analysis have been mined with chemometrics and encouraging preliminary data have been obtained, which shows that multivariate techniques are able to discriminate organic and conventional soils from each other despite the heterogeneity induced by the 4 different geographical locations. This indicates that organic agricultural systems might induce a unique multi-elemental fingerprint with major classification power. These findings will during the autumn 2007 be further explored by including harvested plant products. In addition isotope ratio analysis of N and C will be initiated to complete the multi-elemental dataset for 2007. A paper is in preparation with the working title: "An ICP-MS based semi-quantitative analytical method to classify organic and conventional plant products using chemometrics"*

#### **09-2007 to 08-2008:**

From all geographical locations (Årslev, Flakkebjerg, Jyndevad and Foulum) soil and plant samples have been collected in March and June 2008, respectively. All experimental plots were visited in July 08 and photographed.

During autumn 2008, plant material from all locations were harvest, sorted and prepared for chemical analysis and the human and rat experiments. In general the harvested products were of very high quality and were received in sufficient quantities; except for faba beans (see WP4). Sorting and sample preparation was a very labour intensive procedure and consumed significantly more time than originally planned. As a consequence, more hands (Three Master students from LIFE, KU) have been recruited and allocated to this crucial task in 2008.

The procedures for cultivation and enrichment of carrots and onions with  $^{77}\text{Se}$  were optimized and subsequently the plant material needed for the human intervention study were greenhouse cultivated in autumn 07 to winter 08 (See WP3 and WP5).

In WP1 the key-methodology is the ICP-MS based semi-quantitative analysis, which successfully has been optimized in order to classify samples from the different cultivation systems in the OrgTrace project. Using the limit of detection (LOD) as inclusion criteria, the fingerprints generated generally consisted of 30-40 elements of which 10-20 were determined with an accuracy >70%. In conjunction with chemometrics, the semi-quantitative method achieved a comparable ability to classify samples as the traditional full-quantitative analysis but at a much higher sample throughput rate. Thus, the large amount of elemental information obtained with semi-quantitative ICP-MS fully outweighed the inherent lower accuracy. It is concluded that in combination with chemometrics, semi-quantitative ICP-MS provides a novel and strong analytical tool to be used in OrgTrace and it constitutes a powerful alternative to traditional full-quantitative methods. The developed methods are currently being used to analyse the plant products from the 2007 harvest. The chemical analysis is expected to be completed by 10-2008 and the chemometric data analysis by 03-2009.

## **WP2 Bioactive plant constituents (Pia Knuthsen):**

### **01-2007 to 08-2007:**

*The development of LC, LC-MS and LC-MSMS methods has been started with the aim of developing and optimizing methods tailored for determination of selected bioactive metabolites in crops. The work is presently focusing on polyphenols in relevant crops. The identification and characterization of unknown bioactive metabolites is ongoing and will continue further when the crops are harvested during autumn 2007.*

*An ICP-MS analytical method has been developed and validated for quantitative (FullQuant) element determination. For compound identification on the molecular level an ESI-MSMS method has been developed, which enable determination of Se and S containing species in the plant samples growing on the four different geographical locations.*

### **09-2007 to 08-2008:**

The development of LC, LC-MS and LC-MS-MS methods has continued, thus methods have been tailored and optimized for determination of selected bioactive metabolites in crops and biological fluids. The work has focused on polyphenols, flavonoids and proanthocyanidins, and on polyacetylenes. A paper is in preparation with the suggested title: "Accelerated solvent extraction for analysis of flavonoids in vegetables".

## **WP3 Analysis of Crops (Erik H. Larsen):**

### **01-2007 to 08-2007:**

*A pilot study has been initiated to define the Se species and its safe concentration used for isotopically labelling of*

*some plant materials that will be further used in the human intervention study. In the frame of this experiment two plants (onion and carrot) have been cultivated in a growth chamber and have been sprayed with different selenium solutions.*

*Analysis of crops is awaiting harvest and will start during autumn 2007.*

#### **09-2007 to 08-2008:**

For compound identification the developed LC-ESI-MSMS method has been successfully applied for separation and detection of sulphur-containing bioactive compounds in crops. The long-term aim is that both selenium and sulphur-containing molecular species are determined by the hyphenated LC-ICP-MS/ESI-MSMS analytical platform.

Based on a pilot study, the enrichment of crops via foliar application has been successfully conducted in a greenhouse experiment (see WP1). An upper tolerance limit for foliar application of plants with selenium as selenite or selenate has been identified. This paves the road for efficient application of the costly enriched stable  $^{77}\text{Se}$  isotope for the human experiments.

Sulfur determination by ICP-MS has proved difficult and much effort has been devoted to this activity. The achievement is, however, that we have an analytical method based on ICP-MS for detection of sulphur at the concentration level naturally occurring in crop plants of the OrgTrace study. Finally, the method has been used for detection of sulphur in addition to Se, Zn and Cu in plants harvested from the 2007 field trials.

The ICP-MS based speciation method for analysing Fe and Zn speciation in the cereal grain is almost completed and a paper on the methodology is expected to be submitted ultimo November 2008.

Analysis of crops harvested 2007 for bioactive metabolites and vitamins are ongoing.

#### ***WP4 Health and immunity (Charlotte Lauridsen):***

##### ***01-2007 to 08-2007:***

*The options for pressing the oil from the rape seeds are being considered and pressing are awaiting harvest.*

##### **09-2007 to 08-2008:**

Ingredients from harvest year 1 have been prepared, i.e. potatoes have been boiled and freeze-dried, and rapeseed oil has been produced, and overall the ingredients are ready for the production of complete diets in the rat experiment. However, initiation of the rat experiment is awaiting as described in detail below and under point D (Deviations).

Problems in delivering some ingredients from harvest year 2 (2008) have been announced by partner 1 (WP1). In WP4, we are especially concerned about the lack of faba beans from Flakkebjerg, and the expected reduced availability of this ingredient from Foulum in 2008. We have therefore decided to postpone the planning of diet compositions from harvest 2007 until an overview can be provided on the available amounts of ingredients from harvest 2008, since harvest 2007 and 2008 are repetitions, and the recipe of the complete diets (amount and type of ingredients) should be the same. A meeting has been held with the statistical experts of OrgTrace in order to revise the statistical design of the rat experiment. From a statistical point of view exclusion of faba beans from the experimental diets would be the best solution. However, from a nutritional point of view we would like to keep the faba beans in the recipe because of its protein content. Thus, the decided strategy is to await the information on the amount of faba bean har-

vested from Foulum in 2008, and to assess the possible amount in the recipe of this ingredient. Furthermore, it is planned to use harvest 2007 faba beans in complete diets containing Flakkebjerg ingredients. In this way the interaction between harvest year and location cannot be determined, however, this parameter can be assessed by either exclusion of Flakkebjerg data or by using data from harvest 2007 only.

### ***WP5 Human Bioavailability (Susanne Bugel):***

#### ***01-2007 to 08-2007:***

*The human bioavailability study has been planned in details and an application for the Ethical Committees for Frederiksberg and Copenhagen has been prepared and send.*

#### **09-2007 to 08-2008:**

##### *Volunteers*

Ethical approval for the human study was obtained in October 2007. The volunteers were recruited from the Frederiksberg and Copenhagen area using advertisements in local education centers and local newspapers. 41 men fulfilled the inclusion/exclusion criteria when assessed during the telephone screening and were invited to the oral information meeting for further details. 20 men agreed to participate and gave the written consent. 2 men dropped-out from the project due to personal reasons before the beginning of the dietary intervention, thus 2 other persons from the waiting list were included to the study. In total, 20 men, mean age 24.7 (SD 6.4) years, with a mean body mass index 21.8 kg/m<sup>2</sup> (SD 1.6) were recruited. 2 persons did not come to the first day of the first intervention period and one person dropped-out after the second intervention period due to personal reasons.

##### *Diets*

After harvest and sampling of the crops (WP1) in 2007, the plants were transported to the Department of Human Nutrition (November 2007) and stored in appropriate storage rooms until further preparation of the diets. Three intervention diets were composed from two menus and consumed during each intervention period on day 1, 3, 5, 8, 10 and 12 (menu 1) and 2, 4, 6-7, 9 and 11 (menu 2). Most meals were prepared in advance in the metabolic kitchen and stored at -20 °C, until they were thawed and heated on the day of consumption.

##### *Stable isotopes*

Isotopically enriched copper chloride (<sup>65</sup>CuCl<sub>2</sub>) and zinc chloride (<sup>67</sup>ZnCl<sub>2</sub>) prepared from elemental <sup>65</sup>Cu and <sup>67</sup>Zn, respectively, were administered to the subjects on day 8 of each period. The <sup>77</sup>Se isotope was administered to the subjects during breakfast as carrot marmalade and during dinner as baked potatoes with carrot and minced meat with vegetables.

##### *Blood samples*

Fasting blood was drawn from a forearm vein puncture and stored at -80°C and delivered to the contact persons from WP2 and WP3 for further analyses. Faeces were collected, pre-treated and digested by microwave energy and delivered to WP2 and WP3 for further analyses. Urine (24-h) was collected and delivered to the contact persons from WP2 and WP3 for further analyses. Duplicate portions of all six diets were collected, homogenised, freeze-dried, stored at -20°C and delivered to the contact person from WP3 for further analyses.

## **C.1 Description (summary) of main results and conclusions for each year**

*2007: The project was started in January 2007 and the time until then has mostly been used to develop and optimize analytical methodologies to be used in OrgTrace (WP1, WP2, WP3). Plants are being harvested from august-november and gradually they will be analysed and used to compose diets – no results are planned and expected*

*until 2008.*

*According to the research plan the intervention trials in WP4 and WP5 will be undertaken in 2008 and consequently no results should be expected from these WP's until then.*

**2008:** Plant products from the 2007 harvest were sorted, prepared and distributed to the individual WP's. The plant products were generally of very high quality and homogeneous samples representing the experimental plots were carefully prepared and stored for final analysis/feeding trials. Harvest 2008 is not yet completed.

Most efforts so far have been devoted to the development of appropriate methods suitable for the analysis of the crops from OrgTrace

The first annual OrgTrace coordination meeting was held in Helsingør (Elinore) 11-13/3 2008.

**WP1 (Multi-elemental analysis):** Soil samples for 2007 and 2008 have been analysed and compared. As expected data differs between locations and experimental plots and highly consistent data have been obtained between the years.

The semi-quantitative ICP-MS based method was successfully developed and is ready to be used on harvest 2007 and 2008 material. A paper has been submitted for publication.

Leaf samples from 2007 have been analysed using ICP-MS and IR-MS and a clear classification between systems and locations was possible.

IR-MS analysis of N and C isotope composition in leaf samples from 2007 and 2008 as well as harvest products from 2007 have been undertaken and clear differences between cultivation systems have been identified.

**WP2 (Identification of major bioactive constituents):** An LC-UV and MSMS method has been developed and optimized for analysis of the comprehensive number of flavonoids compounds in different crops. Furthermore methods for analysis of flavonoids in urine have been optimized. And optimizing of a method for analysis of polyacetylenes in crops is almost completed.

A LC-ESI-MSMS method has successfully been developed and applied for separation and detection of sulphur-containing bioactive compounds in crops.

**WP3 (Analysis of Crops):** Crops harvested 2007 have been analysed for vitamin C, and no clear differences between the cultivation systems were found.

The Se isotope enrichment of crops via foliar application has been successfully conducted in a greenhouse experiment (see WP1). An upper tolerance limit of spraying the plants with selenium as selenite or selenate has been identified.

A paper on Se speciation in foliar enriched carrots and onions has been submitted.

An ICP-MS based method for detection of sulphur at the concentration level usually found in crops has been developed and used for detection of sulphur in addition to Se, Zn and Cu in plants harvested from the 2007 field trials.

**WP4 (Immune response in rats):** No main results yet

**WP5 (Human bioavailability):** First human intervention study has successfully been completed, but no main results yet.

## C.2 Fulfilment of deliverables and milestones

Deliverables list (from application)

Workpackage 1						
Deliverable No	Deliverable title	Lead scientist	Delivery date	Allocated scientific person moths	Type of deliverable	Fulfilled (ok) or deviations (d)*
D1.1	Multi-elemental classification of organic and conventional agricultural systems	SHu	12-2010	12	S	
D1.2	Mobilization of Se and S by green manures and catch crops in organic agriculture	SHu	12-2010	6	S	
D1.3	“Er der forskel på økologisk og konventionelt dyrkede planter i Danmark” Popular scientific contribution in Danish	SHu	12-2010	0	P	
D1.4	Presentation of primary data at a suitable international congress	SHu	08-2010	0	C	
D1.5	Ph.D thesis - Multi-elemental classification of crops from organic and conventional agricultural systems using inorganic mass spectrometry and multivariate statistics	SHu	12-2010	4	O	

\* *Deviations are to be further discussed in D*

Milestones list (from application)

Workpackage 1			
Milestone No	Milestone title	Delivery date	Fulfilled (ok) or deviations (d)*
M1.1	Classification of soil based on data from sequential extraction (year 1)	07-2007	OK
M1.2	Classification of multi-elemental data from plant samples (year 1)	03-2008	Deviation
M1.3	Classification of soil based on data from sequential extraction (year2)	07-2008	Ongoing
M1.4	Classification of multi-elemental data from plant samples (year 2)	03-2009	
M1.5	Multivariate data analysis	12-2010	

\* *Deviations are to be further discussed in D*

(The nature of the deliverables must be indicated by S = publication in scientific journal with peer review; P = publication in journals without peer review; R = reports; C = presentation at meetings and congresses or O = other types of deliverables, e.g., prototypes, models, websites, etc.).

## Deliverables list (from application)

<b>Workpackage 2</b>						
<b>Deliverable No</b>	<b>Deliverable title</b>	<b>Lead scientist</b>	<b>Delivery date</b>	<b>Allocated scientific person moths</b>	<b>Type of deliverable</b>	<b>Fulfilled (ok) or deviations (d)*</b>
D2.1	Presentation of results on identification/characterization of selected unknown bioactive trace elements at international symposium	EHL	02-2008	2	C	OK
D2.2	Presentation of results on identification/characterization of selected unknown bioactive secondary metabolites at international symposium	PK	12-2008	3	C	OK
D2.3	Paper on S and Se speciation in crops	EHL	06-2008	8	S	Ongoing
D2.4	Paper on characterization of selected bioactive metabolites in crop plants	PK	10-2008	9	S	
D2.5	Ph. D thesis on S and Se in crops and diets, and their bioavailability	EHL	12-2009	3	O	Deviation (reported 07)
D2.6	Ph. D thesis on bioactive metabolites in crops and diets, and their bioavailability	PK	01-2010	3	O	
D2.7	Identification of key Fe and Zn species in cereal grains grown in different agricultural systems	SHu	12–2010	8	S	
D2.8	Ph.D thesis – Speciation and bioavailability of Fe and Zn in the cereal grain (see also D3.6)	SHu	12-2010	4	O	

\* Deviations are to be further discussed in D

## Milestones list (from application)

<b>Workpackage 2</b>			
<b>Milestone No</b>	<b>Milestone title</b>	<b>Delivery date</b>	<b>Fulfilled (ok) or deviations (d)*</b>
M2.1	HPLC and ICP-MS systems optimised for simultaneous determination of Se and S species	09-2007	OK
M2.2	LC, LC-MS and LC-MS-MS methods tailored for determination of selected bioactive metabolites in crops	12-2007	Ongoing
M2.3	Selected unknown trace element species characterized or identified	12-2007	OK
M2.4	Selected unknown bioactive metabolites characterized or identified	01-2008	Ongoing
M2.5	Key Fe and Zn species identified	01-2008	Ongoing

\* Deviations are to be further discussed in D

<b>Workpackage 3</b>						
<b>Deliverable No</b>	<b>Deliverable title</b>	<b>Lead scientist</b>	<b>Delivery date</b>	<b>Allocated scientific person moths</b>	<b>Type of deliverable</b>	<b>Fulfilled (ok) or deviations (d)*</b>
D3.1	Paper on <sup>74</sup> Se enrichment of crop plants via foliar application	EHL	04-2009	7	S	OK
D3.2	Paper on composition of bioactive metabolites and vitamins in organic and conventional crops	PK	07-2009	11	S	
D3.3	Presentation of results on D3.2 at international conference	PK	08-2009	3	C	
D3.4	Paper on results for Se and S species in conventional and organic crops	EHL	12-2009	12	S	Ongoing
D3.5	Speciation of Fe and Zn in cereal grains	SHu	12–2010	8	S	

	grown in different agricultural systems					
D3.6	Ph.D thesis – Speciation and bioavailability of Fe and Zn in the cereal grain (se also D2.8)	SHu	12-2010	7	O	

\* Deviations are to be further discussed in D

#### Milestones list (from application)

<b>Workpackage 3</b>					
<b>Milestone No</b>	<b>Milestone title</b>		<b>Delivery date</b>	<b>Fulfilled (ok) or deviations (d)*</b>	
M3.1	Crops 2007 analysed for S and Se molecular species		04-2008	Ongoing	
M3.2	Crops 2007 analysed for selected bioactive metabolites and vitamins		04-2008	Ongoing (D)	
M3.3	Speciation analysis of Fe and Zn in cereal grains (year 1)		08-2008	Awaiting harvest	
M3.4	Crops 2008 analysed for S and Se molecular species		04-2009		
M3.5	Crops 2008 analysed for selected bioactive metabolites and vitamins		04-2009		
M3.6	Speciation analysis of Fe and Zn in cereal grains (year 2)		08-2009		

<b>Workpackage 4</b>						
<b>Deliverable No</b>	<b>Deliverable title</b>	<b>Lead scientist</b>	<b>Delivery date</b>	<b>Allocated scientific person moths</b>	<b>Type of deliverable</b>	<b>Fulfilled (ok) or deviations (d)*</b>
D4.1	Differences among cultivation systems with respect to the development of the immune function	CLA	12-2010	18	S	
D4.2.	The effect of cultivation systems with respect to health, antioxidant and nutritional status, and physical activity	CLA	12-2010	18	S	
D4.3.	Preference test between diets of different cultivation systems	CLA	12-2010	10	S	
D4.4	Ph.D. –thesis with the suggested title: “Influence of organic food on health using the rat as a model for humans”	CLA	10-2010	4	O	

\* Deviations are to be further discussed in D

#### Milestones list (from application)

<b>Workpackage 4</b>					
<b>Milestone No</b>	<b>Milestone title</b>		<b>Delivery date</b>	<b>Fulfilled (ok) or deviations (d)*</b>	
M4.1	Complete diets of ingredients from Cropsys of cultivation year 1 are prepared		01-2008	D	
M4.2	Analytical methods regarding the development of the immune function of rats are available		02-2008	D	
M4.3	Rat experiments including chemical analyses of cultivation year 1 is performed		01-2008	D	
M4.4	Complete diets of ingredients from Cropsys of cultivation year 2 are prepared		01-2009		
M4.5	Rat experiments including chemical analyses of cultivation year 2 are performed		01-2010		
M4.6	Overall data-analyses are performed		07-2010		

<b>Workpackage 5</b>						
<b>Deliverable No</b>	<b>Deliverable title</b>	<b>Lead scientist</b>	<b>Delivery date</b>	<b>Allocated scientific person</b>	<b>Type of deliverable</b>	<b>Fulfilled (ok) or deviations</b>

				moths		(d)*
D5.1	Presentation of results at international conference	SHB	12-2010	0	C	
D5.2	Draft Scientific publication "Bioavailability of micronutrients from different organic production systems in humans"	SHB	12-2010	14	S	
D5.3	Draft Scientific publication "Bioavailability of bioactive metabolites from different organic production systems in humans"	SHB	12-2010	14	S	
D5.4	Draft Scientific publication: Interaction between micronutrients and bioactive metabolites from different production systems in humans	SHB	12-2010	14	S	
D5.5	Ph.D thesis	SHB	12-2010	4	O	D (Not changed since last progress report)

\* Deviations are to be further discussed in D

#### Milestones list (from application)

<b>Workpackage 5</b>			
Milestone No	Milestone title	Delivery date	Fulfilled (ok) or deviations (d)*
M5.1	Ethical approval obtained	05-2007	Ok
M5.2	Production of diets for the intervention study, year 1, including the stable isotope labelled diet	10-2007	Ok
M5.3	Recruitment of study population for the 1.year intervention study	10-2007	Ok
M5.4	Complete diets for human and rats of 2007 analysed for Fe, Zn, Se and S	02-2008	Ongoing
M5.5	Complete diets for human and rats of 2007 analysed for bioactive metabolites and vitamins	04-2008	Ongoing (D)
M5.6	Diet (2007) analysed for phytates	05-2008	Ok
M5.7	Completion of dietary intervention study year 1	06-2008	Ok
M5.8	Collection and distribution of human samples from 1.year intervention study	09-2008	Ok
M5.9	Analyses of human samples from 1.year intervention study	02-2009	
M5.10	Production of diets for the intervention study, year 2	10-2008	Awaits harvest
M5.11	Recruitment of study population for the 2.year intervention study	10-2008	Ongoing
M5.12	Urine, plasma and faeces (2007 harvest) analysed for Fe, Zn and Se and enriched stable isotopes	02-2009	
M5.13	Urine and plasma (2007 harvest) analysed for bioactive metabolites and vitamins	02-2009	
M5.14	Complete diets for human and rats of 2008 analysed for Fe, Zn, Se and S	02-2009	
M5.15	Complete diets for human and rats of 2008 analysed for bioactive metabolites and vitamins	04-2009	
M5.16	Diet (2008) analysed for phytates	05-2009	
M5.17	Dietary intervention study year 2 completed, J	06-2009	
M5.18	Collection and distribution of human samples from 2.year intervention study,	09-2009	
M5.19	Analyses of human samples from 2.year intervention study	02-2010	
M5.20	Estimation of absorption of trace elements	06-2010	
M5.21	Urine, plasma and faeces (2008 harvest) analysed for Fe, Zn and Se and enriched stable isotopes	02-2010	
M5.22	Urine and plasma (2008 harvest) analysed for bioactive metabolites and vitamins	02-2010	
M5.23	Estimation of absorption of bioactive metabolites	06-2010	
M5.24	Multivariate data analysis of data	12-2010	

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

### **01-2007 to 08-2008:**

*WP1,D1.2: Corporation with Kristian Thorup-Kristensen (coordinator of VegCure) has been initiated in order to further strengthen the scientific aspects of crop rotation on mobilization of micronutrients with potentially interesting human health effects. Results from the first growing season 2007-08 are expected to be available in the spring 2008.*

*In the budgets from the CropSys field stations (Lyndvad, Foulumgaard, Flakkebjerg), the CropSys coordinator Jørgen E. Olesen have recently stated that OrgTrace is expected to cover sorting and storage costs, which apparently were not included in the current budgets for these field stations. This extra cost was unexpected to the members of OrgTrace who believed that all costs were included to provide market quality products. In order to solve this issue of misunderstanding, it has been agreed that a joint application will be send to the FØJOIII board in order to have these unaccounted costs covered. Jørgen E. Olsen has agreed to initiate and submit this application and Søren Husted has agreed to co-sign it. No further action has been taken to this date.*

*WP2, D2.5: A Post Doc. has been employed instead of a PhD student.*

*WP2, D2.6: PhD project was started on February 2007 (originally planned January 2007) – due to delay of grant.*

*WP5, D5.1: Application for the ethical approval has been sent in the first week of September and acceptance of the ethical approval is awaited within 4-6 weeks from the date of submission.*

*WP5, D5.5: Ph.D thesis. This deliverable has been changed as we have decided to apply for a post.doc/ assistant professor instead of a Ph.d student. It is expected that post.doc Alicja Budek will be appointed assistant professor and will be the daily responsible for the project.*

### **09-2007 to 08-2008:**

WP1. Status: problem solved. The application to ICROFS was granted. The extra costs to handle, sort and prepare the harvested products have been allocated to the experimental stations.

WP1, M1.2 Status: delayed. The sample preparation time was much more comprehensive than originally anticipated and as a consequence we have to move the deadline for M1.2 to primo 2009. However, the methodology is in place and no complications are anticipated. The outcome of OrgTrace will by no means be affected by this delay.

WP3, M3.2. Status: delayed. Due to the excessive time requirements for the planning and pre-treatment of the large number of crop samples, incl. careful and continuous protection against oxygen, light, high temperatures, metals etc. this part is delayed. The analyses of all vitamins are expected to be completed ultimo Dec. 2008, and analyses of all bioactive metabolites are expected to be completed ultimo Jan. 2009.

WP4, M4.1 – 4.3 Status: delayed. The employment of a phd-student in WP4 has been delayed due to a combined announcement of several phd-positions under the Research School SAFE (DJF, AU). The phd-position was announced in May, and a candidate has been recruited (start Oct.1, 2008). The head of ICROFS, Niels Halberg, has been informed on this matter, which will postpone the submission of the phd-thesis to 2011 instead of 2010 as originally planned. However, it is expected that most of the WP4 results can be presented in the final project report despite that the phd-thesis is not submitted until the following year. Regarding the budget (see appendix 1 for details), a solution has been negotiated between Niels Halberg and Charlotte Laurid-

sen and it has been agreed that the phd-grant will be co-financed primarily in year 2011, whereby the delay should have no consequences for the overall budget of OrgTrace. In addition, we are currently planning on running one rat experiment containing both harvest years (36 diets) instead of two subsequent rat experiments (18 diets) in order to cope with the project delay as described under point C.

*WP5, M5.5.* Status: delayed. As the total number of diets and feed samples is quite limited, it is optimal to analyse diets and feed in a row thus making the laboratory work more efficient. When receiving the rat feed, the analyses are expected to be completed within two months.

## **Project publications and other products:**

### **1. Papers, Submissions and Products from Organic Eprints archive**

#### **Submitted papers with peer-review:**

Kristian Holst Laursen, Thomas Hesselhøj Hansen, Daniel Pergament Persson, Jan Kofod Schjoerring and Søren Husted (2008) Multi-elemental fingerprinting of plant tissue by semi-quantitative ICPMS and chemometrics. *Journal of Analytical and Atomic Spectroscopy*.

Emese Kápolna, Peter R. Hillestrøm, Kristian H. Laursen, Søren Husted, Erik H. Larsen Effect of foliar application of selenium on its uptake and speciation in carrot. *Analytical and Bio-analytical Chemistry*

Pia Knuthsen is co-authoring: Maiani G et al. Review. Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Accepted for publication in *Mol.Nutr.Food Res*.

PK co-author of: Maiani G et al. Review. Carotenoids: Actual knowledge on food sources, intakes, stability and bioavailability and their protective role in humans. Accepted for publication in *Mol.Nutr.Food Res*

#### **Popular papers, reports and columns:**

Alicja Budek: Short press column in a local newspaper "Frederiksberg Lokaltidningen" about the human intervention

Susanne Bügel and Søren Husted: Short press column in popular science magazine "Helse" and two short press columns on the home page [www.foodoflife.dk](http://www.foodoflife.dk)

PK contributed to a background chapter – Baggesen DL et al. Fødevarer og sundhed i relation til økologiske fødevarer - to Økologisk Hvidbog: Udvikling, vækst og integritet i den danske økologisektor. ICROFS, June 2008.

#### **Other products (oral presentations, public meetings, field days, etc.)**

##### **Meetings:**

SHu and KHL (KU-LIFE) participated in steering meetings organised by "sister projects" VegCure (Odense Kongres Center Jan 30, 2007) and CropSys (Sandbjerg Gods, Feb. 28,

2007). At these meetings oral presentations were given by SHU.

KHL participated in steering meetings organised by “sister projects” VeqCure 29/1 (KU-LIFE) and CropSys 27/2 (Sandbjerg Gods) and 28/5 (Flakkebjerg).

The first annual OrgTrace meeting for all project participants were held at LO-Skolen Ellsnore, 11-13/3 2008

### **Oral Presentations:**

SB: Short oral presentation at FQH meeting in Modena in June 2008

KHL: Multielemental fingerprinting by semi-quantitative ICP-MS and chemometrics. 4<sup>th</sup> Nordic Conference on Plasma Spectrochemistry, Loen, Norway June, 2008.

DAP: Zinc, iron, sulfur and phosphorous interrelationships in the barley grain analyzed by SEC-ICP-MS and LC-ESI-MS. 4<sup>th</sup> Nordic Conference on Plasma Spectrochemistry, Loen, Norway June, 2008.

### **Conference participation and Posters:**

EmeKa presented the poster: „Se-enrichment of carrot and onion via foliar application” poster presentation at 4<sup>th</sup> Nordic Conference on Plasma Spectrochemistry, Loen, Norway June, 2008. The poster was awarded a prize as winner of the poster section.

MaSol: Poster by M Søltoft, P Knuthsen and J Nielsen: Bioactive metabolites in crops, diets and human samples. Receto Enviro Symposium, Nov. 5-6 2007, Denmark.

MaSol: Poster by Malene Søltoft, Pia Knuthsen, John Nielsen: Comparison of extraction methods for analysis of flavonoids in onions. T1.43, p.207-208, Polyphenols Communications 2008 Vol.1, presented at XXIVth International Conference on Polyphenols, 7-11 July 2008, Salamanca, Spain

### **Scientific education:**

SHU, KHL: Nordtest Workshop on Uncertainty in Sampling. 12-13 April, 2007, Hillerød.

KHL: PhD kursus: Representative sampling of solids-characterization and analysis, 27-31 august 2007, DTU

MaSol: PhD-course in mass spectrometry at University of Southern Denmark passed by Malene Søltoft.

KHL: Chemometrics for environmental sciences, Inst. for Grundvidenskab, ved Giorgio Tomasi og Jan Christensen, 28-29 februar 2008

MaSol participated in the course “Interpretation of MS and MS-MS mass spectra – Small molecules”, by Prof. Wilfried Niessen, NL, at Waters, Hedehusene, DK, 10-11 Oct. 2007. MaSol attended in the ENVIRO symposium “Environmental Strategies and Solutions Symposium” Nov. 2007.

MaSol joined the “Chemometric Workshop” at Faculty of Life Sciences, KU-LIFE, 28-29 Febr. 2008.

## **G. National and international cooperation**

Charlotte Lauridsen has conducted a sabbatical (Oct., 2007-May, 2008) at Bristol University, United Kingdom, by Prof. Chris Stokes, which was granted by OECD through a research fellowship entitled “Establishment of immunological methods for investigating the effect of organic and conventional farming systems on food quality and health”.

International cooperation is also performed by Charlotte Lauridsen and co-workers of WP4 through their participation in the Core organic project entitled “Quality analysis of critical control points within the whole food chain and their impact on food quality, safety and health (QACCP)”. The results of our sub-project in the Core-organic project will be highly relevant for the Orgrtrace project in the sense that the same food (carrots of the Vegquire-project) will be used.

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**8. Budget**
**Account for any change in budgets**
**B. Total budget for the whole project (1.000 DKK)**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel	72	48	63	74	17	202
Technical personnel	35	6	30	25	0	61

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel	2394	1586	2182	2377	633	6779
Technical personnel	949	147	815	701	0	1664
Other operational costs	1238	760	911	1250	179	3101
Equipment		8				
Others (please specify)	156	184	156	51	46	437
Direct costs	4738	2685	4066	4380	859	11990
Indirect costs (20% of direct costs)	947	537	813	876	171	2398
Total	5686	3222	4879	5256	1031	14388

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## 9. Signatures and stamps

Name	Institute	Date	Signature
Head of project  Søren Husted, Ph.D, Assoc. Professor	KU-LIFE-PSSL	29/9-2008	

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 Appendix I. Detailed budget

**A. Budget for each participating institute (1.000 DKr)**
**KU-LIFE (PSSL)**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel	16	15	19	16	2	52
Technical personnel		1				1

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel	510	493	510	523	110	1636
Technical personnel						
Other operational costs	293	404	293	116	56	869
Equipment						
Others (please specify)	43	21	43	51	46	161
Direct costs	846	935	846	690	212	2683
Indirect costs (20% of direct costs)	169	187	169	138	42	536
Total	1015	1123	1015	828	254	3221

**DTU-FOOD (Chem1)**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel	13	12	13	16	4	45
Technical personnel	4	1	4	3	0	8

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel	409	318	409	510	124	1361
Technical personnel	118	15	118	91	0	2235
Other operational costs	126	48	126	106	28	308
Equipment						
Others (please specify)						
Direct costs	653	381	653	707	152	1894
Indirect costs (20% of direct costs)	130	76	130	141	30	379
Total	783	457	784	848	182	2272

One scientific man-month has been moved from 2007 to 2010.

**DTU-FOOD (Chem2):**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel	12	13	12	12	1	38
Technical personnel	2	2	2	2		6

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel	426	437	426	443	38	1334
Technical personnel	59	61	59	60		181
Other operational costs	85	111	85	65	43	305
Equipment						
Others (please specify)						
Direct costs	570	609	570	569	81	1831
Indirect costs (20% of direct costs)	114	121	114	113	16	366
Total	684	731	684	683	97	2197

A postdoc has been employed instead as a phd student who started April 2007. The residual funding has been transferred to 2008. The salary for the hired postdoc has been established by combining the budgeted PhD salary with the costs budgeted for courses etc. for the PhD student. Therefore, the budgeted salary and man months for scientific personnel are different from that in the budget. Similarly, the budgets for scientific personnel for 2009 and 2010 are different from those in the original application.

The budgeted operational expenses for 2008-2010 have been reduced by the costs for PhD courses and academic supervision.

**KU-LIFE-HuNut:**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel	10	4	10	2		16
Technical personnel	20		19	6		25

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel	390	154	390	78		622
Technical personnel	528		504	162		666
Other operational costs	247	114	300	97	25	536
Equipment		8				8
Others (please specify)						
Direct costs	1166	276	1194	337	25	1832
Indirect costs (20% of direct costs)	233	55	238	67	5	366
Total	1399	332	1432	404	30	2199

Originally it was planned that a phd student should be appointed to the study, but it has been decided that a more experienced researcher was needed. However, assistant professors are more expensive and therefore we have changed the number of man month according to the available resources.

**AU-DJF (DAH):**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel	20	4	8	27	10	49
Technical personnel	9	2	5	14	0	21

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel	659	182	388	761	361	1692
Technical personnel	244	55	134	388	0	577
Other operational costs	464	59	83	864	28	1034
Equipment						
Others (please specify)						
Direct costs	1367	296	605	2013	389	3303
Indirect costs (20% of direct costs)	273	59	121	402	77	660
Total	1640	355	726	2415	466	3963

The employment of the ph.d.-student was delayed, which caused the major budget deviation for 2008. The unused resources have been transferred to 2009.

**AU-DJF-GBI:**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months	1,14		1,14	1,14		2,28
Scientific personnel						
Technical personnel						

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries	60		60	62		122
Scientific personnel						
Technical personnel						
Other operational costs	1		1	2		3
Equipment						
Others (please specify)						
Direct costs	61		61	64		
Indirect costs (20% of direct costs)	12		12	12		
Total	73		73	76		150

**AU-DJF-Jyndeivad:**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel						
Technical personnel						

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel						
Technical personnel						
Other operational costs						
Equipment						
Others (please specify)	38	54	38			92
Direct costs	38	54	38			92
Indirect costs (20% of direct costs)	7	11	7			18
Total	45	65	45			110

**AU-DJF-Flakkebjerg:**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel						
Technical personnel						

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel						
Technical personnel						
Other operational costs						
Equipment						
Others (please specify)	38	54	38			92
Direct costs	38	54	38			92
Indirect costs (20% of direct costs)	7	11	7			18
Total	45	65	45			110

**AU-DJF-Foulumgård:**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel						
Technical personnel						

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel						
Technical personnel						
Other operational costs						
Equipment						
Others (please specify)	38	54	38			92
Direct costs	38	54	38			92
Indirect costs (20% of direct costs)	7	11	7			18
Total	45	65	45			110

**AU-DJF-Årslev:**

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months						
Scientific personnel						
Technical personnel						

Year:	Original budget 2008	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries						
Scientific personnel						
Technical personnel						
Other operational costs						
Equipment						
Others (please specify)	22	22	22			47
Direct costs	22	22	22			47
Indirect costs (20% of direct costs)	5	4	5			9
Total	27	26	27			54

**Co-financing by institutions:****Total-contribution all participants**

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months					
Scientific personnel	12	9	14	15	50
Technical personnel	7	5	6	6	24

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries					
Scientific personnel	457	433	565	662	2117
Technical personnel	201	145	148	152	646
Other operational costs	59	193	189	51	492
Equipment					
Others (please specify)					
Direct costs	717	771	902	865	3255
Indirect costs (20% of direct costs)	143	154	199	192	689
Total	860	925	1101	1057	3944

Name of Institute and department: IHE-LIFE-KU (HumNut)

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months	4	2	1	1	
Scientific personnel	2	1	1	1	
Technical personnel	2	1			

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries					
Scientific personnel	90	45	45	45	225
Technical personnel	60	30			90
Other operational costs	30	50	40	11	131
Equipment					
Others (please specify)					
Direct costs	180	125	85	56	446
Indirect costs (20% of direct costs)	36	25	17	11	89,2
Total	216	150	102	67	535

**Comments:**

Name of Institute and department: **DTU-Food (Chem1)**

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months	2	2	3	3	10
Scientific personnel	2	2	2	2	8
Technical personnel			1	1	2

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries	94	96	128	132	450
Scientific personnel	94	96	98	101	389
Technical personnel			30	31	61
Other operational costs	10	10	13	13	46
Equipment					
Others (please specify)					
Direct costs	104	106	141	145	496
Indirect costs (20% of direct costs)	21	21	28	29	99
Total	125	127	169	174	595

**Comments:**

**Co-financing**Name of Institute and department: **DTU-FOOD (CHEM2)**

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months					
Scientific personnel	2	2	3	2	9
Technical personnel	1	0	0	0	1

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries					
Scientific personnel	109	125	142	117	493
Technical personnel	29	0	0	0	29
Other operational costs	14	13	15	12	54
Equipment	0	0	0	0	0
Others (please specify)	0	0	0	0	0
Direct costs	152	138	157	129	576
Indirect costs (20% of direct costs)	31	28	32	26	116
Total	183	166	189	155	692

**Comments:**

**Co-financing**Name of Institute and department: **KU-LIFE-PSSL**

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months					
Scientific personnel	4	4	4	4	16
Technical personnel	4	4	4	4	16

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries					
Scientific personnel	164	167	171	174	676
Technical personnel	112	115	118	121	466
Other operational costs					
Equipment					
Others (please specify)					
Direct costs	276	282	289	295	1142
Indirect costs (20% of direct costs)	55	56	58	59	228
Total	331	338	347	354	1370

**Comments:**

**Co-financing**Name of Institute and department: **AU-DJF-DAH**

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months	0	0	4	8	12
Scientific personnel	0	0	4	8	12
Technical personnel	0	0	0	0	0

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries					
Scientific personnel	0	0	109	225	334
Technical personnel					
Other operational costs	5	120	121	15	260
Equipment					
Others (please specify)					
Direct costs	5	120	230	240	594
Indirect costs (20% of direct costs)	1	24	46	48	119
Total	6	143	276	288	713

**Comments:**

**Co-financing**Name of Institute and department: **AU-DJF-GBI**

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Man-months					
Scientific personnel					
Technical personnel					

Year:	Consumption 2007	Expected consumption 2008	2009	2010	Total
Salaries					
Scientific personnel					
Technical personnel					
Other operational costs					
Equipment					
Others (please specify)					
Direct costs					
Indirect costs (20% of direct costs)		18,4	19,1		
Total		18,4	19,1		