



## **Progress Report 2005 and Application for Continuation in 2006**

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

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### 1. Research program

Research in organic farming 2000-2005 (DARCOF II)

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### 2. Project title and number

Production of organic milk of high quality considering the future demands for use of organically produced feed and natural vitamins (II-2)

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### 3. Head of project

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### 4. Participating institutes

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## 5. Other project staff

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6. Project period (month, year)

Start of project:0102

End of project:1206

## 7. Midterm description of the project, its results and progress, and application for continuation in 2005

### A. Project summary

Future regulations for organic production of milk stipulate that all feed must be organically produced, and that the cow can only be fed natural vitamins and antioxidants. This leads to feeding changes of such importance that it must be presumed to influence the antioxidative capacity and thus the shelf-life of the milk. The project aims at elucidating the nature of the consequences that these new regulations for organic feeding and use of natural vitamins and antioxidants for cows in organic milk production will have on the antioxidative capacity of the milk. New studies show that uric acid in milk is an important antioxidant of significance for the oxidative stability of milk. During the project, it is the aim to increase the supply of selenium and the development of endogeneous antioxidants (uric acid and glutathione peroxidase) in order to produce oxidatively stable milk and dairy products.

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

No.	Work package title	Participants*	Budget (1.000 DKK)	Start	End	Deliverable no(s):
1	Antioxidative capacity of raw milk	<u>Jacob Holm</u> <u>Nielsen</u>	1668	0102	1205	D1-D11
2	Cheese production and oxidative stability of cheeses with high levels of uric acid and selenium	<u>Leif H. Skibsted</u>	330	0104	1205	D5, D8 &D12

\* Responsible participants are underlined

### B. Objectives and expected achievements

To elucidate the effect of introducing new rules for organic feeding and use of natural vitamins and antioxidants on the antioxidative capacity of the milk from cows producing milk under organic conditions. Furthermore to increase the selenium supply and the formation of endogenous antioxidants (uric acid and glutathione peroxidase) of the cow in order to produce oxidatively stable milk and dairy products.

The aim consists of the below intermediate aims:

- To study the variation in content of pro- and antioxidants in organic milk from a number of herds in relation to regional variation, feeding and season
- To study whether the natural isomer of vitamin E is a more effective antioxidant than the synthetic isomer of vitamin E
- To study whether the natural carotenoid content in milk has an antioxidative effect
- To study whether cheese exposed to light shows improved antioxidative characteristics if it is based on milk with a high uric acid content
- To make guidelines on how to improve the oxidative stability of the organic milk through feeding

### Achievements

The project aims at investigating the possibility to create processed dairy products (cheese) with an oxidative stability up to the same standards as conventionally produced dairy products. The project will introduce the possibility of using endogenously formed antioxidants (uric acid and glutathione peroxidase) and natural vitamin E to improve shelf-life of organic dairy products. The project is holistic and comprises the chain from stable to table. It involves inter-institutional cooperation and is based on expertise at both KVL and at DIAS.

## C. Midterm results and progress

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

#### WP1 Antioxidative capacity of raw milk, DIAS

##### Glutathione peroxidase activity in milk

A method for analysis of the selenium-dependent glutathione peroxidase (GPX) in milk has been developed. It has not been possible to detect any specific activity of these enzymes in milk. We have tried to induce expression of GPX in milk through a feeding experiment where dairy cows were fed seleno-methionine. The amount of selenium increased with a factor 5, however, it was still not possible to detect any GPX activity in milk. Further, there is very little endogenous glutathione in milk to be used as reducing substrate for glutathione peroxidase. In fact, glutathione added to milk is rapidly metabolized, probably by the concerted action of  $\gamma$ -glutamyl transpeptidase and sulfhydryl oxidase. Thus, if activity of GPX is of importance for the oxidative stability of milk, alternative substrates must be utilized. The results have recently been accepted for publication in International Dairy Journal (see publications)

For the first time glutathione S-transferase (GST) has been isolated and identified (by MALDI-TOF MS) in milk. This enzyme is normally synthesized in the liver, and recent results where cows were fed a high content of tannins (often used by organic farmers to increase bypass of proteins in the rumen of the cow) indicate that this induces a higher level of GST in the milk. This enzyme is a potential candidate as antioxidant in milk, however, as GPX will it need glutathione as substrate. A paper on this subject will be published in the near future.

##### Development of a method to detect stereoisomers of $\alpha$ -tocopherol

A method for detection of the stereoisomers of  $\alpha$ -tocopherol in milk has been developed. It was possible to extract  $\alpha$ -tocopherol from the milk and afterwards derivatize the stereoisomers of  $\alpha$ -tocopherol with ethylenglycol dimethylether and dimethylsulfat as described by Riss *et al.* (1994). The derivatives of the stereoisomers were separated by chiral-chromatography and separated into five peaks, where the first one was all of the 2*S*-stereo-isomers and the next four peaks were the 2*R*-stereo-isomers in the following order: *RSS*-, *RRS*-, *RRR*- and *RSR*- $\alpha$ -tocopherol. By this method we have been able to differentiate between the four 2*R*-stereoisomers of all-rac  $\alpha$ -tocopherol and the 2*S*-stereo-isomers. It was found that milk from cows contains about 85-95% *RRR*- $\alpha$ -tocopherol and about 5-15% of the synthetic 2*R*-stereoisomers (*RSS*-, *RRS*- and *RSR*- $\alpha$ -tocopherol). It was not possible to find any 2*S*-stereo-isomers in milk from cows.

##### Content of synthetic stereoisomers of $\alpha$ -tocopherol in milk

Organic and conventional milk from retail were examined for the content and distribution of the stereoisomers of  $\alpha$ -tocopherol. The results indicate that the ratio of *RRR*- $\alpha$ -tocopherol was

higher in organic milk (92-97%) compared with conventional milk (85-87%). This result indicates that the use of synthetic  $\alpha$ -tocopherol was higher in the conventional milk production. Furthermore the results have provided a basis for a survey of the composition of organic and of conventional milk.

### **Survey of composition of conventional and organic milk from a dairy plant**

In the conventional milk production in Denmark, the use of corn silage has increased during recent years at the expense of the use of grass silage. This change in feeding strategy has affected the composition of the milk in a direction, where milk fat is more saturated while the content of important antioxidants as tocopherols and carotenoids has declined. However, in the organic milk production the use of grass silage is still very important, and on this background we have found it interesting to make a survey of the general composition of untreated conventional and organic raw milk samples collected from silo tanks on organic and conventional dairy plants. The milk has been examined for the content of the following important anti- and prooxidants: Fatty acid composition, Tocopherols and Carotenoids. The results clearly show that organic milk has a lower content of the synthetic isomers of  $\alpha$ -tocopherol. That organic milk contains significantly higher concentrations of *RRR*- $\alpha$ -tocopherol,  $\beta$ -carotene, lutein and zeaxanthine. There is no significant difference in the content of CLA in the two milk types.

### **Survey of milk composition in relation to shelf life in milk from different herds**

There is overall a positive correlation between the content of pasture in the cows' feed and the content of  $\beta$ -carotene in the milk. There is also a positive correlation between the amount of grass silage in the feed and the content of  $\alpha$ -tocopherol in the milk. High amounts of cereal in the feed promote de novo syntheses of the short chain fatty acids in the udder, and the amount of the short chain fatty acids in the milk correlate positive with the amount of corn in the feed. Unsaturated fat from for example pasture is contributing to high amount of CLA and trans fatty acids in the rumen, and these contributes to a low fat percent in the milk, when CLA and the trans fatty acids contribute to low de novo syntheses. Pasture is positively correlated to unsaturated fatty acids as linolenic acid and CLA but opposite correlated to the fat percent of the milk.

Pasture, grass silage and cereals are counting for a high part of the feed for the organic cows, where the feed for the conventional cows mainly consist of corn silage, concentrate, by-products, minerals and hay/straw, and this correlation is also seen in these data. Organic milk contains more  $\alpha$ -tocopherol, *RRR*- $\alpha$ -tocopherol and  $\beta$ -carotene than conventional milk, and these parameters have an opposite correlation to the incident of mastitis and to the overall health status. Many mastitis incidents and high negative health status are mostly widespread between the conventional cows. The content of  $\alpha$ -tocopherol in the milk is opposite correlated to mastitis incident and health status. There is variation over the season for the organic farms especially in respect to the composition of the feed and composition of the milk. The results will be published in the near future.

### **Detection of $\alpha$ -tocopherol in isolated MFGM**

Data from oxidation studies indicate that  $\alpha$ -tocopherol in the milk is located in different compartments. To investigate and explain these observations we have isolated the milk fat globule membrane (MFGM) from the core of the milk fat globule, and the fatty acids and  $\alpha$ -tocopherol are being quantified in the membrane and in the core of the globule prior and after light oxidation. It seems like there is more unsaturated lipid in the membrane than in the triglycerides of the core of the fat globule. There is also a tendency towards protection of  $\alpha$ -

tocopherol in the membrane of the fat globule by light, when the milk has been exposed to light for 24 hours. Maybe the temperature of 4°C makes the fat more crystalline, so there is not so much movement between the membrane and the core of the fat globule and within the membrane. The research is still on going.

### **Oxidation in milk with high and low content of $\alpha$ -tocopherol**

An experiment was conducted to investigate the effect of synthetic  $\alpha$ -tocopherol on the oxidative stability of milk. The content of the  $\alpha$ -tocopherol was determined in the milk from cows, which had been fed with normal feed (barley whole crop 10%, rapeseed cakes 8%, maize silage 36%, grass silage 34%, rolled barley 6%). The contribution of synthetic  $\alpha$ -tocopherol to the feed was removed for three days, and the content of  $\alpha$ -tocopherol in the milk was analysed. After depletion, 3.400 IU of  $\alpha$ -tocopherol were supplied to the feed per day. The content of  $\alpha$ -tocopherol in the morning milk was subsequently followed during the period, of both depletion and after and at individual level every day. The secretion of  $\alpha$ -tocopherol to the milk was found to vary between the individual cows. The content of  $\alpha$ -tocopherol in the milk was declining during the period where the feed was depleted for synthetic  $\alpha$ -tocopherol, and the content of  $\alpha$ -tocopherol in the milk increased when synthetic  $\alpha$ -tocopherol (3.300 IU) was added to the feed again.

The ratio (in percentage) of the synthetic 2*R*-stereo-isomers (*RSS*-, *RRS*- and *RSR*-) in the milk was found to decrease during the depletion period of vitamins from the feed. However, after supplementation of 3.400 IU per day of synthetic  $\alpha$ -tocopherol to the feed it increased again. It was relevant to investigate the oxidative stability of milk with high (700  $\mu$ g/L) and low (400  $\mu$ g/L) content of  $\alpha$ -tocopherol. Milk with high and low content of  $\alpha$ -tocopherol was exposed to fluorescence light with an intensity of 3.200 lux for 24 hours, and the degradation of  $\alpha$ -tocopherol and accumulation of hexanal in the milk was followed for 24 hours. The accumulation of hexanal in the two types of milk was identical, indicating that the high concentration of  $\alpha$ -tocopherol could not inhibit the lipid oxidation.

### **Oxidation in milk with high content of unsaturated fatty acids and high content of $\alpha$ -tocopherol**

A study of the effect of synthetic  $\alpha$ -tocopherol on the oxidative stability of milk with a high content of unsaturated fatty acids has been conducted. After 16 days of supplementation with *all-rac*- $\alpha$ -tocopheryl acetate the ratio of the synthetic  $\alpha$ -tocopherol stereo-isomers increased to 11.2% of the total  $\alpha$ -tocopherol content in the milk. The cows were receiving in average 907 mg natural  $\alpha$ -tocopherol from the feed per cow per day and 2282 mg *all-rac*- $\alpha$ -tocopherol from the vitamin supplement, which gave a total of 3189 mg *all-rac*- $\alpha$ -tocopherol per cow per day. If the contribution of  $\alpha$ -tocopheryl stereo-isomers with no discrimination between the stereo-isomers had to be 37.4%, 26.8% and 35.8% of *RRR*- $\alpha$ -tocopherol, the 2*S*-stereo-isomers, and the synthetic 2*R*-stereo-isomers, respectively. However, the actual distribution of the isomers in the milk was 88.8%, 11.2% and 0.0% of *RRR*- $\alpha$ -tocopherol, the synthetic 2*R*-stereo-isomers, and the 2*S*-stereo-isomers, respectively. This result clearly shows that only a minor part of the synthetic isomers are transferred to the milk. The amount of  $\alpha$ -tocopherol in the milk increased with 29 %, however the milk with the highest concentration of  $\alpha$ -tocopherol was found to be unstable to induce oxidation (light or copper) than the milk with the low  $\alpha$ -tocopherol content. Kamal-Eldin *et al.* (1996) has described that  $\alpha$ -tocopherol can act as a co-prooxidant when present at high concentrations together with known prooxidants like transition metal ions, lipid peroxides or other prooxidants. In the present study

where the milk have a high content of polyunsaturated fatty acids and oxidation is accelerated by storage in light or by addition of copper(II) it can be hypothesed that the combination of oxidative stress and strong prooxidant as linoleic and linolenic acid,  $\alpha$ -tocopherol will act as a co-prooxidant. The results will be published in: "Selective excretion of 2R-stereo-isomers and effect on oxidative stability of milk of *all-rac*- $\alpha$ -tocopheroyl acetate supplementation to feed of dairy cows" by Tina Slots, Ivan Nielsen, Leif H. Skibsted and Jacob Holm Nielsen.

### **Metal catalysed- and photooxidation in milk**

A preliminary study of metal catalysed oxidation and photooxidation in milk has been conducted. When the milk is added copper alone there is a little accumulation of hexanal, but no degradation of  $\alpha$ -tocopherol. When the milk is both added copper and exposed to light, the hexanal accumulation is somewhat greater, but there is still no degradation of  $\alpha$ -tocopherol. Is the milk exposed to light alone the greatest accumulation of hexanal and the greatest degradation of  $\alpha$ -tocopherol is observed. It is necessary to perform further studies of the oxidation mechanism of  $\alpha$ -tocopherol to understand the mechanisms behind that no degradation of  $\alpha$ -tocopherol is observed, when copper is high.

### **Oxidation in milk with supplement of natural $\alpha$ -tocopherol to the feed**

In a study where the cows have received supplement of the natural isomer of  $\alpha$ -tocopherol and selenium as seleno-methionine, the uptake or excretion of  $\alpha$ -tocopherol and  $\beta$ -carotene seems to compete, when the content of  $\beta$ -carotene in milk, where the cows have received  $\alpha$ -tocopherol supplement to the feed is lower than in the milk, where the cows didn't receive any supplement. Selenium seems to have a positive effect on the uptake or excretion of  $\beta$ -carotene. Furthermore have selenium no effect on the uptake or excretion of  $\alpha$ -tocopherol, when the content of  $\alpha$ -tocopherol is the same in these two milk types. There are no significant differences between the oxidation products of the fatty acid oxidation and lipid hydroperoxides, in the milk, when the milk is exposed to fluorescent light at 2,000 lux.

### **WP2 Cheese production and oxidative stability of cheeses with high level of uric acid and selenium**

Acid gels have been produced from four types of milk (supplements of vitamin e, selenium, vitamin e + selenium and control milk) by addition of gluconolactone. The gels were stored 3 weeks in respectively light and dark at 5°C. Samples for riboflavin, secondary oxidation products and dityrosine measurement were frozen until analysis after 1, 4, 7, 14, 21 and 24 days of storage.

Results:

The data are still under processing

Four types of cheese have been produced with different concentration of organic selenium. The cheeses were packaged in a modified atmosphere consisting of approximately 25 % CO<sub>2</sub> and 75 % N<sub>2</sub>. The cheeses were stored at 5°C in a display counter under conditions similar to those in retail stores. Samples were exposed 24 h per day to light (1000 lx) at the surface of the cheese. The samples were rotated regularly to minimize the possible temperature differences in the display cabinets. Chemical, spectroscopic and colour measurements were frozen until analysis after 0, 3, 7, 13, 37, 56 days of storage. Cheeses stored in dark were stored 8 weeks.

Results:

The supplement of organic selenium did not give significant changes in the colour of the cheese surface. It was possible to separate the four types of cheese in relations to the riboflavin content. Other analyses are still under processing.

Liposomes were produced from phosphatidylcholine as a model system of milk. The liposome system allows the lipid soluble components to be present in the lipid phase without the presence of a co-solvent, while the water soluble antioxidants can be added to the aqueous phase of the liposome. Conjugated dienes were measured to compare the oxidation.

Results:

E-vitamin was a very good antioxidant and delayed the lag phase perceptible when 0.5, 1, 2 mol % of the lipid were incorporated. Selenium did not have an antioxidative effect when up to 10 mol % of the lipid was incorporated.

Radical up scavenging:

The ABTS radicals scavenging in the presences of selenium proceed slower than in the presences of E-vitamin. (ABTS radicals is a semi stable radical often used in assays).

## C.2 Fulfilment of deliverables and milestones

*(To be completed for each work package)*

WP1 Antioxidative capacity of raw milk, DIAS	Time schedule	Deviations, if any*
<b>Deliverables</b>		
1 Report for the Danish Dairy Board	10/2002	Finished
2 Report for the Danish Dairy Board	04/2003	Finished
3 Report for the Danish Dairy Board	10/2003	Finished
4 Report for the Danish Dairy Board	04/2004	Finished
5 Report for the Danish Dairy Board	10/2004	Finished
6 Final report for FØJO and the Danish Dairy Board	12/2005	12 months
7 Paper for Mælkeritidende	2003	Published
8 Paper for Mælkeritidende	10/2005	12 months
9 The shelf-life of organic milk in relation to feeding (paper)	8/2003	See publication list

10 The antioxidative capacity of natural vitamin E in milk (paper)	6/2005	Submitted
11 Mechanistic study of vitamin E i MFGM (paper)	8/2005	Submitted
13 Report for the Danish Dairy Board	4/2005	
14 Report for the Danish Dairy Board	10/2005	
15 Report for the Danish Dairy Board	4/2006	
16 Report for the Danish Dairy Board	10/2006	
17 Defence of Ph.D. thesis	12/2006	
<b>Milestones</b>		
1 Characterization of milk from organic herds finished	5/2005	Finished
2 Variation in the oxidative stability of milk	5/2005	Finished
3 Feeding experiment with natural vitamin E finished	3/2005	Finished
4 Model experiments with vitamin E incorporated in the fat globule membrane of milk	3/2005	12 months
5 Light-induced processes in cheese are described in relation to the level of natural antioxidants	12/2004	12 months
10 The antioxidative capacity of natural vitamin E in milk (paper)	6/2005	6 months
11 Mechanistic study of vitamin E i MFGM (paper)	8/2005	6 months
13 Report for the Danish Dairy Board	4/2005	
14 Report for the Danish Dairy Board	10/2005	
15. Report for the Danish Dairy Board	4/2006	
16 Report for the Danish Dairy Board	10/2006	
17 Defence of Ph.D. thesis	12/2006	

WP2 Cheese production and oxidative stability of cheeses with high level of uric acid and selenium	Time schedule	Deviations, if any*
Deliverables		
5 Report for the Danish Dairy Board	10/2004	Finished
8 Paper for Mælkeritidende	10/2005	12 months
12 Influence of urate and selenium on light-induced oxidative changes in cheese	12/2004	12 months
<b>Milestones</b>		
5 Light-induced processes in cheese are described in relation to the level of natural antioxidants	12/2004	12 months

\* Deviations are to be further discussed in D

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

Due to maternity leave of the ph.d-student Tina Slots (formerly known as Tina Lund-Nielsen) will the project be prolonged with 12 months to the end of 2006

## E. Project publications and other products

### 1. Products from Organic Eprints archive

[Produkter under 1 skal kopieres fra Organic Eprints. Dette gælder også for produkter, som kun delvist er finansieret af FØJO. Listen fra Organic Eprints kan findes på hjemmesiden <http://www.okoforsk.dk/projekt/index.html> under "Project publications" på de enkelte projekter.]

Stagsted, Jan and Nielsen, Jacob H. (2004) [Purification of glutathione-binding proteins from bovine milk and identification of glutathione S-transferase](#). [preprint]\*

Østdal, Henrik; Weisbjerg, Martin; Skibsted, Leif and Nielsen, Jacob H. (2004) [Antioxidative capacity of milk with a high urate content](#). Working Paper.\*

Lund-Nielsen, Tina; Nielsen, Jacob H.; Skibsted, Leif and Nielsen, Ivan (2004) [The effect of a-tocopherol supplementation to the feed of dairy cows on the oxidative stability of milk](#). Submitted International Dairy Journal.\*

Nielsen, Jacob Holm; Lund-Nielsen, Tina and Skibsted, Leif [Higher antioxidant content in organic milk than in conventional milk due to feeding strategy](#). Online at <http://www.darcof.dk/enews/sep04/milk.html>. Newsletter

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

\* 25-75% financed by DARCOF

\*\* 5-25% financed by DARCOF

## F. Scientific education

Ph.d-student Tina Slots is employed at the project.

## G. National and international cooperation

Since March 2004 participating in the EU programme "Improving quality and safety and reduction of cost in the European organic and "low input" food supply chains" coordinated by professor Carlo Leifert, University of Newcastle upon Tyne. Jacob Holm Nielsen and Tina Slots will be responsible for a survey of milk composition in relation shelf life in Danish, Swedish, English and Italian milk.

## **H. Critical reflection on the project**

The dairy industry is very interested in the results from the survey of organic and conventional milk and there will in the future be applied for money to continue developing feeding strategies in order to produce organic milk with another composition than the conventional milk. An application to FØJO III has been submitted **“Organic Milk of High Quality – Development of Production Concepts Based on grazing of the Dairy Cows and Gentle Treatment of the Milk during Handling and Processing”**

## 8. Budget

### A. Account for any change in budgets

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

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

Year:	Consumption before 2004	Consumption 2004	Expected Consumption 2005	Budget 2006	Budget 2007	Total
Man-months						
Scientific personnel	8.5	4.3	9.3	7.3		29.4
Technical personnel	7.4	3.6	3.			14.

Year:	Consumption before 2004	Consumption 2004	Expected consumption 2005	Budget 2006	Budget 2007	Total
Salaries						
Scientific personnel	288	150	337	256		1,031
Technical personnel	163	81	87			331
Other operational costs	98	73	112	20		303
Equipment						
Others (please specify)						
Direct costs	549	304	536	276		1665
Indirect costs (20% of direct costs)	119	67	101	46		333
Total	668	371	637	322		1998

#### Comments:

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## 9. Signatures and stamps

Name	Institute	Date	Signature
Head of project  Jacob H. Nielsen	Dept. of Food Science, DIAS		

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## Appendix I. Detailed budget

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

Name of Institute and department: DIAS, Dept. of Food Science

Year:	Consumption before 2004	Consumption 2004	Expected Consumption 2005	Budget 2006	Budget 2007	Total
Man-months						
Scientific personnel	8.5	4.3	3.3	7.3		23.4
Technical personnel	7.4	3.6	3.			14.

Year:	Consumption before 2004	Consumption 2004	Expected consumption 2005	Budget 2006	Budget 2007	Total
Salaries						
Scientific personnel	288	150	117	256		811
Technical personnel	163	81	87			331
Other operational costs	98	73	57	20		248
Equipment						
Others (please specify)						
Direct costs	549	304	261	276		1390
Indirect costs (20% of direct costs)	119	67	46	46		278
Total	668	371	307	322		1668

**Comments:**

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

Name of Institute and department: KVI, Department of Food Science

Year:	Consumption before 2004	Consumption 2004	Expected consumption 2005	Budget 2006	2007	Total
Man-months						
Scientific personnel			6			6
Technical personnel						

Year:	Consumption before 2004	Consumption 2004	Expected consumption 2005	Budget 2006	2007	Total
Salaries						
Scientific personnel			220			220
Technical personnel						
Other operational costs			55			55
Equipment						
Others (please specify)						
Direct costs			275			275
Indirect costs (20% of direct costs)			55			55
Total			330			330

**Comments:**

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

Name of Institute and department:

Year:	Consumption before 2003	Consumption 2003	Expected consumption 2004	Budget 2005	Total
Man-months					
Scientific personnel					
Technical personnel					

Year:	Consumption before 2003	Consumption 2003	Expected consumption 2004	Budget 2005	Total
Salaries					
Scientific personnel					
Technical personnel					
Other operational costs					
Equipment					
Others (please specify)					
Direct costs					
Indirect costs (20% of direct costs)					
Total					

**Comments:**