## 16 Project description

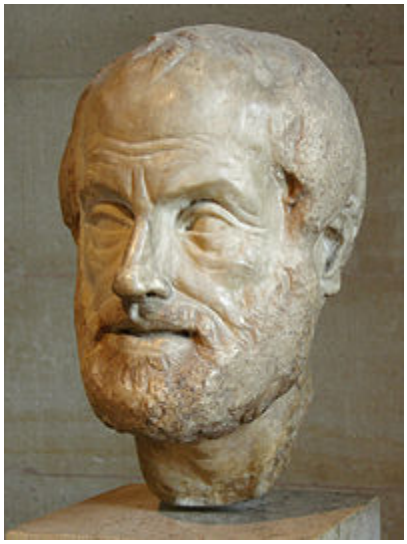
### 1) Summary

Safe drinking and recreational waters are the expected norm in Denmark, but pathogens like *Cryptosporidium* and *Salmonella* and estrogens from pig manure have been shown to leach at high concentrations through intact clay soils. These observations are of general environmental concern, but also a specific problem in the context of fulfilling the EU Water Frame Directive, which requires that there be no ecotoxicological effects of substances leached to freshwaters.

The proposed project will investigate the persistence, dissemination and potential threat to our ground- and recreational waters of these contaminants as influenced by manure management and pretreatment. Due to recent progress in molecular techniques for Real-Time PCR of pathogens, and new developments in high-throughput chemical analysis of steroid estrogenes, the spread of these contaminants can now be traced and quantified in environmental samples.

Today manure is often treated by mechanical separation or additives to provide a range of processed materials. The mechanisms controlling distribution and degradation of pathogens and estrogens during storage and following application to arable soil will be studied, both for manure and selected separation products. The potential for contamination of ground- and recreational waters from manure and processed manure will be investigated via leaching experiments and field validation, using the new techniques available for identification and quantification.

Manure treatments to prevent zoonoses and mitigate ecological risks are a worldwide concern. While the use of state-of-the-art molecular and chemical methods will provide new basic understanding of the regulation of pathogen survival and estrogen persistence in the environment, the research will also serve as documentation of environmental technologies which could support policy development and export of Danish know-how to fight this “worldwide water quality problem number 1”.



**About the acronym PATHOS**

Aristotle's define rhetoric as consisting of three part ethos, pathos and logos. Strategic research programs have a commitment to communicate, and we have chosen our acronym to reflect the emotional appeal of the subject: “leaching of pathogens and estrogens to freshwater”.

“Ethos” is the first part in rhetoric being the credibility of the storyteller (scientist), and the last part “logos” is the use of reasoning to construct an argument.

Within the timeframe of the project we expect to move from “pathos” towards “logos”, and hope to expand our “ethos”.

## 2) Purpose of the project– scientific and in relation to societal/commercial aspects

The overall purpose of the project is to determine to what extend different manure separation techniques can reduce the contamination of surface and groundwater with unwanted contaminants including pathogens, and steroid estrogenes

The immediate objectives of the project are to

1. Optimize and develop new techniques for the quantification of major pathogens and natural estrogenes in manure, soil and water samples.
2. Assess the impact of state-of-the-art, commercial manure separation technologies and storage on distribution and persistence of contaminants.
3. Determine how soil type and separation method affects the transport and fate of pathogens, and natural estrogens from processed manure to freshwater.

The commercial aspects relates primary to immediate objectives 1 and 2. In immediate objective 1 the continued improvement of fast and robust molecular methods for quantification pathogenic microorganisms has a commercial application in also non-environmental science, like food science. The commercial aspect in immediate objective 2 is clearly linked to the verification of manure treatment plants in relation to the documentations of treatment protocol on the fate of pathogens and estrogens. Finally the combined societal and commercial aspects relate primarily to immediate objectives 3 with the focus on drinking water production and safe recreational waters.

## 3) Expected main results from the project

The project will provide knowledge on to what extend the separation technologies currently being developed by Danish companies besides removing nutrient and solids from liquid manure will reduce risks of contaminating fresh water bodies with pathogens and other unwanted compounds present in manure. The entire manure management chain will be examined in order to predict the ultimate fate of contaminants in the environment, and to determine if some separation technologies are superior in avoiding contamination with pathogens and natural estrogenes compared to the present handling of manure.

The project will significantly increase the ability of commercial and governmental research laboratories to quantify a range of important pollutants found in manure. These priority pollutant compounds include microbial pathogens, and natural estrogenes. Detection and quantification techniques will be based on state-of-the-art technologies including DNA/RNA based quantification of pathogens by RT-PCR and mass spectrometry (MS) based methods for quantifications of estrogenes..

The project will further more describe how different separation technologies for livestock manure will redistribute contaminants between a solid fraction and a liquid fraction and investigate the persistence of these contaminants in the different fractions.

The project will determine how the physical and chemical characteristics of the solid and liquid phase as well as the soil will affect the redistribution of contaminants after application in the field

Finally, the possibility decreasing the risk of contaminating freshwater systems following application of different separation products of manure will be investigated using a suite of advanced soil column methods, and state of the art instrumented field sites and quantitative risk assessment.

**4) Concept and premise of the project including state of the art**

While the contamination risk posed by nutrients leaching from manure-treated fields is well recognized, that posed by leaching of steroid estrogenes and pathogens has received much less attention. Nevertheless, leaching though soil has recently been identified as contamination pathways leading these contaminants to the aquatic environment. High numbers of the large *Crytosporidium* sp. as well as *Salmonella* sp. and virus were found to leach through clayey soil columns (Dalsgaard et al in prep.), and field experiment suggest that estrogens originating from manure can contaminate our freshwater bodies in concentrations exceeding the effect concentration towards water-organisms (Kjær et al 2007). Given the massive production of animal waste in Denmark and worldwide (Steinfeld et al. 2006) these findings are of broad environmental concern indicating an urgent need for further research into technology/management practices minimizing the contamination risk posed by these contaminants.

Currently, manure separation technologies, are increasingly seen as a solution to the problems of nutrient surplus in areas of intensive animal production. In Denmark, around 3% of the animal slurry is currently being separated and 4 out of 5 facilities were established in 2006 (Birkmose, 2007). While we know that anaerobic digestion (for methane production) reduces or even eliminates the problems encountered with pathogens and natural estrogens, at present there is no knowledge on how slurry separation technologies, may affect the risk of pathogens and natural estrogenes leaching to the aquatic environment. There is an imminent need to investigate how these changes in animal manure management may affect the spreading of the aforementioned contaminants to the aquatic environment.

A prerequisite for conducting this type of research is fast, highly sensitive and selective analytical chemical methods and microbiological assays that analyze mixtures of compounds in matrices as manure, soil, surface water. Pathogens can now be quantified using molecular technologies based on DNA for enumerating numbers of specific microrganisms and mRNA for quantification active microorganisms (Jacobsen and Holben; 2007). The development of highly selective MS based technologies for drugs and natural hormones is another area where new possibilities in analytical chemistry would improve our knowledge on the quality of environmental waters (Jacobsen and Halling Sørensen 2006 and Jacobsen et al 2004). Methods to quantify drugs and their metabolites in environmental samples are virtually non-existing, why the understanding of the fate of such compounds in soil and groundwater environments are limited.

The ultimate fate of contaminants in slurry, and their potential for leaching to aquatic environments, will depend on the characteristics of the slurry or slurry separates, the application method, as well as on soil conditions. We find that important knowledge of these factors is currently lacking since most fate studies were conducted without addition of manure and have not taken into account the potential impact of preferential transport (Kjær et al. 2007; Lægdsmand et al., submitted).

Separation of liquid manure (slurry) will not in itself remove contaminants or pathogens, but it will produce a liquid fraction that will infiltrate better in the soil (Sommer et al., 2004; Petersen et al.,

2003a), and a solid fraction that is readily composted. In a study of contaminants in sewage sludge it was shown experimentally that several contaminants persisted for 6-12 months within an anaerobic sludge string, but were metabolized effectively at the aerobic waste-soil interface (Petersen et al., 2003b). This suggests that dry matter removal to facilitate slurry infiltration will also promote aerobic conditions in the soil hereby enhancing the degradation of contaminants. Preferential flow (e.g. macropore flow) will enhance the transport of strongly sorbing and easily degrading contaminants, as the contact between the contaminants and the soil matrix will be reduced (Kjær et al. 2007; Lægdsmand et al. submitted). To the extent that contaminants have infiltrated into the soil matrix after application (as with liquid separates) preferential flow may reduce the potential for leaching due to flow bypass of the contaminants located in the soil matrix. Preferential transport enhances both the colloid and colloid-facilitated transport (Lægdsmand et al. 1999; MacCay et al. 1993), both being of relevance for many pathogens as well as for strongly sorbing contaminants such as estrogens and some pharmaceuticals.

The current storage technique will also influence the fate of these contaminants. The bacterial pathogens (*Escherichia coli* O157, *Salmonella* sp., *Campylobacter*sp. and *Listeria* sp) were found to die out quickly in composting manure heaps, while in dairy slurry the pathogens survived for up to six months (Nicholson et al. 2005). Pharmaceuticals and estrogenes are degraded relatively fast in aerobic environment but may endure in slurry tanks. Manure stored under anaerobic conditions (as in slurry tanks) were found to have a high estrogenic activity, which is related to type, sex, age and reproductive status of the animals (Lorenzen et al., 2004).

**5) Innovative value, impact and relevance of the project**

In general it is believed that our groundwater resources are protected from contaminants, since the transport through soil is hampered by degradation and sorption processes. However, leaching though structured soil has recently been identified as a contamination pathway, transporting contaminants like pathogens and estrogenes to the aquatic environment through soil cracks and bio-pores. Given the massive production of animal manure as well as the increasing intensification of livestock production in Denmark and worldwide these findings are of general environmental concern, but also a specific problem in the context of fulfilling the EU Water Frame Directive. There is thus an urgent need for further research into new technologies/management practices minimizing the contamination risk posed by these contaminants.

The PATHOS project will be the first to study in a chain perspective how manure separation technologies currently under rapid development with Danish companies in the forefront, may reduce the environmental impact of these emerging contaminants (natural estrogenes and pathogens). Such knowledge will be very valuable and give the industries within this area a competitive advantage and a research-based foundation for expansion and future export.

The project will provide a very well defined area of research linking to the quantitative detection of pathogens in environmental samples. Molecular based detection of pathogens is an emerging business area in environmental, food and medical research. However, a great challenge exists in the further development, validation and commercialisation of highly specific molecular tools suited for environmental samples. The society impact of more precise determination of transport and survival of pathogens would be of great importance also for other areas, e.g. within food production and processing.

The society interest for highly precise and robust methods for the quantification of drug and metabolites of these are evident. Methods to be used in environmental samples are virtually non-existing and thus governmental monitoring programs can not currently include these compounds. Also as a consequence of the lack of proper methodology the understanding of the fate of such compounds in soil and groundwater environment is limited.

The assessment of the fate of contaminants following manure separation and application in agriculture as well as any associated risks for freshwater contamination will be of direct relevance and interest to national authorities, the EU as well as consumers of drinking water.

**6) Project's methodology and anticipated results**

**Work Packages 1. Quantification techniques for pathogens, and steroid estrogenes**
(KU-FARMA (head, ½ post-doc), KU-LIFE-IVP (1/3 PhD), GEUS (1/2 post-doc), DIANOVA (1 PhD), COBIO)

WP1.1 Validation of established methods on new matrices. In WP1 we will continue the development of cutting edge technologies providing the ability to detect and quantify not only the presence, but also the activity of pathogenic microorganisms and organic contaminants in environmental waters using new mass spectrometric methods capable of analyzing steroid estrogenes, pharmaceuticals, antibiotics and antiparacetics and their degradation products simultaneously and introducing modern microbiological techniques to the field.. The methods will further more be used for analysis the compounds in the different matrices formed using the new manure handlings techniques in WP 2 and WP 3 for assessing the fate of compounds and microorganisms. Similarly, established quantitative PCR techniques for *Campylobacter* spp. developed for chicken dung (Keramas et al. 2004, Lund et al. 2004, Lund & Madsen 2006, Li et al. *in press*) will be adapted and employed for the matrices soil, manure and water.

WP 1.2 Methods to quantify viable and infective pathogens. We will exploite the potential of using molecular quantification technologies based on quantitative PCR of cDNA generated from soil mRNA. Our present technology targeting *Salmonella* spp. is using a sequence specific purification technology (Jacobsen and Holben, 2007). This technology confers high specificity, but the capture technology will due to its principles exclude genes not used as capture probes. We have recently successfully used another purification system to quantify indigenous soil microbial functional genes on mRNA level (Bælum et al unpublished). Using this new approach we will make a cDNA copy of the full transcript from microorganisms present in environmental waters since the reverse transcript is generated using random hexamer primers. The potential for using this technology in automated high throughput systems targeting not only *Salmonella* sp. *inv*A genes will be pursued.

WP 1.3 Fate of steroid estrogens or metabolites. The fate of these compounds may be very different from that of the parent compounds. In many cases metabolites are more polar than the parent compound, and thus are more mobile. Especially the need of expanding the methods for also analyzing the conjugates in existing methods will be pursued (Lykkeberg et al 2006). GC-MS-MS will be used for the analysis of transformation processes and metabolite formation in model systems (known bacteria and sorbents), abiotic degradation and liver homogenates (microsomes of pigs) under different redox conditions (Lykkeberg et al 2006). Accelerated Solvents Extraction will be used combined with solid phase extraction and other clean up methods before analysis (Jacobsen

and Halling-Sørensen 2006). Main metabolites will be identified by use of NMR and included in the final analytical method if possessing activity.

Deliverables:
1.1 A scientific article description of a method to create cDNA copies from environmental water samples allowing the potential assessment of activity of various pathogens.
1.2 A report and scientific articles describing the development and validation of primer sets for DNA and mRNA based Real time quantification of pathogens as *Salmonella* sp., C*ampylobacter* sp. and *Cryptosporidium* sp.
1.3 A scientific article describing the development and validation of analytical methods for estrogenes, and important metabolites in soil, drainage and manure matrices.

**Work Packages 2: Impact of manure separation technologies and storage on contaminants**
(KU-LIFE-IJV (head, 1 PhD), KU-LIFE-IVP (1/3 PhD), KU-FARMA (1/4 post-doc), GEUS (1/4 post-doc), Kemira Waters Denmark A/S, Grundfoss New Business (InFarm) A/S, Novozymes)

This work package will determine the distribution of contaminants following different manure separation and treatment technologies and their persistence during storage of the liquid fraction.

WP 2.1 Information on distribution of contaminants. Information about pathogens and natural estrogenes as well as chemical (nutrients, particle size distribution) and biological (biochemical composition, degradability) parameters in liquid and solid fractions from operating farm slurry separation units with a range of different technologies, slurry types and animal species (pig, cattle, mink, raw/biogassed slurry) will be collected and evaluated. This will be based on an existing database and sample archive of about 40 operating slurry separation facilities (from a concurrent VMP-III project) as well as information from the participating industrial partners, previous projects and the literature.

WP 2.2 Separation technology and storage effects on contaminants. Comparisons of contaminant distribution, fate and chemical and biological properties of separates between commercially available manure separation technologies from the industry partners of the project will be carried out at a few farm operations Treatments with slurry spiked with different contaminants will also be included. To address special contaminant problems, laboratory studies will be conducted on differently contaminated animal slurries, applying newly emerging separation methodologies (e.g. various enzymes and biological additives, *Novozymes A/S*). Test pathogens of different size and with different surface properties will be spiked into manure samples for precise determination of distribution.

Knowledge on the persistence of contaminants in the liquid fractions of separated manure will be obtained in storage experiments with different fractions of separated slurry including degradation of natural estrogenes and survival of pathogens. Experiments will be carried out in partner laboratories.

The following parameters will be determined in WP 2.1 and WP 2.2 . Chemical parameters: C, $N_t$/$NH_4$, P, VS, pH, biochemical composition (van Soest) and degradability (NIR, Stenberg et al., 2005). In addition speciation of P in micro-particulates, which have a high leaching potential and possible association with some contaminants, will be investigated in the liquid fraction using $^{31}$P-NMR and isotopic dilution techniques (providing information regarding the stability of the P-particulate complex (Hamon and McLaughlin, 2002). Pathogens and fecal indicators: Standard culture-based

phenotypic microbiological methodologies will be used for the detection and distribution of bacterial pathogens and fecal indicators, i.e. *E. coli* and enterococci, in separated manure fractions. The bacteriophage, *Salmonella* Thyphimurium 28B, will be used as a model indicator for fate of viruses. Steroid estrogens will be analysed using MS-based techniques. Extraction methods will be optimised to the different biosolids and wet fractions.

Deliverables:
2.1. A report describing the distribution of selected contaminants and other constituents in liquid and solid fractions of animal slurry following a number of different fractionation technologies
2.2. A publication describing possible relationships between chemical and biological properties of fractions and the distribution and fate of contaminants after slurry separation and storage.

**Work Package 3.** Persistence and leaching of contaminants in field-applied manure
(AU-DJF) (head, 1 PhD), KU-LIFE-IVP (1/3 PhD), KU-FARMA (1/4 post-doc), GEUS (1/4 post-doc), Kemira Waters Denmark A/S, Grundfoss New Business (InFarm) A/S

This work package deals with the redistribution and leaching to the aquatic environment of contaminants when applied to agricultural soils in slurry or slurry separation products. We hypothesize that the redistribution of contaminants after application to the soil will significantly affect the potential for transport to the aquatic environment and ultimate fate. This will be analysed using different scales of investigation including micro-scale studies of redistribution of slurry and contaminants; transport studies using undisturbed soil columns; and for pathogens field scale experiments. Different slurry separates will be used in the investigations to ensure the broadest span of physical, chemical and microbiological characteristics in the separates, based on the knowledge achieved under WP2. Analysis of contaminants in water, slurry and soil will be done using established methods as well as methods developed in WP1.
WP3.1: Redistribution**:** Identify mechanisms controlling the redistribution of slurry or separation products and associated contaminants as affected by dry matter content of separation products and soil water potential. This will be accomplished by quantifying effects of and interactions between slurry properties and soil water potential using dialysis (Petersen et al., 2003a). The mobilization and fate of estrogens will be studied in incubation experiments, and by amendment of slurry to repacked soil adjusted to different soil water potentials.
WP3.2: Leaching: Investigate the effect of soil type and slurry properties on the potential for leaching of contaminants through intact soil. Leaching of natural estrogens and pathogens will be investigated in intact soil columns in a set-up with realistic lower boundary conditions in either small columns in the laboratory or large soil monoliths in a semi-field set-up (Laegdsmand et al. in prep. or Leagdsmand et al., 2007). The intact soil will be amended with slurry/separates and then subjected to realistic leaching events.
WP3.3. Field experiment**:**
Transport of manure applied pathogens will be assessed through a controlled field experiment monitoring the leaching of pathogens for 1 year after manure application. The field experiment will take place at two experimental field sites already used for monitoring of pesticide leaching within the frames of the Danish Pesticides Leaching Assessment Programme (Lindhardt et al., 2001). This project can thus benefit from on-going monitoring activities, including state-of-the-art monitoring equipment and a well-proven sampling strategy for analyzing leaching processes of contaminants (Kjær et al., 2005; Kjær et al., 2007). The field sites are managed by normal agricultural practice and the type of slurry or slurry separates and application method applied will be selected based on the prelininary results of WP3.1 and WP2.2.

| 7) Projectplan | 2008 | | | | 2009 | | | | 2010+2011 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **WP 1 Quantification techniques for pathogens, veterinary drugs and estrogenes** | | | | | | | | | | | | |
| 1.1: Established methods in new matrices | | | | | | | | | | | | |
| - *MS methods* | | | M1.1.1 | | | | | | | | | |
| - *PCR methods* | | | | | | M1.2.1 | | | | | | |
| - *reporting* | | | | | | | | | | M1.3.1 | | |
| 1.2: Pathogens | | | | | | | | | | | | |
| - *DNA based quantification* | | | | M1.2.1 | | | | | | | | |
| - *mRNA of viable cells* | | | | | | | | | M1.2.2 | | | |
| - *reporting* | | | | | | | | | | | | M1.2.3 |
| 1.3: Estrogenes and drugs | | | | | | | | | | | | |
| - *structure main steroids* | | | | | | | | | | | | |
| - *structure drug metabolites* | | | | | | | | | | M1.3.2 | | |
| | | | | | | | | | | | | M1.3.3 |
| **WP 2 Impact of manure separation technologies and storage on contaminants** | | | | | | | | | | | | |
| 2.1: Screening for distribution of contaminants | | | | | | | | | | | | |
| - *collection of separ. fractions* | | | M2.1.1 | | | | | | | | | |
| - *sample analyses* | | | | | M2.2.1 | | | | | | | |
| - *reporting* | | | | | | | M2.3.1 | | | | | |
| 2.2: Separation technologiy and storage effects on contaminants | | | | | | | | | | | | |
| - *slurry separation experiments* | | | | | M2.2.1 | | | | | | | |
| - *sample analyses* | | | | | | | M2.2.3 | | | | | |
| - *lab-scale experiements* | | | | | | | | | | M2.2.2 | | |
| - *storage experiment* | | | | | | | | M2.2.4. | | | | |
| - *reporting* | | | | | | | | | | M2.2.5. | | M2.2.6. |
| **WP 3 Persistence and leaching of field applied contaminants to ground and surface water** | | | | | | | | | | | | |
| 3.1 Redistribution | | | | | | | | | | | | |
| - *dialysis and incub. exp.* | | | | | | M3.1.1 | | | | | | |
| - *repacked soil experiments* | | | | | | | | M3.2.1 | | | | |
| - *reporting* | | | | | | | | | | M3.3.1 | | |
| 3.2: Column experiments | | | | | | | | | | | | |
| - *soil sampling and setup* | | | | M3.2.1 | | | | | | | | |
| - *leaching experiments* | | | | | | | | | M3.2.2 | | | |
| - *reporting* | | | | | | | | | | | | M3.2.3 |
| 3.3: Field experiment | | | | | | | | | | | | |
| - *monitoring design* | | M3.3.1 | | | | | | | | | | |
| - *field monitoring* | | | | | | | | | | M3.3.2 | | |
| - *reporting* | | | | | | | | | | | | M3.3.3 |

**Milestones:**

**WP1**

M1.1.1: Extraction of mRNA from watersamples
M1.1.2: Validation of mRNA based quantification to viability of cells
M1.1.3: Publication of cDNA based method
M1.2.1: Design of primersets for efficient simultaneous PCR amplification of the target pathogens
M1.2.2: Validations of primers in environmental samples
M1.2.3: Publication of quantitative PCR method to simultaneous detection of pathogens
M1.3.1: Development of analytical tool to quantify steroid estrogens and metabolites.

M1.3.2: Quantifications of steroid estrogen and metabolites in soil, drainage and manure matrices.
M1.3.3: Publications of steroid estrogens quantification methods.

**WP2** M2.1.1: Screening of liquid and solid fractions from commercial farm operations
M2.1.2: Analysis of samples from screening completed
M2.1.3: Publication of screening study results
M2.2.1: Full-scale experiment with existing, commercial manure separation technologies
M2.2.2: Lab-scale experiments with newly emerging separation methodologies
M2.2.3: Analysis of samples from manure separation experiments completed
M2.2.4: Storage experiment with manure and liquid fractions
M2.2.5: Publication of results on different existing and new technologies effect on contaminant distribution.
M2.2.6: Publication of storage experiment results

**WP3** M3.1.1: Redistribution of manure components
M3.1.2: Leaching of contaminants in manure
M3.1.3: Publication of dialysis and incubation studies
M3.2.1: Soil sampling and test of experimental setup
M3.2.2: End of leaching experiments and analysis
M3.2.3: Publication of columns experiment results
M3.3.1: Drainage sampling equipment installed and tested
M3.3.2: End of field monitoring and analysis
M3.3.3: Publication of monitoring results

**8) Legal and ethical aspects**

With increased demand on the quality of our ground- and recreational waters, all members of society should do their best to protect this limited resource. Danish farmers are concerned about this demand and will do their outmost to avoid new environmental problems related to their agricultural practise, including the very economically profitable livestock production.

The project provides new insight into the spreading of pathogens, and natural estrogenes into Danish freshwaters. The use of molecular techniques on environmental samples to quantify pathogens using mRNA technique is on the forefront of science and validation of these emerging technologies are needed in relation to future high throughput techniques.

The Danish industry for environmental technology is currently expanding their efforts on solutions to the problem of excess manure nutrients in relation to the land available for disposal. The project will contribute significantly by shedding light on possible other benefits in terms of sanitary and health issues of their technology, issues which from an international perspective has strong potential for promoting export of the developed technologies.

**9) Publication and dissemination strategy**
Scientific results will be published in the best peer-revieved journals within the respective fields of study. As many papers as possible should have authors from two or more partners to encourage exchange of ideas and approaches.

Communication to the public will have high priority, and it will be channelled through popular science journals, press releases and a center homepage that will be established as one of the centers first activities. The participation of a private organisation within the water sector (DANVA) will enable more direct communication lines to the end user of the knowledge produced. The private companies involved in the project should facilitate the dissemination of knowledge in the industry, and therefore agreements on intellectual property rights of innovation created in the project will be negotiated and signed at the start of the project. .
The center intends to arrange one larger international symposium (start of 2011) where the press will be invited and results communicated to the public.

**10) Innovation**
The MANUTECH project will be the first to

- Document whether manure separation technologies currently under rapid development with Danish companies in the forefront, may reduce the environmental impact of newly identified emerging contaminants (veterinary drugs, natural estrogens and pathogens).
- Establish method that can quantify the content of Campylobacter spp. Salmonella sp., Cryptosorium sp. in soil, manure and water. (eller måske:Establish quantitative techniques determining Campylobacter spp. Salmonella sp., Cryptosorium sp. in the soil, manure and water samples. )
- Developed separation technique based on enzymes and biological additives

These innovative contributions are highly relevant with large societies and commercial impact as described in section 5.

**11) Participating parties' scientific competencies and contributions to the project**

The Department of Geochemistry at The **Geological Survey of Denmark and Greenland GEUS** are providing in-house cross disciplinary competence in assessing chemical and microbiological contaminants transport to groundwater based on combinations of geomicrobiology, analytical chemistry and field and soil core based experimental design. The geomicrobiology group is lead by professor Carsten Suhr Jacobsen and is world leading in use of molecular based methods to assess survival and activity of microorganisms in soil and water. The field validation and leaching testing facility (VAP) is lead by senior scientist Jeanne Kjær and provides an unmatched facility to allow testing of leaching of chemicals, natural estrogenes and pathogens transport to surface and groundwater in real size field facility.

The environmental zoonotic microbiology group at **University of Copenhagen, Faculty of Life Science**s**,** are lead by professor Anders Dalsgaard and are world leading in assessing fate and survival of various pathogens in the external environment including water, soil and foods. Using a range of traditional and molecular-based technologies pathogens (bacteria, vira, and parasites) and fecal indictors are detected and their viability and infectivity assessed. The group has undertaken research funded by various Danish bodies as well as internationally funded research, in particular the EU, see e.g. the Early Stage Training Network (Marie Curie), TRAINAU (www.trainau.dk).

The environmental chemistry group at **University of Copenhagen, Faculty of Pharmaceutical Sciences**, is lead by Professor Bent Halling-Sørensen. This group are world leading in development of chemical based detection systems for quantification of natural estrogenes in environmental samples. The group will facilitate the use and implementation of the GC-MS based technologies for the detection of selected important natural estrogenes in manure, processed manure and

environmental samples from soil and water. ERApharm project (www.erapharm.org) and CREAM project http://www.veluxfondene.dk, funded by the Velux foundation.

The Department Agroecology and Environment at **Aarhus University, Faculty of Agricultural Sciences (FAS), Department of Agroecology and Environment** is working with the agricultural soil and its interaction with the plants and atmosphere. One of the key facilities in the Institute is the soil physical laboratory with a long history of in measuring the structural and hydrological characteristic of structured soil. The newest development in the laboratory is a method for investigating transport of contaminants in structured soil using realistic boundary conditions which has been developed by Project Scientist Mette Lægdsmand. Senior Scientist Søren O. Petersen has developed the dialysis set-up and described an algorithm for determining the effect of slurry organic matter and soil moisture on contaminant redistribution and turnover. Academic employee Preben Olsen is part of the project group around the leaching testing facility (VAP) and has been involved in the initiation and construction of the VAP facilities and the ongoing monitoring activities. He has diverse experience in dealing with environmental problems related to the agricultural production.

The soil fertility and plant nutrition group at **University of Copenhagen, Faculty of Life Sciences**, led by professor Lars Stoumann Jensen, has extensive research experience with both agricultural (manures, crop residues) and urban (sewage sludge, MSW composts) waste products, their quality, utilisation and effects on soil nutrient cycling, soil quality and losses of N and P to the environment. Associate Professor Enzo Lombi, is an expert on soil biogeochemistry and bioavailability of phosphorus, trace elements and metalloids, including natural attenuation and ecotoxicology of inorganic contaminants.The group has significant expertise in stable and radio-isotopic tracer methodologies, new spectroscopic techniques (e.g. NIR, NMR, synchrotron) and chemometry for characterising organic matter quality or element speciation.

The company **Kemira Waters A/S** (a subsidiary of the international Kemira Oy corporation) has a long standing position as producer of sewage and waste water chemicals, but has recently developed activities on animal manure and slurry separation technologies, and now markets several different concepts, based on simple technologies and environmentally harmless additives.

The company **Grundfos New Business A/S** is Grundfos' cooperate venture company.
Infarm as well as Grundfoss Biobooster, Grundfos Sensor Division, and Microrefinery are all companies owned by GNB. GNB is owned 100 % by Grundfos Holding. The goal of GNB is to find and develop business areas that in the long run will strengthen Grundfos core activities. In the current project the company Infarm will collaborate by supplying large scale treatment facilities for manure treatment.

The company **Novozymes** is producing a large suite of different biological based additives including compounds to facilitate manure treatment. The activities relating to the current project is to plan experimental setup and provide reagents for small scale experiments on manure treatment.

The company **Dianova** collaboration with governmental institutions as well as private companies and supply services in a very broad field of research, both as consultancy, training courses, diagnostics and laboratory analyses. Dianova sells sera, vaccines, diagnostic analyses and consultancy on behalf of the National Veterinary Institute and the National Food Institute. Dianova represent the institutes in business activities and innovation.

The company **Cobio** (Copenhagen Biotech Supply ApS) is a recent established company aiming at developing and providing kits for DNA and RNA based quantification of microorganisms in environmental samples.

The Danish Water and Waste Water Association (Danva) is a national association of water and sewerage suppliers. The association primarily comprises the larger municipal and private ones, local authorities and counties, suppliers to the water and sewerage industry, the joint municipal environmental centres, advisors and institutions. The object of the association is to look after the common interests of Danish water and sewerage suppliers in promoting a steady and high-quality water and sewerage supply on an environmentally sustainable basis

## 12) Organisation and management of the project

The project will be running for 4 years and will be organised with core activities at GEUS, University of Copenhagen and University of Aarhus. The project will be coordinated by the project leader (Carsten Suhr Jacobsen, research-professor GEUS and University of Copenhagen Life). The project leader will be responsible to the strategic research council. He will make use of a steering board consisting of the project leader, four scientist from the project also being WP leaders (Mette Laegdsmann Scientist University of Aarhus; Lars Stoumann Jensen, professor, University of Copenhagen and Bent Halling-Sørensen, professor University of Copenhagen) and two persons outside the project but working in related areas: Bjørn Kaare Jensen (GEUS, vice-director); Anne Marie Zink (Danish Farmers Association). One of the post-docs in the project will assist the board.

Work package leaders and deputy

| **Workpackage, WP** | **Head of WP** | **Deputy of WP** |
|---|---|---|
| WP1 | Bent Halling Sørensen, KU | Carsten Suhr Jacobsen, GEUS |
| WP2 | Lars Stougaard Jensen, KU-Life | Jens Lund Pedersen, Kemira Waters |
| WP3 | Mette Lægdsmann, AU | Jeanne Kjær, GEUS |

PATHOS will stimulate direct collaboration between companies involved in development and marketing of manure separation technologies. The direct collaboration with the strong research groups on quantification of pathogens and chemical substances contaminating manure would provide a strong and unique opportunity to have the Danish manure treatment technologies tested in relation to no harmful effect on the water environment.

PATHOS supports 4 PhD projects and two post doctoral research associates. Strong candidates for the post docs have been identified but a requitement process from national and international researchers with specialised qualifications within the area. The PhD positions will be partly financed by the Technical University of Denmark (1/3 PhD scholarships), Aarhus University (1/3 PhD scholarship) and University of Copenhagen (2 * 1/3 PhD schlorships). The core scientists have ample experience in PhD education. Candidates will obtain an internationally recognised education with a clear focus on dissemination of results in highly cited journals and at international conferences. The international group of scientist and companies involved will guarantee a high standard of the work and form a strong basis for international networking.

The PhD students in PATHOS will be part of the Research School of Environmental Chemistry, Microbiology and Ecotoxicology (RECETO) or the International research school of water resources (FIVA). The students will be able to take advantage of PhD courses regularly offered by these schools. PhD courses on topics of direct relevance to the project will be anticipated in relation to visits by the international scientists during meetings in the knowledge exchange group.

PATHOS will be a significant player in the development of new techniques for sustainable treatment of manure to avoid contamination problems of our freshwater bodies. Further Ph.D students and post docs will work on molecular based quantification techniques for pathogen quantification. The PhD-candidates and post docs will be able to interact directly with new companies that will be developing and marketing cutting edge techniques. The participation of the waterworks organization DANVA will stimulate efficient knowledge transfer and open new perspectives for development of technologies and tools.

A amount of 150.000 has been reserved for international activities of the center. Professor William E. Holben (University of Montana) has accepted an invitation to work with the center for 6 months in 2009 spending a part of his sabattical in Copenhagen. He has strong background in DNA based analysis of fecal and soil based microbial community. He will further be involved in a focused activity on the use of molecular techniques in environmental samples comprising of Ph.D. courses, International meetings and summerschools.

A small amount has been reserved to host a 2 day project meeting for all participants at least once every year. For a small project like this, the use of full project meetings for communication will be given a highest priority. On top of this, project economy as well as overall research structure will be communicated via project web-site. The project leader has used a similar structure with great success in the former research centre BIOPRO.

The centre leader is cofounder of the small company Copenhagen Biotech Supply ApS (Cobio). The interest of Cobio is to take part in developing and marketing of new molecular based detecting tools, but the wages related to this activity within the projects is paid 100% by Cobio.

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