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Expert Review of Project Description:

Experimental Studies Investigating the Risks of Select Amines (Part A: Human Toxicity)

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Norsk institutt for luftforskning
Norwegian Institute for Air Research
NILU

Expert Review of Project Description

Experimental Studies Investigating the Risks of Select Amines

(Part A: Human Toxicity)

Review Committee Members:

Marit Kopangen
Norwegian Climate and Pollution Agency (KLIF)

Dan Costa
U.S. Environmental Protection Agency (USEPA)

Aage Haugen
National Institute of Occupation Health (STAMI)

Review Committee Coordinator:

Scott Randall
Norwegian Institute for Air Research (NILU)

Executive Summary

Post-combustion CO_2 capture based on amines is currently being developed in Norway. The technology is viewed as effective and promising in terms of CO_2 capture capabilities, however, the effects of amine emissions to human health and the environment are relatively unknown. To address the environmental impacts of amines, NILU and its research partners performed the "CO_2 and Amines Phase I" project from 2008-2009. Based on the Phase I results, the consortium submitted new proposals to Gassnova (CLIMIT) and the Norwegian Research Council. The project proposals have the common name of ExSIRA (Experimental Studies Investigation Risks of select Amines), Phase II. ExSIRA is comprised of three subprojects: Part A (Human Toxicity), Part B (Aqueous and Gas Phase Chemistry), and Part C (Environmental Risks and Modeling).

This report is an expert review of the **Part A** project proposal, which was established for an independent assessment of the quality and feasibility of the proposal. The review committee consisted of 3 experts in the field. Each expert carefully reviewed background material (including Phase I reports) and the Part A project description; based on this work, each expert wrote an individual review of the project plan. These individual reviews were used to create a consensus review for the committee in which this report is based upon.

The consensus review states that the project description as proposed is feasible and that the project team is competent to perform these investigations. The committee also identified a total of 11 specific comments and recommendations throughout the 4 tasks for the project. The committee recommended that these comments should be addressed before the project is funded. The committee's recommendations and comments focused upon the following general points for improvement:

- Additional detail should be added to the research plan to improve clarity.
- More detailed Information should be provided on types of methods to be followed, and how the project team is experienced in these established methods.
- Administrative issues need clarification - such as project management, detailed budget, and collaboration with other related projects.

This report is being delivered to Gassnova (CLIMIT) to complete the pre-project requirements, and in hopes that the ExSIRA Part A project can be funded once the necessary review committee recommendations are addressed by the project team.

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Expert Review of Project Description:
Experimental Studies Investigating the Risks of select Amines
(Part A: Human Toxicology)

1 Introduction

1.1 Background

Currently the most popular technology to capture CO_2 in Norway is through the use of injecting the compound "amines" in industrial processes before the emissions are released to air. Amines are chemical components derived from ammonia, where the hydrogen atoms are replaced by organic groups. While amines are the most efficient and effective technology currently available to capture CO_2, the effects of amine emissions to human health and the environment are relatively unknown.

A theoretical study was performed in 2008 to preliminarily investigate the potential effects of amine emissions to human health and the environment – this project was called "Amine Emissions to Air During Carbon Capture, Phase I: CO_2 and Amines Screening Study for Environmental Risks", partnered by the Norwegian Institute for air Research (NILU), the Norwegian Institute for Water Research (NIVA), the Norwegian Institute for Nature Research (NINA), the University of Oslo Centre for Theoretical and Computational Chemistry (UiO-CTCC), and the Norwegian Institute of Public Health (FHI). A series of reports from this project were released early in 2009, see: http://co2.nilu.no.

Results from the Phase I project revealed that information was lacking regarding the effects of amine emissions, and that experiments would need to be conducted to fill these knowledge gaps. Three proposals representing Phase II were then developed to address these issues, and they were submitted in Summer/Fall 2009:

1. *Amine Emissions During Carbon Capture*
   Experimental Studies Investigating the Risks of select Amines (ExSIRA),
   Part A: Experiment Preparations and Human Toxicity
   Submitted to Gassnova (CLIMIT): May 2009; Partners: NILU, FHI
2. *Amine Emissions During Carbon Capture*
   Experimental Studies Investigating the Risks of select Amines (ExSIRA),
   Part B: Aqueous and Gas Phase Chemistry
   Proposal not submitted.
3. *Amine Emissions During Carbon Capture*
   Experimental Studies Investigating the Risks of select Amines (ExSIRA),
   Part C: Environmental Risks and Modeling
   Submitted NFR (KMB): August 2009; Partners: NILU, NIVA, NINA

ExSIRA Part C was accepted as a partial NFR/industry funded project, which begins work May 2010. ExSIRA Part A was not fully accepted, but a **pre-project** was approved in order to receive input from experts in the field regarding the goals, purpose, and methods set forth in the proposal. It was determined that the most appropriate way to receive constructive input was to establish a review committee made up of relevant experts. This report presents the results of the pre-project,

which consists of the work of the review committee to give input to the ExSIRA Part A proposal.

1.2 Review Committee Members

A total of three members sat on the review committee (see table below). These experts were selected due to their prestigious work in the field, and numerous years of research within human toxicological issues. Selection criteria for the members also included the necessity for a wide range of international expertise in the field.

Review Committee Members:

Name	Position	Organization
Marit Kopangen	Chief Engineer	Norwegian Climate and Pollution Agency (KLIF)
Aage Haugen	Research Director	National Institute for Occupational Health (STAMI)
Dan Costa	National Program Director	United States Environmental Protection Agency (US-EPA)

Review committee members were selected with assistance from Marit Låg (FHI) and Maria Ducinska (NILU). The review committee was coordinated by Scott Randall (NILU).

1.3 Review Committee Mandate

The task of the committee was to provide scientific input in the form of individual recommendations based on the project proposal. The individual recommendations can be found in Appendices A-C, and the project proposal can be found in Appendix D. A summary sheet was also given to the committee to make this mandate clear for all members (Appendix E). These individual recommendations from each member were used to compile a general consensus for the committee which is presented below in Section 2. The review committee was established in January 2010, and concluded in April 2010.

2 Review Committee Consensus

The three individual reviews have been compiled below into a consensus review for the greater committee. This consensus review is broken down into overall comments collected, as well as detailed comments accumulated for each tasks. Any specific points which the committee believes requires attention or modification to the proposal are specifically noted as "**POINT *X*"** below.

2.1 Overall comments

The aim of the proposal is clear and targeted. The study is feasible, with appropriate timelines.

Phase I theoretical work was an extensive literature review which gives a good introduction and basis for this proposal, and in turn provides a good rationale for this study.

The study will contribute with highly important information that can be used in the risk assessment of amine photo-oxidative products.

The project team should be aware that although many data gaps do exist for amines today, more information will become available in 2010 through amine registration under the REACH program.

The project team is a capable group to carry out this work, with the necessary expertise, qualifications, and skills. The team is composed of highly experienced researchers, with excellent publication records in their respective sub-fields. The team has a good track record with this type of work, but the problem to be investigated is relatively new, and the team should be prepared to address many chemical nuances during the study due to this fact.

- **POINT 1**: The description of work lacks a level of necessary detail, which diffuses the proposal in some parts, making a careful review difficult. At some point, preferably in the proposal, specific details for the research plan need to be documented before work begins.

- **POINT 2**: Connection to other projects (especially in regards to the most relevant potential photo-oxidative products) should be mentioned, and how this potential collaboration will influence the timeline and progress of the project.

- **POINT 3**: It is not clear what the potential is for other effects or specific mechanisms to influence the results, but several pathways are possible. This point needs to be discussed and/or prepared for in the proposal.

- **POINT 4**: Project management is not discussed; need to include details regarding this.

- **POINT 5**: The budget *should* be more detailed and include additional discussion.

2.2 Task 1 Comments

The committee believes that Task 1 is a reasonable approach to take to screen for the induction of *in vitro* pro-inflammatory responses, DNA damage, and cell death in lung cell cultures from the photo-oxidative products of amine emissions. This is especially true since the most relevant exposure route for humans will be via inhalation.

- **POINT 6**: Further detail regarding dosimetry and study plans *should* be incorporated (see also POINT 1).

2.3 Task 2 Comments

The committee believes that Task 2 is appropriate in order to establish if amine photo-oxidative products have genotoxic and carcinogenic potential *in vitro*. The task employs standard OECD recommended test systems which are suitable for this work.

- **POINT 7**: Although these assays are standardized, there is no evidence in the proposal that these methods are established at NILU/FHI, in addition, it may also be appropriate for these tests to be performed at test laboratories according to GLP. It will be useful to provide detail regarding this point.

2.4 Task 3 Comments

The committee believes that Task 3 is a good approach in order to investigate if amine photo-oxidative products have the potential to induce specific types of DNA lesions in organ tissue after *in vivo* and *in vitro* exposure, and if such lesions are repaired. It is also reasonable to use the proposed 8-oxoguanine DNA glycosylase (OGG1) targeted knock-out mice because they are sensitive to oxidative stress, and are prone to chemical carcinogenesis by accumulating oxidized DNA bases.

- **POINT 8**: It is unclear how the animals will be exposed and for what duration to allow for an appropriate and meaningful assessment, more detail *should* be added here (see also POINT 1).

- **POINT 9**: It *could* be valuable to perform parallel assessments to enrich the data from the comet assay.

2.5 Task 4 Comments

The committee believes that Task 4 is generally an appropriate way to study the toxic effects of amine photo-oxidative products on the immune system after *in vivo* exposure in mice models.

- **POINT 10**: Exposure routes for this task are vague, and the assays proposed do not explicitly address T1 and T2 cells – although flow cytometric methods are noted, some detail is missing here (see also POINT 1).

- **POINT 11**: It is not clear whether the methods described for this task are established methods used at NILU/FHI. It is not justified why the methods given for this task are being used instead of the well established OECD LLNA test method (OECD TG 429). This point needs to be explained and justified.

3 Conclusion

Overall, the committee is delivering a positive review of the proposal in which concrete recommendations are given to improve the scientific quality. The recommendations were compiled and specifically outlined in Section 2 above. The committee's recommendations and comments focus upon the following general points for improvement:

- Additional detail should be added to the research plan to improve clarity.
- More detailed Information should be provided on types of methods to be followed, and how the project team is experienced in these established methods.
- Administrative issues need clarification - such as project management, detailed budget, and collaboration with other related projects.

The committee proposes that all of the specific recommendations should be incorporated and/or addressed before funding is granted.

This report completes the task of the review committee, and the committee is from this point dissolved. This review report will then be submitted to Gassnova (CLIMIT) for them to further assess the proposal and its potentiality for funding. In addition, the NILU/FHI team will respond to the review and make changes to the proposal where necessary in order to resubmit the proposal for funding consideration.

4 Acknowledgements

The FHI and NILU team would like to sincerely thank the thorough reviews provided by review committee members Dan Costa, Aage Haugen, and Marit Kopangen. Their generous efforts will add to the strength and quality of the proposal.

Appendix A

Individual Review: Dan Costa (USEPA)

REVIEW: AMINE EMISSIONS DURING CARBON CAPTURE.
Phase II: Experimental study investigating risks of selected amines

Phase I of the project area involved extensive literature review of the amines in question. The aim of this proposal is targeted, focusing on the proinflammatory and potential genotoxic, carcinogenic and immunotoxic (including allergy) effects of photo-oxidative products of fugitive amines associated with carbon capture. The topics areas are proposed to be investigated in vivo and in vitro as aligned with the expertise and laboratory capabilities of the applicants. The potential for other effects or specific mechanisms underlying the outcomes of these studies should positive results emerge is not clear at this point, but may serve as the core for further investigation.

Task 1
This study task focuses on the screening of various photo-oxidative amines, nitrosamines and nitramines in lung cell culture. A range of in vitro pro-inflammatory responses (cytokines etc.), DNA damage and pathways to cell death in lung cells are planned. The dosimetry and details of the studies planned are not spelled out but in essence this part of the study appears to be essentially a screening activity incorporating the expertise and historic databases of the investigators involved.

Task 2
This study task addresses the potential in vitro genotoxic and carcinogenic activities of photooxidative products of amines. This section uses the micronucleus assay and mammalian cell gene mutation OECD test (HPRT). Carcinogenicity in vitro will utilize the morphological transformation Syrian hamster embryo (SHE) assay (also OECD). Again no details as to study design or past experience is provided by the investigators but these assays are standardized and seem to be within the expertise of the applicants.

Task 3
The "Organ toxicity: DNA damage and repair" study plan focuses on the 8-oxoguanine DNA glycosylase (OGG1) targeted knock-out mice considered to be sensitive to oxidative stress, and prone to chemical carcinogenesis by accumulating oxidized DNA bases. The research is not unreasonable but it is unclear how the animals will be exposed and for what duration to allow for appropriate and meaningful assessment. The investigators plan to use the comet assay to evaluate DNA-damage in genotoxicity testing. It would be nice to see some other parallel assessments to enrich the data from the comet assay. This study seems to be a unique animal study meriting a fuller analysis.

Task 4
This Immunotoxicity study will examine for effects on the immune system after in vivo exposure. Again the exposure route is in question as are other details, but the follow-on study suggests that instillation of inhalation methods will be used. The overall goal of the proposed research is to explore whether the photooxidative products have immunotoxic potential and can promote allergic responses in mice models. The assays proposed however seem rather routine and don't explicitly address T1 and T2 cells – although flow cytometric methods are noted. The group

may have all necessary infrastructure needed to perform the project but for review more information would be helpful.

Overall assessment
The study is feasible as described but the absence of more detail makes a careful review difficult. The investigators have a track record but this problem is new and has many chemical nuances that be addressed. It would seem that the details at some point need to be put down on paper and carefully assessed to ensure that the best and most meaningful data emerge.

Applicant capabilities: The researchers of this proposal seem to have the necessary expertise to conduct the proposed project and address the questions posed. The research group consists of highly experienced researchers with publication record in relevant fields.

Project management is not described.

Budget
The overall the financial plan need to be discussed

Appendix B

Individual Review: Aage Haugen (STAMI)

AMINE EMISSIONS DURING CARBON CAPTURE.
Phase II: Experimental study investigating risks of selected amines

The aim of the proposal is to investigate the photo-oxidative products of amines in vivo and in vitro. The question of inflammatory, toxic/genotoxic/immunotoxic, carcinogenic and allergy-promoting effects are of great current interest. Previous documentation has provided a good rationale for this study. Mechanisms are not clear, but several pathways are possible.

Task 1
Screening of relevant nitrosamines and nitramines in lung cell culture.
The applicants are planning to study pro-inflammatory responses, DNA damage and different types of cell death in lung cells in vitro. Each participant will mainly continue to focus on his/her current expertise and area of interest.

Task 2
Establish whether photooxidative products of amines have genotoxic and carcinogenic potential in vitro.
The second component of the proposal is to examine genotoxicity in vitro using standard OECD recommended test systems such as micronucleus test and mammalian cell gene mutation test (HPRT). Depending on the results a few compounds will be tested using the Syrian Hamster Embryo transformation assay. No evidence is provided that these methods are established at NILU.

Task 3
Organ toxicity: DNA damage and repair
The study plan focuses on the 8-oxoguanine DNA glycosylase (OGG1) targeted knock-out mice. Mice from this line are considered to be sensitive to oxidative stress and chemical carcinogenesis by accumulating oxidized DNA bases. The research plan is based to a large extend on the groups expertise. The comet assay is widely used for evaluation of DNA-damaging effects in genotoxicity testing.

Task 4
Immunotoxicity: Toxic effects on the immune system after in vivo exposure.
The overall goal of the proposed research is to explore whether the photooxidative products have immunotoxic potential and can promote allergic responses in mice models. The group has all necessary infrastructure needed to perform the project.

Overall assessment
The study is certainly feasible and timelines are provided.
Weakness: The project has clearly defined objective, however there is a lack of details in the research plan. The description of work tasks lacks experimental detail and the proposal is diffuse in some parts. I am therefore not able to fully evaluate the experimental approaches and how the objectives are met.

The researchers submitting this proposal have the necessary expertise in the field and are well qualified for the project and their skills match well with the tasks. The research group consists of highly experienced researchers with publication record in relevant fields.

Project management is not described.

Budget
The overall the financial plan need to be discussed.

Appendix C
Individual Review: Marit Kopangen (KLIF)

AMINE EMISSIONS DURING CARBON CAPTURE.
Phase II: Experimental study investigating risks of select amines
Part A: Experiment Preparations and Human Toxicity
General comments

The phase I projects has reviewed currently available information about health effects of several amines that could potentially be used for CO2 capture. There are data gaps but more information concerning effects of the amines themselves are expected to be available as, among others, a registration of amines are expected under REACH in 2010 according to the REACH registration requirements.

This phase II project focus on photooxidative products of amines, these types of compounds may be mutagenic and carcinogenic. The aim of the project is to establish whether the photooxidative products of amines have inflammatory potential, allergy-promoting, immunotoxic, genotoxic and cytotoxic effects. Information from this project will contribute with highly important information that can be used in the risk assessment of the amines with regard to their potential photooxidative products.

The connection to other amine projects as regards the choice of the most relevant potential photooxidative products has not been described in the proposal. This seems to be a crucial point that could also influence the timeline and progress of this project.

The test proposals consist of both currently used laboratory procedures as well as studies following OECD test guidelines.

Specific comments

Task 1
The aim of this task is to establish the potential of photooxidative products of the amines to induce pro-inflammatory responses, DNA damage and cell death in lung cells.

As inhalation is considered the most relevant exposure route, lung cells toxicity studies are a central part of this project. This is reasonable and the outcome of this task will add useful information to the overall assessment of amines with regard to their potential photooxidative products.

Task 2

The aim of this task is to establish whether photooxidative products of amines have a genotoxic and carcinogenic potential *in vitro*.

Three different OECD tests have been chosen for initial screening. The tests are relevant and are also part of the REACH testing strategy for mutagenicity and carcinogenicity. Gene mutation tests in bacteria have not been chosen as the starting point as in the REACH strategy, however, the choice of in vitro tests with

mammalian cell cultures is relevant. The proposal does not provide information concerning the past experience by the investigators with these particular tests. From a regulatory point of view such tests should be performed at test laboratories according to GLP.

Task 3

Tha aim of this task is to establish whether photooxidative products of amines have the potential to induce specific types of DNA lesions in organ tissue after *in vivo* and *in vitro* exposure and whether such lesions are repaired.

The work will be based on currently used methods in this laboratory. The studies are relevant and the *in vivo* Comet assay is also under consideration in EU as recommended for *in vivo* genotoxicity testing, including application within REACH.

Task 4

The aim is to establish whether the photooxidative products have a general immunotoxic potential, and whether they can promote allergic responses (adjuvance effect) in mice models.

Investigation of the immunotoxic potential is an important aspect. Among other tests “the popliteal lymph node assay” will be used. It is not clear whether this is already an established method used in this laboratory. The preference for this study compared to the well established OECD LLNA test method (OECD TG 429) is not justified.

Appendix D

ExSIRA Part A Project Proposal Description

Project Description

Amine Emissions during Carbon Capture

Phase II: Experimental Study Investigating Risks of select Amines

(ExSIRA)

Part A: Experiment Preparations and Human Toxicity

Norwegian Institute of Public Health (FHI)

Norwegian Institute for Air Research (NILU)

Contents

1 Amine Emissions during Carbon Capture

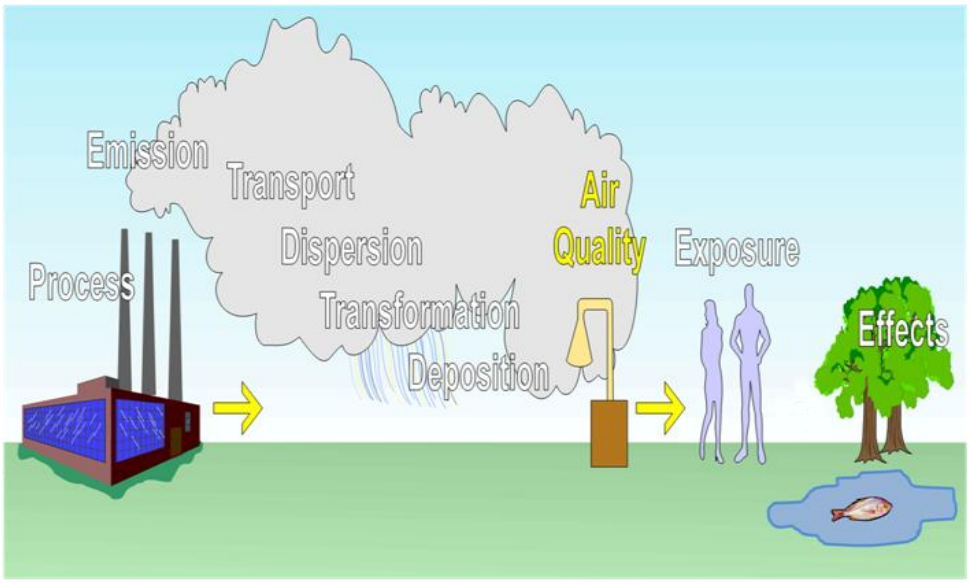
Phase II: Experimental Study Investigating Risks of select Amines

1.1 Background

New technology for CO_2 capture using amines is being implemented in Norway. Emission from the CO_2 capture plants may have effects on the environment and on human health, but their precise nature and magnitude is largely unknown. Toxicological evaluations of the chemicals to be used for CO_2 capture – as well as their degradation products – are therefore needed.

This proposal is a continuation of the Phase I project "Effekter på helse og miljø av utslipp til luft av aminer som følge av CO_2 fangst" (Health and environmental effects of amine emissions from CO_2 capture). The Phase I part of the project was a literature-based study to identify potential effects of amines and secondary compounds generated in the atmosphere due to the CO_2 capture process. It was concluded that there is a significant lack of information on both the chemical transformation of amines and their secondary compounds in the atmosphere and of the effects of amines and secondary compounds on the environment and human health.

In order to evaluate environmental loads and impacts, experiments have to be designed and carried out, and a model framework needs to be developed. This project proposal takes advantage of the results on the findings in Phase I. This project will receive important input from the project "Atmospheric Degradation of Amines" (ADA), which in the spring 2009 was performed in the EUPHORE reaction chamber to determine photochemical reactions in the atmosphere; the chemical analytical methods to be employed will take advantage of methodologies developed in the Phase I project are applied. This information will give valuable information to the experimental studies in phase II.



A model framework capable of simulating chemical reactions in the atmosphere will be established, and the gas phase, water phase and particulate phase will be coupled. Experimental methods will be developed for the atmospheric measurement of amines and their degradation products in gas phase, water phase and particles (see Figure).

Phase I revealed that emissions of the amines used in this process can have severe effects in the environment and on human health. In the stack and in the atmosphere amines can degrade and transform on short time scales to produce toxic compounds which can potentially damage ecosystems and human health. So far only a rough estimate of the ecological and toxicological consequences of the released amines can be provided. Worst case studies have shown that the effects can be severe for some substances shown to be present. This is, however, dependent on the amounts that are emitted or produced in the atmosphere from the emissions. It is necessary to increase the accuracy in the predictions and quantifications of the effects in order to be able to verify whether unacceptable effects are occurring, alternatively, that these effects are so small that they can be neglected. Secondary reaction products identified in a theoretical investigation of the atmospheric degradation of amines have been found to be more hazardous to the environment and human health than the parent amines. However, the results from the Phase I theoretical study have to be confirmed by toxicological experiments, which are to be described in this Phase II project.

Overall, the phase II project will improve our understanding of the transformation of amines in the atmosphere (air, particles, cloud, rain) and the effects of amines and degradation products on ecosystems and human health (see Appendix I), thus contributing to a better evaluation of possible environmental and health risks.

2 The human health part of phase II:

Part A: Experiment Preparations and Human Toxicity

2.1 Background

The potential exposure of the general population to compounds used and formed during CO_2 capture warrants an investigation of their possible health hazards. Some information about the health effects of the amines MEA, AMP, piperazine and MDEA and selected possible photooxidative products is available from the literature. These data have been compiled and critically reviewed in FHI Rapport 2009:3. However, a significant knowledge gap for some potentially harmful photooxidative products remains. To close this knowledge gap a reaserch project has been proposed by The Norwegian Institute of Public Health (FHI) and the Norwegian Institute for Air Research (NILU) in a collaborative effort. The project proposal is conforms with the fact that FHI is not a research contract institute. The proposed research falls within well-established activities at FHI and NILU. However, we believe that data from this research will contribute with hazard information that is essential for an evaluation of possible health risk associated with exposure to the relevant compounds. Other studies that might be considered necessary for the evaluation of health risk from photooxidative products, such as long-term inhalation toxicity and carcinogenicity experiments, may be shown to be necessary but these could then have to be outsourced to contract laboratories.

2.2 Principal Investigators

Norwegian Institute of Public Health (FHI): Birgitte Lindeman, Gunnar Brunborg, Department of Chemical Toxicology (MIKT); Unni C. Nygaard, Martinus Løvik, Department of Environmental Immunology (MIMI); Per E. Schwarze, Marit Låg, Department of Air pollution and Noise (MILS).

Norwegian Institute for Air Research (NILU): Maria Dusinska, Centre for Ecological Economics (CEE).

2.3 Objective

To examine the toxic potential of photooxidative products of amines in selected cellular and *in vivo* models. The initial focus will be on inflammatory, cytotoxic, immunotoxic, allergy-promoting effects and DNA damage and repair. We will assess genotoxicity and potential carcinogenicity *in vitro* using established laboratory procedures as well as OECD recommended test guidelines. The results from this project will contribute to an increased knowledge and thereby estimation of possible health risk from exposure to the relevant compounds. Moreover, on the basis of these results we will eventually suggest whether *in vivo* carcinogenicity studies should be performed and for which substances.

2.4 Motivation

The use of amines to capture CO_2 leads to the emission of large amounts of the amines into the air, which may result in formation of photooxidative products. The possibility, that the compounds represent a health hazard to the population in the neighbourhood, needs to be explored. From the scientific literature we have more knowledge about the health effects of the amines (MEA, AMP, piperazine and MDEA) than of their photooxidative products. Some of the photooxidative products are suspected to be reactive, such as nitrosamines and nitramines. These types of compounds may be mutagenic and carcinogenic. Dimethylnitrosamine (and diethylnitrosamine) has been demonstrated to be highly carcinogenic in the liver and kidney in virtually all mammalian species tested. The inflammatory potential, immunotoxic, allergy-promoting and genotoxic effects of the relevant photooxidative products the CO_2 capture process of the gas power plants should be investigated before any extensive use of the amines. As inhalation is considered the most relevant exposure route, lung cells toxicity studies are a central part of the proposed project.

2.5 Scope of Work

Establish whether the photooxidative products of amines have inflammatory potential, allergy-promoting, immunotoxic, genotoxic and cytotoxic effects. This knowledge will contribute to the risk assessment of the photooxidative products.

3 Project plan

3.1 Task 1: Toxicity in the lung: Inflammatory response, DNA damage and cell death in lung cells

Goal: Establish the relative potential of photooxidative products of the amines to induce pro-inflammatory responses, DNA damage and cell death in lung cells.

Implementation:

1. Screening of relevant nitrosamines and nitramines in lung cell cultures.
    - Establish concentration –response curves for release of different cytokines (e.g. IL-6, IL-8, eicosanoides)
    - Establish concentration –response curves for some specific DNA lesions
    - Establish concentration –response curves for cell death
    - Comparison to known inflammatory and genotoxic compounds
2. Select the most potent compounds and examine the potential to induce a panel of inflammatory mediators on RNA and protein expression levels.

Methods. Cytokine and eicosanoid measurements by ELISA. Measurements of DNA damages with the comet assay (strand breaks, AP sites, and oxidative lesions). RNA expression will be measured using real-time PCR and microarray technology and protein expression by Western analysis. Different type of cell death will be detected by fluorescence microscopy and flow cytometry.

Responsibility. Marit Låg, Per E. Schwarze, Jørn A. Holme, Magne Refsnes Department of air pollution and noise (MILS); Birgitte Lindeman, Christine Instanes, Ann-Karin Olsen, Gunnar Brunborg, Department of Chemical Toxicology (MIKT)

Expected outcome. Acquired knowledge of the toxicity of the photooxidative products in lung cell cultures

3.2 Task 2: Genotoxicity and carcinogenicity in vitro using OECD recommended tests

Goal: Establish whether photooxidative products of amines have the genotoxic and carcinogenic potential *in vitro*.

Implementation:

1. Examine the potential of 5-10 different photooxidative products to induce genotoxicity *in vitro* using OECD recommended assays for genotoxicity testing, in coherence with the OECD testing Guidelines (positive and negative controls and at least 3-5 concentrations of tested compounds depending on results of Task 1).
2. Depending on genotoxicity *in vitro* to select two to five compounds and investigate their carcinogenicity potential *in vitro* using morphological Syrian Hamster Embryo transformation assay following OECD guideline

Methods for genotoxicity testing in vitro. Various OECD recommended methods will be used to test for genotoxicity testing *in vitro*:

- ***In Vitro* Micronucleus Test (OECD 487)** which is a genotoxicity test for the detection of micronuclei (MN) in the cytoplasm of interphase cells. Micronuclei may originate from acentric chromosome fragments (*i.e.,* lacking a centromere) or whole chromosomes that are unable to migrate to the poles during the anaphase stage of cell division. The assay detects the activity of clastogenic and aneugenic chemicals in cells that have undergone cell division during or after exposure to the test substance. The OECD Test Guideline recommends the use of protocols with and without the actin polymerisation inhibitor cytochalasin B (cytoB). The addition of the cytoB prior to the targeted mitosis, allows for the identification and selective analysis of micronucleus frequency in cells that have completed one mitosis because such cells are binucleate.

- **The *in vitro* mammalian cell gene mutation test (OECD 476)** is used to detect gene mutations induced by chemical substances. Suitable cell lines include the V79 lines of Chinese hamster cells. In these cell lines the most commonly-used genetic endpoints measure mutation at the hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene. HPRT mutation tests detects genetic events on the HPRT locus on X-chromosome. The choice for mutagenicity will depend on mechanistic studies within the other tasks.

Methods for carcinogenicity testing in vitro.

- **The morphological transformation Syrian hamster embryo (SHE) assay (OECD 2006)** for carcinogenicity *in vitro* is a unique assay to detect neoplastic (morphological) transformation *in vitro*. Cell transformation assays (CTA) are based on detecting phenotypic changes induced by chemicals in mammalian cell cultures. The most suitable assay is the Syrian hamster embryo (SHE), the low-pH SHE assay. The SHE assay is believed to detect early steps of carcinogenesis (OECD, 2006). This assay determines both, the cytotoxicity of tested substances by measuring colony-forming ability, and/or growth rate and the potential carcinogenicity by assessing colonies with criss-cross growth of cells as result of contact inhibition loss. Accumulated evidence strongly supports the assumption that cellular and molecular processes involved in cell transformation *in vitro* are similar to those of *in vivo* carcinogenesis (OECD, 2006). As it is already known that certain amines are carcinogenic it would be extremely valuable to include this assay to assess the potential carcinogenicity of the degradation products. These studies depend on the other tasks.

Responsibility. Maria Dusinska, NILU, CEE – Health effect unit

Expected outcome. Acquired knowledge of the genotoxicity and carcinogenicity *in vitro* of the photooxidative products

3.3 Task 3: Organ toxicity: DNA damage and repair

Goal: Establish whether photooxidative products of amines have the potential to induce specific types of DNA lesions in organ tissue after *in vivo* and *in vitro* exposure and whether such lesions are repaired. Depending on the distribution and metabolism of the relevant compounds, it is expected that, in addition to the lung, various organ tissues may be affected including the reproductive organs.

Implementation:

1. Examine the potential of the compounds to induce DNA lesions and repair in primary cell cultures from a repair deficient mouse model ($Ogg1^{-/-}$ mice). Cells from wild-type animals and the repair deficient animals will be utilised to allow detection of different types of DNA lesions with special emphasis of oxidative lesions. The *in vitro* experiments will be performed on somatic and germline testicular cells to complement the lung cell studies described in task 1.
2. Depending on the DNA damaging potential of the photooxidative products *in vitro*, experiments with wt and $Ogg1^{-/-}$ mice will be used to establish whether DNA damage including oxidations – formed directly or via ROS - are of particular importance *in vivo.* DNA damage in cells from

potential target organs (e.g. liver, kidney, lung) will be measured following *in vivo* exposure.

3. Depending on the ability of the compounds to induce DNA damage and the repair of such lesions, DNA damage response genes will be analysed for changes in their expression, at the level of RNA and also with respect to protein expression.

Methods. A currently used method for analysing several tissues from the same animal will be utilised. DNA damage (oxidative damages, strand breaks and Abasic sites) will be measured by sensitive methods established in our laboratory (Comet assay/ alkaline elution). The *in vivo* Comet assay is now under consideration in EU as recommended for *in vivo* genotoxicity testing, including application within REACH.

Responsibility. Birgitte Lindeman, Christine Instanes, Ann-Karin Olsen, Gunnar Brunborg, Department of Chemical Toxicology (MIKT).

Expected outcome. Acquired knowledge of the *in vivo* organ genotoxicity of selected photooxidative products.

3.4 Task 4: Immunotoxicity: Toxic effects on the immune system after in vivo exposure

Goal: Establish whether the photooxidative products have a general immunotoxic potential, and whether they can promote allergic responses (adjuvance effect) in mice models

Implementation:

1. Examine the potential of 4-6 different photooxidative products to enhance allergy towards a common environmental or model allergen (adjuvant effects). The mouse footpad immunization model will be used, a model suitable for screening for adjuvant effects on respiratory allergy. 2-3 concentrations will be tested. Investigate the allergen-specific antibody levels (IgE/IgG1/IgG2a) in serum.
2. Examine the toxic effect on the immune system of some photooxidative products. The popliteal lymph node assay will be used, suitable to study toxic effects on the immune cells in draining lymph nodes, an important site for immune regulation. Six days after footpad injection of the products, the immune cells from the draining lymph nodes are prepared and the distribution of different types of immune cells is measured (using membrane markers). Further, examine the ability of isolated immune cells to respond upon stimulation to known growth stimulants to induce proliferation and cytokine release.

3. Depending on the adjuvance potential of the photooxidative products determined under point 1, examine the adjuvant effects of 1-2 selected compounds in an intranasal immunisation model. Endpoints as described under point 1, as well as determination of allergic airway inflammation.

4. Examine the toxic effect on the immune system after intratracheal instillation of 1-2 selected photooxidative products (once weekly for 4 weeks). General toxic effects will be investigated in liver and kidneys. Analyze effects on immune cells (such as tissue weight, cell type distributions, proliferative capacity and cytokine production) in the blood, in thymus, bone marrow, spleen and lymph nodes.

Methods. The mouse models and assays are methods routinely used in the department. Measurements of antibody production, cytokines and proliferation by ELISA and flow cytometric bead assay. Analyzes of cell type distribution by flow cytometry. Airway inflammation will be determined by performing differential cell counts and determining cytokine levels in the bronchial lavage fluid. General toxic effects will be assessed by determining tissue weight and histological changes. Histological analyses will be performed elsewhere.

Responsibility. Martinus Løvik and Unni C. Nygaard, Department of Environmental Immunology (MIMI)

Expected outcome. Acquired knowledge of how the photooxidative products of the amines will act on immune responsive organs in the body.

3.5 In vivo carcinogenicity and toxicity studies

Based on the results accumulated through the above mentioned tasks we may eventually suggest *in vivo* carcinogenicity and organ toxicity studies to be performed. These studies which should be outsourced to other laboratories and may include the following implementations:

Goal: *In vivo* animal studies should be performed if indicated from the results of the *in vitro* studies and the *in vivo* organ genotoxicity/toxicity.

Implementation:

Methods. Inhalation or instillation exposure. Initial stage of lung cancer and possible cancer in other organs as end points together with organ toxicity.
Responsibility. Laboratory with necessary capability and capacity (probable foreign)
Expected outcome. Lung cancer pre-stage dose-response relationships for the 2-3 most important compounds

3.6 Synthesis of compounds and reference compounds for analytical identification

Principal Investigator
Prof. Yngve Stenstrøm, Department of Chemistry, Biotechnology and Food Science, Norwegian University of Life Sciences, Ås (UMB).

Objective
To synthesise starting materials and stable products formed during atmospheric degradation of amines.

Motivation
The synthesis of reference compounds is necessary for other tasks in this proposal. These include the gas- and liquid-phase degradation studies, studies of the toxicity to humans, and the establishment of the analytical methods in the chemical lab.

Project plan
The synthesis of compounds will take place whenever needed. As a general rule the expected delivery time of about one month.

Implementation
This activity involves advanced organic synthesis of compounds not commercially available. Due to potential toxicity, the nitrosamines and nitramines needed will primarily be synthesised in micro-scale amounts (<10 mg) for analytical applications. Sample authenticity will be documented by NMR and MS. Large scale preparation of these compounds (>500 mg) may require special safety precautions and cannot always be guaranteed. The list of compounds to be synthesised depend on the amines to be studied.

Expected outcome. Non-commercially available reference compounds for degradation studies, analytical work, and effects studies.

Time Line
Chemical compounds will be produced on demand. The organic synthesis of the necessary chemical substances will be available during the whole period of Phase II, i.e. three years.

Cost Estimate

The costs of this tasks are related to the number of substances synthesized. A base cost of 100 000 NOK per year for running and maintaining a laboratory with special security measures is envisaged. The average cost for each compound to be synthesized is set at 50,000 NOK, which corresponds to 1 week full time work + costs for chemicals and laboratory materials. The costs per chemicals will be charged to the other tasks and are included in their budgets.

For both the *in vivo* and *in vitro* experiments 1-3 g of the compounds that are studied most thoroughly are needed. For the other compounds (for screenings) less is needed, approximately 500 mg.

3.7 Publication plan

Mid-term reports, as well as a final report will be prepared. We aim to write six to seven scientific papers based on the cellular toxicity, genotoxicity, potential carcinogenicity and immunotoxicity studies, these will be submitted for publication in toxicological, carcinogenicity or environmental journals. The number of papers and the choice of journal will depend on the outcome of the experiments. Tentative titles describing the topic for six papers, as well as tentative journals for submission, are given below.

1. The potential of relevant nitrosamines and nitramines to induce inflammatory mediators and cell death in epithelial lung cell cultures (Toxicology and Applied Pharmacology).
2. Genotoxic potential of amines and their break-down products, implied in carbon dioxide capture technology (Screening for genotoxicity of relevant nitrosamines and nitramines in epithelial lung cell cultures, and comparing them to known genotoxic compounds) (Toxicology).
3. In vitro and in vivo genotoxicity of break-down products of amines used for CO_2 trapping (Establish whether degradation products of amines have the potential to induce specific types of DNA lesions in different cell types after *in vivo* and *in vitro* exposure and whether such lesions are repaired) (Reproductive toxicology).
4. The ability of different amine degradation products to enhance allergic responses in mouse models (Toxicological Sciences).
5. Assessment of systemic immunotoxicity of amine degradation products in mice after airway exposure (Toxicology).
6. Assessment of potential carcinogenicity of amine degradation products in vitro using morphological transformation assay. Comparison with mutagenicity and other toxicological outcomes (Carcinogenesis)

3.8 Project Personnel

One PhD student and one Post doc will work on the applied project for 3 years.

In addition will the following persons contribute to the project:

FHI, MILS: Marit Låg, Per E. Schwarze, Jørn A. Holme and Magne Refsnes

FHI, MIKT: Birgitte Lindeman, Christine Instanes, Ann-Karin Olsen and Gunnar Brunborg

FHI, MIMI: Unni C. Nygaard and Martinus Løvik

NILU, CEE Health effect unit: Maria Dusinska, Lise Fjellsbø, Alessandra Rinna, Zuzana Magdolenova, Elise Runden-Pran + one PhD/technician for 3 years (in the case of one genotox and one carcinogenicity assay). In coordination with NILU, INBY: Svein Knudsen, and Scott Randall.

4 Time Line

Example of a Time Line:

		Year 1				Year 2				Year 3			
		1	2	3	4	1	2	3	4	1	2	3	4
Task 1	Inflammatory response, DNA damage and cell death in lung cells												
Task 2	Organ toxicity: DNA damage and repair												
Task 3	Toxic effects on the immune system after in vivo exposure												
Task 4	Genotoxicity and carsinigenicity in vitro												

5 Cost Estimate

FHI (MILS, MIKT and MIMI):

Postdoc in 72 man month (Task 1,3,4)	4860 kKr
Activity Task 1(cell cultures, PCR arrays, other materials)	210 kKr
Activity Task 3 (experimental animals, materials)	300 kKr
Activity Task 4 (experimental animals, materials)	370 kKr
Biohazzard bench and incubator for volatile carcinogenic compounds	140 kKr
Totalt FHI	**5880 kKr**

NILU:

PhD/tech 36 man months (Task 2)	4730kKr
Scientist 6 man months (Task 2)	1010kKr
Task 2 (experimentsl animals, cell cultures, materials)	420 kKr
Total NILU	**6160 kKr**

UMB:

Synthesis of compounds (postdoc 12 man months + materials)	800 kKr

Appendix E

Review Committee Project Summary Sheet

Amine Emissions during Carbon Capture

Phase II: Experimental Study Investigating Risks of select Amines (ExSIRA)

Part A: Experiment Preparations and Human Toxicity

- PROJECT SUMMARY FOR REVIEW COMMITTEE -

The main objective of ExSIRA Part A project is to examine the toxic potential of photo-oxidative products of amines in selected cellular and in vivo models. The initial focus will be on inflammatory, cytotoxic, immunotoxic, allergy-promoting effects and DNA damage and repair. We will assess genotoxicity and potential carcinogenicity in vitro using established laboratory procedures as well as OECD recommended test guidelines. The results from this project will contribute to an increased knowledge and thereby estimation of possible health risk from exposure to the relevant compounds. Moreover, on the basis of these results we will eventually suggest whether in vivo carcinogenicity studies should be performed and for which substances.

Project Tasks:

Task #	Description
1	Toxicity in the lung: Inflammatory response, DNA damage and cell death in lung cells
2	Genotoxicity and carcinogenicity in vitro using OECD recommended tests
3	Organ toxicity: DNA damage and repair
4	Immunotoxicity: Toxic effects on the immune system after in vivo exposure

Budget: 12 840 000 NOK for 3 years.

Partners: Norwegian Institute of Public Health (FHI)
Norwegian Institute for Air Research (NILU)

Status: The ExSIRA Part A proposal was submitted to the Norwegian Research Council (NFR) in May 2009. The NFR decision regarding the proposal was to grant a small "pre-project" in order to obtain expert recommendations regarding the proposal before they would further consider the project. The proposal was updated Fall 2009, and an external review committee established in the beginning of 2010 to fulfil the NFR request.

Review Committee members:

Name	Position	Organization	Contact
Marit Kopangen	Chief Engineer	KLIF	marit.kopangen@klif.no
Aage Haugen	Research Director	STAMI	age.haugen@stami.no
Dan Costa	National Program Director	US-EPA	costa.dan@epa.gov

Committee Mandate:

a. To comment on the research needs (human health) for the safe use of amines for CO2 capture based on the Phase I reports, and
b. Review the proposed research project in the light of this information and describe any studies that should be included and/or that are necessary for a risk evaluation.
c. The final deliverable of the committee will be a report outlining the outcomes of the above investigation.

Committee Deadlines:

Date	Description
31.03.2010	Individually review proposal and compile: a) comments, b) applicable studies, c)recommendations
07.04.2010	Discuss individual reviews within committee
12.04.2010	Assemble agreed upon common review for the committee
30.04.2010	Deliver Final Report presenting the committee review and recommendations forward

Review Committe Coordinator: Scott Randall (NILU), sr@nilu.no
Website for the Review Committee: http://co2.nilu.no, login, click on "PARTA RC"

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TITLE	PROJECT LEADER
Expert Review of Project Description: Experimental Studies Investigating the Risks of Select Amines (Part A: Human Toxicity)	Scott Randall
	NILU PROJECT NO. 110089
AUTHOR(S) Dan Costa, Aage Haugen, Marit Kopangen, Scott Randall	CLASSIFICATION * A
	CONTRACT REF. Erik Gjernes

REPORT PREPARED FOR
Gassnova

ABSTRACT
A review committee was established to examine the Phase II ExSIRA Part A project proposal. The committee consisted of 3 experts in the field. Each expert carefully reviewed background material (including Phase I reports) and the Part A project description; based on this work, each expert wrote an individual review. These individual reviews were used to create a consensus review for the committee in which this report is based upon.

The consensus review states that the project description as proposed is feasible and that the project team is competent to perform these investigations. However, one major comment from the committee is the consistent lack of detail regarding the study design and specific methods to be utilized. The committee recommends that this detail be documented at some point before experiments are conducted. The committee also identified a total of 11 specific comments and recommendations throughout the 4 tasks for the project – many of these comments should be addressed before the project is funded.

NORWEGIAN TITLE

KEYWORDS		
CO_2 Capture	Amines	Human Toxicity

ABSTRACT (in Norwegian)

** Classification*
A Unclassified (can be ordered from NILU)
B Restricted distribution
C Classified (not to be distributed)

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