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SINTEF REPORT

TITLE

TQPAmine3
Protocol for Evaluation of Solvents – Emission Compound Toxicity
Call Off No. 01

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ABSTRACT

This report describes the background and rationale for the preparation of a Protocol for Compound Mixture toxicity of compounds in flue gas emissions from CO_2-capture technology. The recommendations made include:

1. According to current information and technology status the protocol should be based on health and environmental information from single chemicals; where quality data are not available these should be provided by laboratory testing according to flow-sheets described in the suggested Protocol (Appendix A).
2. Only validated OECD tests are recommended for the Protocol
3. Based on information from toxicity testing of single flue gas compounds an assessment for hazard should be performed. For human/mammalian effects this should be based on derived no-effect levels (DNEL) / derived minimal-effect levels (DMEL) if enough data are available. If data for determination of DNEL/DMEL are not avaliable, a worst case approach based on occupational exposure limits (OEL) is recommended. We further recommend a hazard assessment for environmental data based on predicted no-effect concentrations (PNEC). All hazard methods will be used as part of a risk assessment.
4. Since methods for determination of exposure concentrations are not yet established a full risk assessment is not possible, and therefore not included in the Protocol
5. Testing of emission mixtures are not recommended for hazard or risk assessment, but may in the future be used for comparison of toxicities of different solvent technologies, or for detection on "unknown" compounds of toxicological importance. Appropriate methods for sampling and toxicity testing of flue gas are not yet established. When such methods are established we recommend that toxicity testing of emissions should be performed by in vitro methods and not be animal testing. Testing of emission mixtures is not included in the Protocol due to the lack of relevant methods.
6. Recommendations for a Call Off 2 and for future research requirements are included in the report

KEYWORDS	ENGLISH	NORWEGIAN
GROUP 1	Health/Environment	Prosessteknikk
GROUP 2	Emissions	Utslipp
SELECTED BY AUTHOR	Absorption system	Absorpsjonssystem
	CO_2 Capture	CO_2 innfanging
	Sampling	Prøvetaking

ABBREVIATIONS

AF – Assessment factor

ASTM – American Standards for Testing of Materials

BCF – Bioconcentration factor

BMDL – Benchmark dose level

BOD – Biochemical oxygen demand

CCM – CO_2 capture Mongstad

CCP – CO_2 capture plant

CHP - Combined heat and power plant

DNEL – Derived No-Effect Level

DMEL - Derived Minimal-Effect Level

DOC – Dissolved organic carbon

EC – European Community

EC_{50} – Effective Concentration causing 50 % inhibition of growth for a population of uniform organism

ECHA – European Chemical Agency

ECVAM – European Centre for the Validation of Alternative Methods

EIF – Environmental Impact Factor

EPA – U.S. Environmental Protection Agency

EU – European Union

GESAMP - IMO/FAO/UNESCO/IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection, 2002. Revised GESAMP Hazard Evaluation Procedure for Chemical Substances Carried by Ships

GHS – Global Harmonization System

GLP – Good Laboratory Practice

HE – Health and Environment Information about a substance

HOCNF - Harmonized Offshore Notification Format

HSS – Heat-stable salts

IC_{50} – Half maximal inhibitory concentration

IC_{max} – Inhibitory concentration of maximum effect

IC_{NOEC} – The Maximum concentration of no effect

ID_{50} – Half maximal inhibitory dose

ICCVAM - Interagency Coordinating Committee on the Validation of Alternative Methods at the National Toxicology Program

IMO – International Maritime Organization

IRIS – Integrated Risk Information System

ISO – International Standardisation Organisation

ITT – Invitation to Tender

IUCLID – International Uniform ChemicaL Information Database

JaCVAM – Japanese Centre for the Validation of Alternative Methods

Klif – Norwegian Climate and Pollution Acency

LD_{50} – Lethal dose causing 50 % lethality for a population of animals

LC_{50} - Concentration causing 50 % lethality for a population of uniform organism

LOAEL - lowest observable adverse effect level

LOEC – Lowest observed effect concentration

NOAEL – No-observable adverse effect level

NOEC – No-observed effect concentration

NOS – No-otherwise specified compound

NT-LAF – Non-threshold "large assessment factor"

OECD – Organisation for Economic Cooperation and Development

OEL-TWA – Occupational Exposure Limit – time-weight average

OSPAR - Oslo and Paris Conventions for the protection of the marine environment of the North-East Atlantic

PEC – Predicted environmental concentration

PNEC – Predicted No-Effect Concentration

Pow – Partition coefficient between octanol and water

QA – Quality Assurance

RA – Risk assessment

RC – Risk characterisation

REACH – European Regulation for Registration, Evaluation, Authorisation and restriction of Chemicals

RTECS – Toxic Effects on Chemical Substances®: Number indicate record number

SAR – Structure-Activity Relationships

SRC – Syracuse Research Company

SST – System Suitability Test

TD_{50} – Toxic Dose

TGD – Technical Guidance Document

$ThCO_2$ – Theoretical CO_2 evolution

ThOD – Theoretical oxygen demand

WFD – European Water Frame Directive

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1 Background

In 2006 the Norwegian government granted permission for building of a combined heat and power plant (CHP) at the Mongstad Refinery site north of Bergen on the west coast of Norway. The permit for CO_2-emissions from the plant was granted by the Ministry of Environment. An agreement on CO_2 handling was signed by the Ministry of Petroleum and Energy and Statoil ASA.

This Implementation Agreement states that CO_2 shall be captured from CHP flue gas in a large scale capture plant. Annual estimated amounts of 1.3 million tonnes of CO_2 is the basis for designing capture plant to condition and compress CO_2 before pipeline transport to geological storage under the Norwegian Continental Shelf.

The CO_2 Capture Mongstad (CCM) Project is in an early development phase of project development. The project is currently organized as a joint effort by Gassnova SF and Statoil, and is funded by the Norwegian government. The purpose of the project is to plan and build a large scale CO_2 capture plant (the CCP).

CCM invited qualified tenderers for the following activities:
Establish Sampling of Analytical Procedures for Potentially Harmful Components from Post Combustion Amine Based CO_2 Capture (TQPAmine1)
Modelling of Atmospheric Dispersion of Components from Post Combustion Amine Based CO_2 Capture (TQPAmine2)
Protocol for Evaluation of Solvents – Emission Compound Toxicity (TQPAmine3)
Protocol for Evaluation of Solvents – Process and Atmospheric Chemistry (TQPAmine4)
Nitramine Analyses and Screening Toxicity Study (TQPAmine5)
(Emission Reducing Technologies (TQPAmine6))
Alternative Approaches to Animal Toxicity Testing (TQPAmine9)

After the Tender Invitation SINTEF was awarded to prepare a protocol for emission toxicity evaluation of solvents (TQPAmine3). This project has been conducted in the period June to November 2010. The results of the services may be used for, but not limited to, the qualification of capture plant vendors and their solvents.

2 Introduction

2.1 Post combustion CO_2 technology and emissions

The emissions routes from a post combustion CO_2 adsorption process is illustrated in *Figure 2.1*. The process consists of three main parts:

1. *The flue gas pre-treatment*. The cooler reduces the flue gas inlet temperature to 40-50 ºC and is important in terms of designing the plant for a neutral water balance.
2. *The CO_2 removal step*. This is performed by an absorber column where CO_2 is absorbed by the solvent. A water wash section is included on top in order to reduce loss and emission of solvent due to evaporation.
3. *The solvent regeneration step*. This consists of a desorption (stripper) column, where CO_2 is stripped off from the solvent and recovered at high purity in the top, downstream from the condenser. The stripping is performed by re-boiling the solvent, so that stripping steam is generated in the stripper bottom and rising upwards through the column. The solvent leaving the stripper is re-circulated to the absorber and thereby closing the solvent circulation loop.

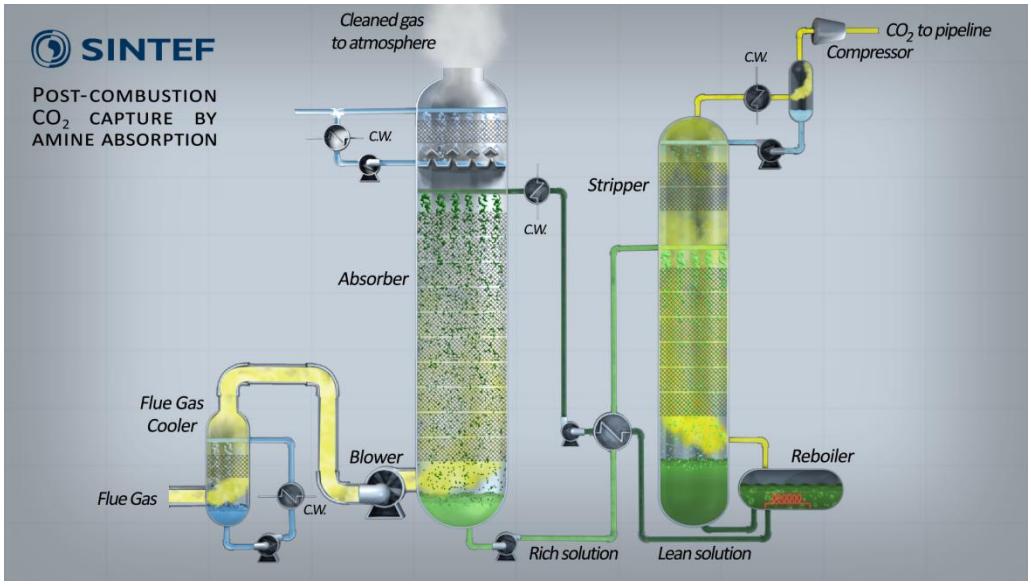


Figure 2.1 Absorption process for post combustion CO_2 capture.

The main sources of emissions from the process can be summarised as followed:

1. Emissions to air
    - Trace amounts of solvent vapour not captured in the water wash unit

   - Continuous emission of solvent components with the treated flue gas has not received significant focus earlier since in conventional applications of absorber technology, the treated gas is not directly emitted but utilized in subsequent processing
2. Reclaimer waste
   - Bottoms product from the solvent reclaimer, aimed at regenerating degraded solvent
   - Semi continuous emission, depending on reclaimer technology and operation
3. Excess desorber condensate (semi-continuous, in order to regulate the water balance of the process) - sent to water treatment plant
4. Excess condensate water from gas precooler if the flue gas is cooled below its saturation point
5. Spent filter cartridges (batch wise) for disposal
6. Solvent leakages and accidental spills – may lead to local pollution of soil

The emissions to air (1) are considered very important since the chemical composition of treated flue gas from absorption plants has not been thoroughly investigated and since the emissions can be dispersed over long distances. The flue gas volumes from gas or coal fired power plants are very large compared to other process streams treated with absorption technology. A 400 MW natural gas fired power plant produces a flue gas flow of 2 mill. Nm^3/hr. It is therefore clear that even trace amounts of pollutants in the gas leaving the absorber will add up to relatively large amounts emitted per year. These emissions can potentially lead to environmental risks.

For reclaimer waste (2) and excess water (3-4), which are in the form of liquid/liquid sludge, there are treatment options. Reclaimer waste can be incinerated, excess water and condensate can be cleaned in a biological waste water treatment plant as conventional spent process water. However, the scale and extent of new post combustion plants will lead to significantly higher volumes of waste materials and emissions than treated earlier.

Solvent leakages and accidental spills (6) may appear on a smaller scale and should be remedied by good operation procedures, plant design and maintenance.

Emissions to air and reclaimer waste are considered the most important emission sources.

2.2 Emissions to air of amine solvents

Due to high vapour pressure of the aqueous amines treated flue gas will contain traces of volatile solvent components and water vapour when leaving the absorber sections. The most important volatile component is the amine itself, and it has traditionally been considered important to work with amines with as low as possible vapour pressure. The water wash at the adsorber top is used to readsorb evaporated amines and transfer these back to the main solvent cycle. However, amine degradation in the combustion process may result in some volatile products, like ammonia (NH_3), which will escape together with the purified gas.

The emissions may include entrained droplets, as well as evaporated substances including –

1. Amines
2. Ammonia
3. Aldehydes
4. Amides
5. Alkylamines
6. Nitrosamines
7. Nitramines

2.3 Water wash

Water wash is used to remove water-soluble flue gas compounds from the emissions. The water wash is placed on top of the absorber section. The primary objective of the water wash is to reabsorb evaporated solvent and transfer it back to the main solvent cycle and thus minimize the amine losses. Treated flue gas exiting from the wash section will be transferred into an exit pipe placed on top of the absorber and wash section structure. However, volatile compounds like ammonia will mainly escape the water wash system.

2.4 Reclaimer waste

Solvent degradation implies that the active component of the circulating solvent, the amine, is degraded to other species, so that it is no longer active in the reversible reaction with CO_2. There are basically three important degradation mechanisms:

1. Presence of other acidic components than CO_2 in the flue gas
    a. SO_2 and the NO_2-part of NO_x will act as stronger acids than CO_2 and bind the amines as sulphate and nitrate salts, so called heat stable salts (HSS)
2. Oxidative degradation of the amine
    a. Due to the presence of oxygen in the flue gas, amines will to some extent be oxidized. The chemistry is complex, but the primary degradation products are ammonia (NH_3) and organic acids (formic and acetic acid and others). The acids will bind to amine cations as additional HSS.
3. CO_2 induced degradation/carbamate polymerization leading the formation of other amine components, e.g. diamines that are stronger bases than the original amine. Also other complex organic molecules can be formed.

3 Objectives and scope

3.1 Description of scope in the Invitation to Tenderer (ITT)

The objectives and scope of this project was stated in the Invitation to Tenderer (ITT) as follows:

The major objectives of the project are described in the Tender Invitation of TQPAmine3, aiming at "*proposing a protocol for toxicity testing of samples of air emissions and water wash that could be used as a toolbox for evaluations of health and environmental risk of emissions from post combustion amine based CO_2 capture*"

The scope was separated in two Call Offs:

1) Call Off 1:

    a. Propose methods on how to sample and handle representative extracts of emissions to air from post combustion amine based CO_2 capture to enable toxicity testing of the extracts. The sampling methods should enable sampling of representative emission extracts, including gaseous, liquid phases and particulates/aerosols, and emissions may include entrained droplets and evaporated substances of compound groups described in the ITT;

    b. Propose method(s) (laboratory experiments) to establish relevant human and ecotoxicity data for one or more end-points, as described in the ITT. The proposed methods should be established for both air emission and water wash samples. The proposed methods should be suited for small emission extracts samples and preferably be in vitro tests.

    This service should include doing an assessment of the suitability of using the proposed methods in a) and b) alone, or together with an individual substance risk approach, for assessing risk of an emission profile and comparison/ranking of emission profiles from different solvent/supplier specific technologies for post combustion amine based CO_2 capture.

2) Later Call Offs (Call Off 2): Experimental toxicity studies according to the recommendations in Call Off 1. These later Call offs should include (but not be limited to) experimental toxicity studies according to methods developed in Call off 1 (a) and/or b)), or as defined by Company with emission samples

This report only concerns Call Off 1, and recommendations in this report will be used for suggesting studies proposed for Call Off 2.

According to the ITTT for Call Off 1 the TQPAmine3 could be separated in the following two objectives:

1) Establish a protocol for comparison of risk of different emission profiles from different solvent/supplier specific technologies

2) Establish a protocol that enables the assessment of a single substance risk approach for an emission profile; i.e. does the sum of the emission profile ‘s individual substances’ risk measure up to the emission mixture overall risk, or are there synergistic effects or effects from non-identified substances.

The major value of this project will be to propose integrated methods for sampling toxicity and risk assessment to be used for health and environmental studies of emissions from the CO_2 Capture Mongstad (CCM).

The ITT describes one or more of the following endpoints as relevant:

> Toxicological information – Mutagenicity, carcinogenicity, reproductive toxicity (including developmental toxicity), actute toxicity, repeated dose toxicity, sub-chronic toxicity, sensitization, corrosion/irritation (skin and eye)
>
> Ecotoxicological information – Acute and/or chronic toxicity, biodegradation

3.2 Important issues to consider

The current project should be able to present a protocol for evaluation of emission mixture toxicity. Based on the recommendations in this Call Off 1 report, the Protocol should be ready for use after a Call Off 2 period. The time frame for a Call Off 2 has been suggested by Company to be approximately 6 months. It was suggested by Company that recommendations made in the current report should be possible to establish within this 6-month period. In addition, Company suggested to identify issues to consider for future investigations beyond the time fram of Call Off 2. Important issues to consider are described below and are further elaborated in later sections of the report, as basis for our recommendations.

3.2.1 Toxicity methods

Within the 6-monthg time frame of Call Off 2 no development of new test toxicity methods or protocols for toxicity will be possible. Only internationally accepted and validated methods, which are “ready to use”, should therefore be recommended. As described later in this report the “*OECD Guidelines for Testing of Chemicals*” represent such a suite of accpted and validated test methods for human/mammal toxicity, ecotoxicity and biodegradation, describing the endpoints requested to be evaluated by Company.

The Company recommends the use of *in vitro* methods, when possible. Advantages and disadvantages of *in vitro* methods are discussed later in the report, and it is concluded *that in vitro* methods can not yet replace *in vivo* methods. In addition, relatively few *in vitro* methods are currently available as validated OECD guidelines. *In vitro* methods are often used as initial screening methods, which are supplemented by *in vivo* tests, and *in vivo* test methods are still essential for hazard and risk assessment. *In vitro* and *in vivo* methods for genotoxicity and reproduction toxicity are discussed later in the report.

3.2.2 Flue gas emission toxicity and single substance approach

The ITT suggests an assessment of the suitability of using toxicity testing of flue gas emissions alone and/or together with an individual substance risk approach.

Testing of flue gas will mainly give information about the toxicity of the whole effluent, but no or limited information about individual compounds (except some predominant compounds). If flue gas toxicity should be compared to single-substance approach it is essential that the toxicities of the individual compounds add up to represent the "toxicity" of the mixture in the flue gas (additive toxicity). Toxicity interactions between compounds will complicate or make the comparison impossible. *In vivo* reactions, like metabolic activation, will also render the comparison between single substance and flue gas toxicity difficult or impossible in animal models.

Validated methods, like the OECD Guidelines, are recommended for single compounds. If flue gas emissions are to be tested by these methods, modifications of test methods must be considered, and these modified test methods may not be regarded as validated methods any more. In addition, all validated toxicity methods are, to our knowledge, recommended for water media, not for gaseous samples.

Risk assessment is normally based on the toxicity of single compounds. Flue gas emission toxicity may therefore be of limited value for risk assessment. In addition, different flue gas compounds may have different fate after emission, so the distribution of compounds exposed to the population or the environment may be different from the flue gas composition out of the stack.

3.2.3 Risk and hazard assessment

The ITT asks for a possible risk approach. A risk assessment requires exposure concentrations. Since exposure concentrations, or methods for determination of these, are not available for this project we do not have the tools to perform or suggest a complete risk assessment. However, a hazard evaluation is possible to perform, based on available toxicity data. A hazard assessment is important to perform before final toxicity test regimes are decided, based on lack of quality toxicity data. Data from a hazard assessment will be used as part of a complete risk assessment.

3.2.4 Sampling methods

In the ITT it is requested a protocol for sampling methods to be used for toxicity testing from water wash and flue gas emission samples.

Water wash samples will normally not be discharged to the environment, but circulated in the system. The need for toxicity testing of water wash samples may therefore be questioned, as discussed and concluded on later in the report.

Flue gas sample extracts will include gaseous and liquid phases, as well as particulates/aerosols. Common methods for emission sampling include steps to *destruct* the sample, while toxicity testing will require *non-destructed* samples. It is also advantageous to sample all flue gas compounds in one phase, preferably as a liquid, since all validated toxicity test methods are recommended for water-based media. This is further discussed in the report, with the recommendation of a sampling approach.

4 Organisation of project and report

4.1 Project organisation

This project was organised by SINTEF Materials and Chemistry as Contractor, and with the following institutions as subcontractors:

- Norwegian University of Science and Technology (NTNU), Faculty of Medicine, department of Neuroscience
- Norwegian Institute for Air research (NILU), Centre for Ecology and Economics (CEE)
- Norwegian Institute for Public Health (FHI), Dept. Air Pollution and Noise

The project was organised thematically:

- Sampling methods and analyses: SINTEF Materials and Chemistry
- Human / mammalian toxicology
    - Mutagenicity/genitoxicity/carcinogenicity: NILU
    - Reproduction toxicology and sensitization: NTNU
- Ecotoxicology and biodegradation: SINTEF Materials and Chemistry
- Technical Adviser for the report: FHI

The work on health methods performed by NTNU and NILU is summarised in the main report, but a full report on this work is shown in Appendix C.

4.2 Report organisation

The main objective of the project was to propose approach(es) and methods which can be used for *evaluations of health and environmental risk of emissions from post combustion amine based CO_2 capture*. The report is organised in different chapters for:

- Selection criteria for emission compounds
- Human/mammalian toxicity methods, including hazard assessment
- Ecotoxicity and biodegradation
- Hazard and risk assessment for health and environment
- Flue gas sampling methods

The various sections include results obtained in the current project and recommendations for Call Off 2.

Recommended research requirements outside Call Off 2 are described in a separate chapter.

Each chapter contains a number of elements, including summaries of relevant methods and recommendations for the protocol. Where several options are available arguments are included for the choices made. For mammalian toxicity and ecotoxicity relevant methods are summarised and discussed in the main part of the report and in Appendix C, while the principles of the individual methods are presented in separate appendices (Appendices D to F).

As a basis for hazard assessment we have surveyed available databases and scientific literature for relevant health and environmental data for a number of selected flue gas components, being representative for amine solvents, aldehydes, amides, alkylamines, nitrosamines and nitramines. Summaries of these data are presented in the main part of the report, while complete data sets and references are found in separate appendices (Appndices .

Based on the summaries and evaluations recommendations are made for the Protocol. This Protocol is placed in a separate Appendix to the report (Appendices H and I).

5 Selection criteria of emission compounds and validated test methods for toxicity testing

According to the ITT this project should suggest toxicity testing strategy for emission mixtures from post combustion CO_2-capture. The request included both absorber **flue gas emissions** and **water wash** systems.

5.1 Water wash systems

In addition to absorption of evaporated amine solvents the water wash system may also trap various water soluble degradation products. However, volatile compounds like ammonia and alkylamines may not be trapped in the water wash.

The water wash efficiency will affect the emissions in the flue gas. Water failure, or saturation of water wash with degradation products, may result in higher emissions than expected in the flue gas.

Compounds in the water wash are removed from the emission. Instead they are recycled back to the main solvent cycle, and will therefore not be released to the environment except by accidental discharges. We therefore do not regard compounds in the water wash system to pose any risk to the environment under normal process conditions. However, the water wash may be used for indirect measurements of toxic effects of the emissions. In theory, toxicity measurements of water wash samples may be used for determination of removal of flue gas toxicity prior to emission, since the waster wash system is placed in the top of the absorber (see Figure 2.1). However, this is very difficult to calculate.

In addition, the water wash may represent water droplet entrained with the flue gas emissions, and the toxicity of the water wash may then in principle represent the toxicity of the water droplets in the flue gas. Once again, this will be very difficult to determine as part of the flue gas toxicity.

We therefore decided not to focus on the effects of the water wash. However, risk assessment of water wash substances may be performd in the same way as described for the flue gas, by combining toxicity data for individual compounds with exposure concentrations, for example after accidental discharge incidents.

5.2 Flue gas emissions

As described above flue gas emissions are complex samples consisting of compounds distributed between gas, particulate and aerosol phases. As discussed in the previous chapter we can not

currently recommend that flue gas is used directly for toxicity testing due to the lack of available sampling methods.

In our view toxicity testing of flue gas samples may be used for comparison of different samples. This may include testing of sample-to-sample variations from individual duct systems (inhomogeneous samples, variations between planes and ports in a duct) and sample reproducibility (variations by time). However, this information may be provided by higher degree of certainty by chemical analyses.

Toxicity analyses may also be used for comparison of emissions from various sources and technologies. These kinds of toxicity data may only be used for comparison of the toxicities of complete effluents. If toxicity data from flue gas samples should be related to the compounds measured by analytical tools extensive knowledge should be available about the toxicity mechanisms related to various endpoints. If experimental studies show that emission compounds elicit additive toxicity for the emission compounds and high-quality data are available for essential emission compounds, these data may also be used for estimations of "unknown" compounds of toxicological importance.

If emission toxicity testing should be performed, we suggest that only simple *in vitro* toxicity test methods should be used, including both mammalian toxicity and ecotoxicity endpoints. This is further discussed in cpt. 11). However, these methods are designed for pure compounds and not for emission mixtures, and revisions of methods may therefore be required.

Toxicity testing of emission mixtures can not be used for risk assessment and for testing of long-term effects. After emissions several processes may change the compositions of the emissions. This includes both dispersion characteristics and degradation/transformation processes. Thus, emission mixture compositions will be changed from the stack to the point of human or animal/plant exposure. The scope requested for risk assessment of flue gas emissions/emission compounds. However, we regard emission samples as unsuitable for risk assessment. We suggest a hazard assessment with the use of single compound strategies in combination with non-threshold approach, or the lowest acceptable exposure limits, as part of a risk evaluation (see cpt. 8). This may be supplemented at a later stage with a complete risk assessment, including emission data in combination with post-emission processes for the predictions of *exposure concentrations*.

5.3 Single compound strategy

From a toxicological point of view there are several reasons for using a single compound strategy instead of testing on real flue gas emissions consisting of multiple compounds, and where one compound (ammonia) is quantitatively predominant:

- Testing mixtures containing high levels of ammonia and low levels of other contaminants is a challenge. Exposure will primarily be limited to the inhalation and possibly oral route. The

irritative properties of ammonia will change the physiology of the animals as well as affecting the uptake kinetics of other components in the test mixture.

- *In vitro* testing of mixtures containing volatile compounds is extremely difficult, since it is difficult to maintain a stable exposure concentration over time. Further, if a major component of the mixture is either acidic or alkaline it will strongly affect most *in vitro* tests. If not corrected through the use of appropriate buffers the test will merely become an indicator of what happens if the pH changes. If strong buffers are use, this has to be controlled through the use of controls.

- Many chemicals in the flue gas mixture may interact with each other with the net result being a change in biological behaviour. Health wise, an important aspect of this is the production of nitrosamines from e.g. amines (Pitts *et al.,* 1978; Challis and Li, 1982) or nitramines (Churakov *et al.,* 1995) in a nitrogen rich atmosphere as well as endogenously (Vermeer *et al.,* 1998, Fraser *et al.,* 1980). However, to what extent this may occur is unknown.

- If samples of flue gas are tested there will always be questions of whether that sample is representative of the effluent over time. There will be inevitable changes in production level, aging of the solvent and other processes which may consequently cause the composition of the flue gas to change.

The single compound strategy is well known from toxicity evaluations of mixtures. For instance, the environmental impact factor (EIF) used for risk assessment of oil discharges to the marine environment is based on toxicity evaluation of the single oil compounds, in combination with the predictions of the environmental concentrations of these compounds. In the EIF the oil is separated into pseudo-compound groups and the toxicity of selected compounds representing each of these groups are used for defining ecotoxicity and fate of each pseudo-compound group (Johnsen et al., 2000)

For human exposure we do test mixtures like e.g. consumer products and pharmaceuticals. However, these are mixture where the product composition over time is carefully controlled and the result of testing is applicable to the product as long as it maintains the composition. For product with changing composition, risk assessments are usually based on evaluation of combinations of compounds where great care is taken to include those which may represent primary importance to public health.

It is also of importance that all validated test methods are described for the testing of single compounds. If testing of emission mixtures or mixtures of single compounds should be performed this may therefore require modifications of test procedures according to the standard guidelines.

5.4 Collecting available data on flue gas compounds

As analytical methods and knowledge about flue gas compound composition are increasing, the list of flue gas compounds may steadily increase. In addition, the use of alternative solvents will also result in different degradation products both before and after emission. A large number of degradation products from different amine solvents have therefore been identified from different measuring campaigns, experimental studies, or by theoretical deductions. Some of the degradation products have been identified in the CO_2-capture system and others after emissions to the air (e.g. Strazisar *et al.*, 2003; Lepaumier *et al.,* 2008, Bråthen *et al.,* 2008).

To open up for new flue gas compounds we suggest that **all compounds detected in monitoring or experimental studies should be considered for toxicity evaluation**. However, we also realise that some priority must be made, since **some compounds are of more toxicological importance than other**. In that respect we suggest a ranking system for toxicological evaluations of flue gas compounds, ranked in importance from higher (top) to lower (bottom):

1. High concentrations possible in emissions and potential effects related to mutagenic, carcinogenic and/or reproduction toxicity (highest importance)
2. High concentrations possible in emissions and potential for persistence, bioaccumulation and high ecotoxicity
3. Low concentrations probable in emissions and potential effects related to mutagenic, carcinogenic and/or reproduction toxicity
4. Low concentrations probable in emissions and potential for persistence, bioaccumulation and high ecotoxicity
5. High concentrations possible in emissions, but no potential effects related to mutagenic, carcinogenic and/or reproduction toxicity, and no potential for persistence, bioaccumulation and high ecotoxicity
6. Low concentrations probable in emissions and no potential effects related to mutagenic, carcinogenic and/or reproduction toxicity, and no potential for persistence, bioaccumulation and high ecotoxicity (lowest importance)

Chemicals ranked within categories 1 to 4 should be tested if relevant HE-data are lacking or judged to be of inadequate quality (expert opinion).

Before testing relevant information should be collected from number of sources:

1. CAS number (if available). Cas number is used as for compound identification when searching for toxicological information

2. Information on data from previous testing. This can be gathered from a number of databases described in Cpt. 6 and Cpt. 7 of this report.

3. Information on data from scientific literature, e.g. PubMed, SciFinder or Web of Science

4. List of chemicals registered or pre-registered in REACH. More than 140000 chemicals arranged by CAS-numbers are currently pre-registered in REACH for toxicity evaluations according to relevant endpoints for human end environmental toxicity. Registry process can be found on the homepages of the European Chemical Agency – ECHA (http://echa.europa.eu/).

If it is impossible to place new compounds within one of the ranking categories 1 to 6 based on available testing information, the potential for the human health or environmental effects may be considered by the use of structure-activity relationships (SAR). Health-related SAR information is further described in another TQP Amine project (TQP Amine 9 Alternative Approaches to Animal Toxicity Testing).

Based on the collection of toxicological data informational gaps will be identified and testing will be suggested to fill these gaps. For further information about testing, see cpt. 6 and 7 for health and environmental effects and methods, respectively.

In the ITT a number of flue gas compound groups are described (see cpt. 2.2). We have no information about all possible compounds within each group. For hazard evaluation of complex mixtures it is common to use compounds representing the various groups. For instance, this is used for hazard and risk assessment of produced water from offshore installations (e.g. Johnsen *et al.,* 2000). In this project we have used this approach and selected a number of substances identified as **a case study for hazard evaluation** (Table 5.1). These substances have all been detected, or are expected, in emissions from post combustion CO_2-capture, and they represent the compound groups described in the ITT:

- Monoethanolamine (MEA) is chosen since this is a common primary solvent amine.
- Ammonia is well documented to be the predominant compound in flue gas from amine-based post-combustion CO_2 capture.
- Aldehydes: Formaldehyde and acetaldehyde are well-known degradation products of MEA (Knudsen, 2009; Sexton and Rochelle, 2008).
- Amides: Acetamide is expected to be a stable degradation product of aliphatic amines (Bråthen *et al*., 2008).
- Alkylamines: Methylamines have been observed experimentally in degradation tests where MEA has been exposed to NOx (Pedersen *et al.* 2010), and methylamine has been a suggested degradation product of amines (Rooney *et al.*, 1998)
- Nitrosamines and nitramines: NOx may react with amines and nitrosamines and nitramines. The nitrosoamines (N-nitrosodimethylamine, N-nitrosodiethanolamine and N-nitrosomorpholine) and nitramines (dimethylnitramine, ethanolnitramine and

methylnitramine) selected may form under process conditions or after emission to air (Bråthen *et al.,* 2008; da Silva *et al.*, in press), although the appearance of all these compounds are not yet shown experimentally.

With the emergence of new solvent strategies and knowledge about new degradation products we suggest that hazard evaluations should be performed on the individual compounds in the same way as described below.

Table 5.1 Chemicals selected for representation of a flue gas emission sample from a CO_2 capture plant

Chemical Group	Name of the chemical	CAS Numbers
Amine	MEA	141-43-5
NH3	NH3	7664-41-7
Aldehydes	Formaldehyde	50-00-0
	Acetaldehyde	75-07-0
Amides	Acetamide	60-35-5
Alkylamines	Methylamine	74-89-5
	Dimethylamine	124-40-3
Nitrosamines	N-nitrosodimethylamine	62-75-9
	N-nitrosodiethanolamine	1116-54-7
	N-nitrosomorpholine	59-89-2
Nitramines	Dimethylnitramine	4164-28-7
	Ethanolnitramine	74386-82-6
	Methylnitramine	598-57-2

5.5 Transformation of compounds after emissions

The flue gas composition may change after emission to the atmosphere. Immediately after emission the compounds are subjected to a number of processes in the atmosphere, including dispersion, partitioning between gas and rain water dropeltes and between gas and aerosols and degradation processes (photolyc degradation, hydrolysis). The compounds may be precipitated by wet or dry processes and subjected to a number of abiotic or biotic processes in terrestrial or aquatic environments. These processes, and their implications for human or environmental toxicity, are outside the scope of the processes (see TQP Amine 4).

However, post-emission processes are of major importance for risk evaluations. It is therefore impossible to describe any realistic exposure conditions or scenarios this report.

5.6 Validated test methods

It is important in this project to describe and recommend methods for health and environmental effects. Since the test methods described here should be used for regulatory purposes we regard

as essential that these are generally accepted by the industry as well as national and international authorities. Probably the most important sources for such accepted methods in European countries are the validated methods presented as Guidelines by the Organisation for Economic Co-operation and Development (OECD) and the International Standardization Organisation (ISO). However, the OECD guidelines are more accessible than the ISO standards, since the latter require pre-payment before accession.

OECD is an intergovernmental organisation with representatives of 30 industrialised countries in North America, Europe and the Pacific, as well as the European Commission. Most of the OECD's work is carried out by specialised Committees and subsidiary groups composed of Member country delegates, and the work of the OECD related to chemical safety is carried out in the Environment, Health and Safety Programme. As part of its work on chemical testing, the OECD has issued several Council Decisions and Recommendations (the former legally binding on member countries), as well as numerous Guidance Documents and technical reports. The OECD Guidelines for the Testing of Chemicals are a collection of the most relevant internationally agreed test methods used by government, industry and independent laboratories to determine the safety of chemicals and chemical preparations, including pesticides and industrial chemicals. They cover validated tests for the physical-chemical properties of chemicals, human health effects, environmental effects, and degradation and accumulation in the environment. The OECD Test Guidelines are recognised world-wide as the standard reference tool for chemical testing. More information about the Environment, Health and Safety Programme and its publications (including the Test Guidelines) is available on the OECD's World Wide Web site http://www.oecd.org/ehs/.

Validated methods (like OECD test guidelines) are thoroughly tested, and by following the given recommendations, it is to be expected good reproducibility, together with the use of recommended references, standards, good laboratory practice (GLP), approved statistical methods, etc.

OECD guideline methods usually provide information about recommended statistical approaches and how the data should be interpolated. The OECD also provides a number of guidance documents on ecotoxicity testing methods (REF: http://www.oecd.org/document/30/ 0,3343, en_ 2649_34377_1916638_1_1_1_1,00.html).

In the EU, the promotion of alternative methods is increasingly favoured at the expense of conventional animal testing. Similar developments for the promotion of alternative methods can be observed in other industrialized regions such as USA and Japan. They are coordinated by OECD at the international level (e. g. OECD 1990, 1996, 2005a). As a consequence of the EU Directive 86/609/EEC, in 1991 the European Centre for the Validation of Alternative Methods (ECVAM) was founded which has become a unit of the Joint Research Centre of the EU Commission in Ispra, Italy, since 1992. Normally, new alternative methods are validated by ECVAM, or by other relevant facilities like the Japanese Centre for the Validation of Alternative Methods (JaCVAM), or the Interagency Coordinating Committee on the Validation of

Alternative Methods at the National Toxicology Program (ICCVAM), before OECD starts the validating process.

In this project we consider only OECD validated methods as acceptable validated methods for the testing strategy (Lilienblum et al., 2008).

5.7 Expert judgment

Expert judgment is recommended for several of several decisions related to hazard assessment and selection of test methods in the chepter below. By "expert judgment" we suggest –

- Competent people on toxicity/ecotoxicity/biodegradation methods, i.e. experts from relevant testing laboratories or actively working scientifiste in toxicology and ecotoxicology
- Competent people on flue gas emissions from CCS, i.e. experts on sampling and analysis, and post-emission processes like dispersion and environmental fate (e.g. atmospheric processes)

6 Human/mammalian toxicity data

6.1 Human/mammalian toxicity data for hazard assessment

The terms "Hazard" has been defined by OECD and by REACH (e.g. http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm). Hazard is commonly defined as "the potential to cause harm" and can be defined as "a property or situation that in particular circumstances could lead to harm" (EEA, 1998).

Health effects are important to consider for hazard assessment in many areas, such as hygiene, pollution studies, workplace safety, nutrition and health sciences in general. Some of the major environmental sources of health effects are air pollution, water pollution, soil contamination, noise pollution and over-illumination.

Health effects related to CO_2-capture technologies are mainly related to emissions of potentially hazardous components through flue gas from the absorber. Flue gas composition and components concentrations will partly depend on solvent technology and cleaning of flue gas prior to emission.

Potential health effects are evaluated by toxicity studies using animals or cell cultures from mammal species. Various endpoints are used to cover a variety of possible toxic responses, as described in the Tender Invitation:

- Mutagenicity/genotoxicity
- Carcinogenetic effects
- Reproduction toxicity and developmental toxicity
- Acute toxicity
- Repeated dose toxicity (chronic toxicity)
- Subchronic toxicity
- Sensitization
- Irritation/corrosion of skin and eyes

6.1.1 Principles of evaluation

In the present project exposure to humans may occur in an occupational setting or as a result of chemicals being dispersed to the neighbourhood of the production plant. Any controversy as to exposure limits etc will most probably be focused on the neighbourhood environment. Exposure to the general population around an industrial facility like Mongstad is characterised by low-level continuous exposure. Thus, acute, oral and dermal toxicity will play a minor role in the final overall assessment. Acute (4 hours) inhalation data will of course be important in any setting where atmospheric exposure is the prime source. However, acute short term exposure at relatively high concentrations may not be a good indicator of health hazards which may occur after low level and long term exposure. We therefore need to focus on compounds causing

effects that may occur after long-term and low-level exposure. At low level and long term exposure the following health hazards will be of prime interest when regulations are set for permissible exposure to population at or near a plant:

- Carcinogenicity (C)
- Mutagenicity (M)
- Reproductive effects (R)
- Sensitization, primarily by inhalation (S)

We therefore regard **mutagenicity/genotoxicity**, **carcinogenicity**, **reproduction toxicity** and **sensitization** as the most important endpoints to consider.

6.1.2 Types of toxicity testing

Acute toxicity deals with the adverse effects of single doses. It establishes the relationship between dose and adverse effects and can be used to compare the relative toxicity and calculate a median lethal dose. Such studies can therefore identify highly toxic chemicals and provide information on the possible hazards. Dosing periods lying between the single dose and 10 per cent of lifespan dosage are often called subacute.

Sub-chronic testing, on the other hand, can identify the toxic effects associated with repeated doses of a chemical over part of an average lifespan of experimental animals. It can detect the delayed effects which may occur due to accumulation of the chemical in tissues or by other mechanisms. These studies will provide detailed information on toxic effects, target organs, reversibility of effects and an indication of a 'no effect level'. The division between subchronic and chronic dosing regimes is sometimes taken as 10 per cent of the test animal's lifespan. In general, the longer the sub-chronic study, the more information is likely to be gained.

6.1.3 Explanation for classification of long term health hazards

Explanation for classification of long term health hazards is adopted from GESAMP-EHS: http://www.gesamp.org/publications/publicationdisplaypages/rs64

The symbol C, M, R and S indicate:

C	Shown to induce or increase cancer in animals or man
M	Shown to cause increased incidence of permanent changes in the amount or structure of the genetic material
R	Shown to cause adverse effects on reproductive ability or capacity, or the development of offspring
S	Shown to be a sensitizer (skin or respiratory)

Carcinogenic

The term carcinogenic denotes substances or mixtures that are presumed to induce cancer or to increase its incidence in humans. Evidence to substantiate the notation "carcinogenic" should be

available from epidemiological studies and/or from well conducted studies in experimental animals. On a case by case basis, scientific judgment may warrant a decision of presumed human carcinogenicity (C) derived from studies showing limited evidence in humans with limited evidence in experimental animals.

Mutagenic
A mutation is a permanent change in the amount or structure of the genetic material in a cell. The term mutation applies to genetic changes both for somatic cells and for germ cells that may give rise to subsequent adverse changes at the phenotypic level. The term mutagenic denotes substances or mixtures that can give rise to an increased occurrence of mutations *in vivo*, in populations of cells and/or organisms. Evidence to substantiate a notation of "mutagenicity" (M) is normally provided from studies conducted *in vivo* on mammalian somatic cells or germ cells. It is recognized that genetic events are central in the overall process of cancer development. Therefore, evidence of mutagenicity indicates that a substance has a potential to induce carcinogenic effects.

Reprotoxic
Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females or on the development of the offspring. The notation "reprotoxic" (R) includes substances for which there is reliable evidence from human experience or from experimental animals of an adverse effect on reproductive ability, capacity, or on development of the offspring in the absence of other toxic effects.

Sensitizer
The term sensitizing denotes substances or mixtures, which can induce a condition of hypersensitivity in individuals following inhalation (respiratory sensitizer) or skin contact (contact sensitizer). Evidence to substantiate a notation of "sensitizing" (S) should be available from human experience and/or from appropriate studies using experimental animals. The term photosensitizing (Sp) denotes substances or mixtures that require light to become active and may subsequently induce a condition of contact sensitivity. Evidence to substantiate the notation of "photosensitizing" should be available from human experience and/or from appropriate studies using experimental animals.

6.1.4 Classification of long term health hazards

There are two internationally recognized hazard classification systems available; GESAMP-EHS and GHS (Global Harmonized System). The first is primarily for maritime transport of chemicals while the last forms the basis of the UN system for recognition of transport hazards as well as R/S-sentences. GESAMP is the oldest system and classifies chemicals for environmental fate, ecotoxicology and human hazards. The GESAMP and GHS classifications are almost identical, the main differences being:

- The classification numbering is opposite (a rating of 4 is the most toxic in GESAMP while it is the least toxic in GHS)

- There are minor differences in cut-off values for acute toxicity
- There are some compounds classified as slightly irritant to skin and eye in GESAMP which are not classified as irritants in GHS
- There are a few compounds classified as C, M or R in GESAMP without similar rating in GHS

The GESAMP classification system was adopted for this report as one member of the project team was member of the GESAMP group of experts for 24 years and have extensive experience with this system. The outcome of the hazard evaluation for relevant flue gas chemicals would be the same regardless of whether GESAMP or GHS is used.

In addition, the CLP (Classification, labelling and packaging) system has been approved in EU and Norway. This system is mainly relevant for classification of compounds to be transported. Therefore, the CLP system is not used for the current project, since it is not relevant for emissions to the environment. Both GESAMP and EHS systems are used to sort and classify chemical hazards and are relevant for the current report.

6.1.5 Flue gas compounds used for hazard assessment

In the current project some chemicals were selected (Refer Chapter 5 for selection of compounds) for the representation of the components of a flue gas emission (Table 5.1). In brief, the chemicals have either been detected in emissions from CO2-capture systems based on amine solvent technologies, or are of concern as potential amine degradation products. Health and environmental data were collected for these chemicals for preliminary hazard assessments.

6.1.5.1 Literature search on potential flue gas chemicals

We did a literature search on flue gas substances chemicals in the following databases:
RTECS: http://ccinfoweb.ccohs.ca/rtecs/search.html
IUCLID data sheet: http://ecb.jrc.ec.europa.eu/esis/
GESAMP-list: http://www.imo.org/includes/blastDataOnly.asp/data_id%3D25672/Report-BLGCirc.29annex6doc.pdf
GESAMP background info: http://www.gesamp.org/publications/publicationdisplaypages/rs64
CPDB: http://potency.berkeley.edu/chemicalsummary.html
EPA-IRIS: http://www.epa.gov/ncea/iris/index.html
CCRIS: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS
Hazmap: http://hazmap.nlm.nih.gov/
SciFinder: http://www.cas.org/products/scifindr/index.html
PubMed: http://www.ncbi.nlm.nih.gov/pubmed

We also included search on the TOXNET (TOXicology Data NETwork). This is a cluster of databases covering toxicology, hazardous chemicals, environmental health and related areas. It is managed by the Toxicology and Environmental Health Information Program (TEHIP) in the Division of Specialized Information Services (SIS) of the National Library of Medicine (NLM).

The database links are: HSDB, IRIS, ITER, CCRIS, GENE-TOX, ToxTown, Household Products Database, Haz-Map, TOXMAP, LactMed and CPDB. **The literature links are:** TOXLINE, DART/ETIC, Toxics Release Inventory. **The Chemical Information links are:** ChemIDplus.

TOXNET databases are accessible free of charge at: http://toxnet.nlm.nih.gov/

From these databases, information was obtained for all the chemicals (see Table 5.1) except ethanolnitramine (74386-82-6). The following table (Table 6.2) shows a list of chemicals and source of information.

6.1.5.2 Preparation of summary sheets

The information obtained from the databases was used to prepare a summary sheet for each of these compounds (Appendix H). The summary sheet contains information about oral, percutaneous, inhalation, skin and eye irritation and other long term effects. The source of databases and GESAMP profile is also given in the summary sheets for each chemical.
GESAMP is an organization for cooperation between several UN organizations (UNEP, FAO, UNESCO, IOC, WHO, WMO, IMO, IAEA). GESAMP/EHS was established in 1974 and have carried out detailed examination on roughly 3000 compounds and products carried at sea. The GESAMP classification is carried out by a team of international experts on chemistry, marine ecotoxicity and human health hazard assessment. The experts are invited by IMO (International Maritime Organization) on behalf of GESAMP. Their classification has been published as the GESAMP Composite list by IMO. The GESAMP/EHS review is based on public records as well as confidential company information. In some cases laboratory reports from the toxicity studies have been examined. The files supporting the decisions of the GESAMP/EHS group is located at IMO, London, UK.

Table 6.2 List of flue gas chemicals and source of information[A)].

Chemical Group	Name of the chemical	CAS Numbers	RTECS	IUCLID	GESAMP	CPDB	IRIS	Toxnet	Other
Amin	MEA	141-43-5	+	+	+	-	-	+	+
NH3	NH3	7664-41-7	+	+	+	-	+	+	+
Aldehydes	Formaldehyde	50-00-0	+	+	+	+	+	+	+
	Acetaldehyde	75-07-0	+	+	-	+	+	+	+
Amides	Acetamide	60-35-5	+	X	-	+	-	+	+
Alkylamines	Methylamine	74-89-5	+	+	+	-	-	+	+
	Dimethylamine	124-40-3	+	+	+	-	+	+	+
Nitrosamines	N-nitrosodimethylamine	62-75-9	+	+	-	+	+	+	+
	N-nitrosodiethanolamine	1116-54-7	+	+	-	+	+	+	+
	N-nitrosomorpholine	59-89-2	+	-	-	+	-	+	+
Nitramines	Dimethylnitramine	4164-28-7	+	-	-	+	-	+	+
	Ethanolnitramine	74386-82-6	-	-	-	-	-	-	-
	Methylnitramine	598-57-2	+	-	-	+	-	+	+

[A)] Explanations +: Information available; x: Chemical is listed but no data sheet available; -: No data available
Other databases are: CCRIS, Hazmap, SciFinder

6.1.5.3 Preparation of C, M, R and S sheets

In addition to summary sheets, Carcinogenicity (C), Mutagenicity (M), Reproduction (R) and Sensitization (S) sheets were prepared for each individual study (refer appendix H).

Carcinogenicity and mutagenicity sheets:

For this toxicity, results from individual studies should be divided into two broad categories: mutagenic (genotoxic) and carcinogenic.

Reproductive effects sheets:

Broadly, reproductive toxicology is defined as any adverse effect on any aspect of adult male or female sexual function and fertility, or on the conceptus or on lactation, which would interfere with the production or development of normal offspring which could be reared to sexual maturity, capable in turn of reproducing the species. For the Amine 3 project, individual studies were divided into three broad categories: male/female reproduction capacity, fetotoxicity and fetal development which can be defined as follows: (i) male/female reproduction capacity: adverse effects on reproductive ability or capacity in adult males and females, that is, effects on sexual behaviour and fertility; (ii) fetotoxicity: adverse effects on maintenance of pregnancy (which includes no. of implantations, post implantation loss, counting of corpora lutea, duration of gestation, still births, runts etc.) (iii) fetal development: developmental outcome (teratogenicity and postnatal evaluations) of the offspring.

Sensitization sheets:

For sensitization, individual studies should be categorized into inhalation and percutaneous studies.

6.1.5.4 Ratings for the chemicals

All the chemicals were classified and rated for each of the endpoints. For classification and numerical rating, the definitions used by GESAMP/EHS have been used. Individual studies from RTECS, IUCLID and other databases were rated in Columns C1, C2, C3, D1, D2, D3 according to GESAMP classification. From the GESAMP profile and ratings obtained from individual studies for each chemical, conclusions on oral, dermal, inhalation and long term effects were made which were presented as expert judgment at the end of summary sheets for each individual chemical (Refer appendix H for summary sheets).

A GESAMP classification system used for preparing summary sheets for potential flue gas compounds is shown below (Table 6.3).

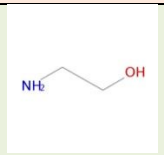
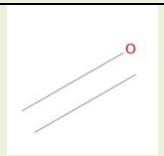
Table 6.3 Hazard system according to GESAMP

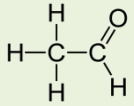
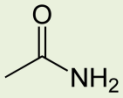
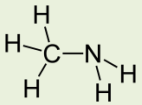
Column Label	Column heading	Explanation
C1	**Oral toxicity LD50 rating codes**	0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw
C2	**Percutaneous toxicity LD50 rating codes**	0: >2000 1: 1000-2000 2: 200-1000 3. 50-200 4: <50 mg/kg bw
C3	**Inhalation toxicity LC50 4 hours exposure rating codes**	0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)
D1	**Skin irritation / Corrosion**	0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive 3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min
D2	**Eye irritation / Corrosion**	0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury
D3	**Long term effects**	Full description of rationale for rating given at bottom of table. Short form rating code: C: Shown to induce or increase cancer in animals or man M: Shown to cause increased incidence of permanent changes in the amount or structure of the genetic material R: Shown to cause adverse effects on reproductive ability or capacity, or the development of offspring
	GESAMP/EHS rating	GESAMP ratings for each column (C1, C2, C3, D1. D2, D3) and date.
	Expert Judgement	A summary expert opinion on the chemical is given in the comments column. For oral/dermal/inhalation the numbers in respective columns indicate: • Negligible toxicity: 0 • Slight toxicity: 1 • Moderate toxicity: 2 • Moderately high toxicity: 3 • High toxicity: 4 Ratings in brackets: Provisional ratings based on limited or no data. Expert judgment. OEL: Occupational exposure level (Taken from RTECS: Lowest Scandinavian values. If not available then lowest OECD countries values are taken). **Conclusions and recommendations written in bold**

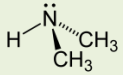
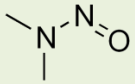
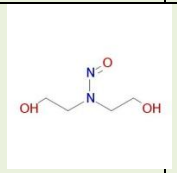
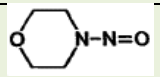
The expert judgment from individual summary sheet (Refer appendix H) of potential flue gas compounds is collected here and shown as a summary table for potential flue gas compounds (Table 6.4).

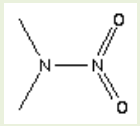
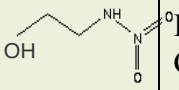
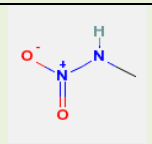
We have not performed any quality assessment of the toxicity data which is used for the basis of OEL

Table 6.4 Summary table for potential flue gas compounds. (Refer appendix C-3.1.2 for detailed explanation on these compounds and appendix H for summary sheets)

Chemical group	Name of the chemical	CAS Number	Structure	Data bases examined	Oral tox	Derm tox	Inhal tox	Long Term	Comment on human health
Amine	Ethanol, 2-amino- (MEA)	141-43-5		RTECS+ IUCLID+ GESAMP+ CPDB- IRIS- TOXNET+	1	1	3	M? S?	OEL: 2.5 mg/m3 Skin and eye: Severely irritating **C: No data available** **M: Need more data** **Confirm no R** **S: Need more data** **Need further testing for M and C[A)]**
NH3	Ammonia	7664-41-7	H3-N	RTECS+ IUCLID+ GESAMP+ CPDB- IRIS+ TOXNET+	1	(2)	3	C M?	OEL: 14 mg/m3 Skin: Severely irritating Eye: Severely irritating **C: tumor promoter** **M: Not enough data** **Confirm no R** **Confirm no S** **No further testing needed**
Aldehydes	Formaldehyde	50-00-0		RTECS+ IUCLID+ GESAMP+ CPDB+ IRIS+ TOXNET+	2	2	4	C M R S	OEL: 0.37 mg/m3 Skin and eye: Severely irritating **Confirm C** **Confirm M** **Confirm R** **Confirm S** **No further testing needed**

Chemical group	Name of the chemical	CAS Number	Structure	Data bases examined	Oral tox	Derm tox	Inhal tox	Long Term	Comment on human health
	Acetaldehyde	75-07-0		RTECS+ IUCLID+ GESAMP- CPDB+ IRIS+ TOXNET+	1	0	0	C M R S	OEL: 45 mg/m3 Skin: Mildly irritating Eye: Severely irritating **Confirm C** **Confirm M** **Confirm R** **Confirm S** **No further testing needed**
Amides	Acetamide	60-35-5		RTECS+ IUCLIDx GESAMP- CPDB+ IRIS- TOXNET+ Hazmap+	0	-	-	C M? R	OEL: 25 mg/m3 **Confirm C** **M: Need more data** **Confirm R** **S: No data available** **No further testing needed**
Alkylamines	Methylamine	74-89-5		RTECS+ IUCLID+ GESAMP+ CPDB- IRIS- TOXNET+	2	(2)	3	M	OEL: 6.4 mg/m3 Skin and eye: Severely irritating Moderate acute toxicity. **C: No data available** **Confirm M** **Confirm no R** **S: No data available** **Need further testing for C**

Chemical group	Name of the chemical	CAS Number	Structure	Data bases examined	Oral tox	Derm tox	Inhal tox	Long Term	Comment on human health
	Dimethylamine	124-40-3		RTECS+ IUCLID+ GESAMP+ CPDB- IRIS+ TOXNET+	2	0	2	M S	OEL: 3.5 mg/m3 Skin and eye: Severely irritating **Confirm no C** **Confirm M** **Confirm no R** **Confirm S** **No further testing needed**
Nitrosamines	N-nitrosodimethylamine	62-75-9		RTECS+ IUCLID+ GESAMP- CPDB+ IRIS+ TOXNET+	3	-	4	C M R	OEL: 0.001 mg/m3 **Confirm C** **Confirm M** **Confirm R** **S: No data available** **No further testing needed**
	N-nitrosodiethanolamine	1116-54-7		RTECS+ IUCLID+ GESAMP- CPDB+ IRIS+	0	(0)	-	C M	OEL: 0.001 mg/m3 Non-toxic by oral or dermal route. **Confirm C** **Confirm M** **R: No data available** **S: No data available** **Need further testing for R**
	4-nitroso-morpholine	59-89-2		RTECS+ IUCLID- GESAMP- CPDB+ IRIS- TOXNET+	2	-	-	C M	OEL: 0.001 mg/m3 Moderate toxicity by oral route **Confirm C** **Confirm M** **R: No data available** **S: No data available**

Chemical group	Name of the chemical	CAS Number	Structure	Data bases examined	Oral tox	Derm tox	Inhal tox	Long Term	Comment on human health
									Need further testing for R
Nitramines	Dimethylnitramine	4164-28-7		RTECS+ IUCLID- GESAMP- CPDB+ IRIS- TOXNET+ CCRIS+ Scifinder+	1	-	-	C M	**Confirm C Confirm M R: No data available S: No data available Need further testing for R**
	Ethanolnitramine	74386-82-6		RTECS- IUCLID- GESAMP- CPDB- IRIS-					No data available **Need further testing for C, M and R**
	Methylnitramine	598-57-2		RTECS+ IUCLID- GESAMP- CPDB+ IRIS- TOXNET+ CCRIS+	-	-	-	C M?	**Confirm C M: Need more data R: No data available S: No data available Need further testing for R and M**

[A)] *It should be stressed that though we suggest MEA for further testing (due to lack of knowledge,) this is temporary decision as further check with ongoing REACH dossiers which are still not known should be done.*

6.2 Methods available for toxicity testing

6.2.1 Method sources

Standard validated methods for human related mammalian toxicity studies are described in a number of databases:

- OECD Guidelines for Testing of Chemicals http://www.oecd-ilibrary.org/environment/oecd-guidelines-for-the-testing-of-chemicals-section-4-health-effects_20745788
- ECVAM - European Centre for the Validation of Alternative Methods (http://ecvam.jrc.ec.europa.eu/)
- JaCVAM - Japanese Centre for the Validation of Alternative Methods (http://jacvam.jp/en/index.html)
- ICCVAM - Interagency Coordinating Committee on the Validation of Alternative Methods at the National Toxicology Program (http://iccvam.niehs.nih.gov/)

6.2.2 Search for methods for carcinogenicity and mutagenicity

We searched ECVAM, JaCVAM and ICCVAM for validated methods (both for *in vitro* and *in vivo* methods) suitable for testing acute toxicity, mutagenicity and carcinogenicity of the compounds listed in table 6.2. The tables 6.5 and 6.6 lists validated test methods (*in vitro* and *in vivo*) for testing carcinogenicity and mutagenicity. Also methods for oral/chronic oral/inhalation were assessed in order to get an overview of adverse effects and an estimate of dosages for further toxicity assays (6.7). It should be noted that a few methods have passed ECVAM, JaCVAM and ICCVAM validation, and are in waiting list to obtain OECD guideline number. We also searched for methods or their modifications for specific type of toxicity (phototoxicity, non-genotoxic carcinogens, etc).

Table 6.5 Validated methods for carcinogenicity

OECD validated – In vivo assays	
451	Carcinogenicity studies
452	Chronic Toxicity Studies
453	Combined Chronic Toxicity/Carcinogenicity Studies (alternative to 451 and 452)
ECVAM validation process – In vitro assays	
OECD draft review paper (No 31)	Cell Transformation Assays; Combination of different assays: 1.Syrian hamster embryo (SHE) assay 2.Balb/c assay 3.C3H/10T1/2 assay (Alternative to 451)

Table 6.6 Validated methods for mutagenicity

OECD validated - In vivo assays	
474	Mammalian Erythrocyte Micronucleus Test
475	Mammalian Bone Marrow Chromosome Aberration Test
477	Genetic Toxicology: Sex-Linked Recessive Lethal Test in Drosophila melanogaster
478	Genetic Toxicology: Rodent Dominant Lethal Test
483	Mammalian Spermatogonial Chromosome Aberration Test
484	Genetic Toxicology: Mouse Spot Test
485	Genetic toxicology, Mouse Heritable Translocation Assay
486	Unscheduled DNA Synthesis (UDS) Test with Mammalian Liver Cells in vivo
JaCVAM/ECVAM/ICVAM validation process – In vivo assays	
JaCVAM ECVAM/ICVAM	In vivo Comet Assay (Single-Cell Gel Electrophoresis [SCGE] Technique)
OECD validated – In vitro assays	
471	Bacterial Reverse Mutation Test
473	In vitro Mammalian Chromosome Aberration Test
476	In vitro Mammalian Cell Gene Mutation Test
479	Genetic Toxicology: In vitro Sister Chromatid Exchange Assay in Mammalian Cells
480	Genetic Toxicology: Saccharomyces cerevisiae, Gene Mutation Assay
481	Genetic Toxicology: Saacharomyces cerevisiae, Miotic Recombination Assay
482	Genetic Toxicology: DNA Damage and Repair, Unscheduled DNA Synthesis in Mammalian Cells in vitro
487	In Vitro Mammalian Cell Micronucleus Test
JaCVAM validation process – In vitro assays	
JaCVAM	In Vitro Comet Assay (Single-Cell Gel Electrophoresis [SCGE] Technique)

Table 6.7 Validated methods for acute oral, chronic oral and inhalation toxicity

OECD validated - In vivo assays	
423	Acute Toxic Class (ATC) Method for acute oral toxicity testing (replacing 401)
420	Fixed Dose Procedure (FDP) for acute oral toxicity testing (replacing 401)
425	Up-and-Down Procedure for acute oral toxicity testing (replacing 401)
407	Repeated Dose 28-day Oral Toxicity Study in Rodents
408	Repeated Dose 90-Day Oral Toxicity Study in Rodents
409	Repeated Dose 90-Day Oral Toxicity Study in Non-Rodent
403	Acute Inhalation Toxicity
412	Subacute Inhalation Toxicity: 28-Day Study
413	Subchronic Inhalation Toxicity: 90-day Study
436	Acute Inhalation Toxicity - Acute Toxic Class Method
OECD validation process In vitro assays	
OECD Draft Guidance document 129	Using cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests (a combination of the two NRU tests below) (NHK NRU assay and mouse 3T3 fibroblasts) (3T3 NRU assay)

6.2.3 Search for methods for reproduction toxicity

We searched for OECD and ECVAM validated methods suitable for testing reproduction toxicity of these compounds. Table 6.8 gives a list of validated test methods (*in vitro* and in *vivo* methods) for testing reproduction toxicity.

Table 6.8 Validated methods for reproduction toxicity

OECD validated - In vivo assays	
415	One generation
416	Two generation
414	Prenatal developmental
426	Developmental neurotoxicity
New TG Draft	Extended one generation (415+416+414+426)
407	Repeated dose 28 day oral
421	Rep/dev toxicity
422	Combined 407+421 (422 is a screening for reproductive effects)
440	Uterotrophic Bioassay in Rodents: A short-term screening test for oestrogenic properties
441	Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties
OECD validated – In vitro assays	
455	The Stably Transfected Human Estrogen Receptor-alpha Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals
New TG Draft	Steroidogenesis Assay
ECVAM validated – In vitro assays	
Embryonic development	Whole embryo culture: Embryo (malformation, retardation and death) after over a 48 hr exposure
	Embryonic stem cell test: Growth and differentiation of murine ESC D3, into spontaneously contracting cardiomyocytes (adjunct- OECD TG 414)
	Micromass assay: Detect inhibition and cell differentiation in rat micromass cultures of limb bud

6.2.4 Search for methods for sensitization testing

Inhalation sensitization: To our knowledge, there are no validated methods for testing sensitization from exposure via inhalation. OECD has TG 413, which measures subchronic inhalation toxicity and is a 90 day study which measures subchronic inhalation toxicity after repeated exposure via inhalation route for 28 days. It can be used for determining NOAEL (no-observable adverse effect level) and LOAEL (lowest observable adverse effect level), but does not provide any indication of sensitization.

Skin sensitization: For sensitization via skin exposure, OECD recommends a two tier testing. The first tier consists testing of chemical with TG 429 using Lymph node assay or Mouse ear swelling test. If no definite answer is obtained from the above then TG 406- Guinea pig maximization test is recommended.

6.3 Consideration of methods for mammalian/human toxicity testing

6.3.1 Important points to consider for selecting appropriate methods

There are few important points to consider for the selection of appropriate methods for testing genotoxicity, carcinogenicity and reproductive toxicity concerning potential flue gas compounds like **nitrosamines** and **nitramines**:

- Nitrosamines can be converted to nitramines in the atmosphere primarily via reaction with the hydroxyl radical, reaction with ozone, and photolysis (Tuazon *et al*., 1984)

- Nitramines can be reduced (deaminated) to formaldehyde and ammonia both *in vivo* and *in vitro*, and this process is reported to be responsible for the carcinogenicity of nitramines, (Frie *et al*., 1984)

This indicates the need for a metabolic activation system or appropriate metabolic system (metabolically active cells or feeder cells) for assessing the toxicity of nitramines and nitrosamines.

Most of the compounds described in Table 6.4 are likely to be mutagenic. If they are carcinogenic they will be mutagenic carcinogens except for ammonia (tumour promoter) and formaldehyde which may act genotoxic, but also likely as promoter. It is important to choose methods and endpoints which may identify genotoxic endpoints on different levels (DNA breakage, base damage, point mutations, larger mutagenic and clastogenic changes as well as disruptions in cell divisions). For testing of carcinogenicity it is important to address both initiation of carcinogenicity (genotoxic effects) as well as non-genotoxic carcinogenicity (promotions, proliferation, progression). Literature reviews have shown that these compounds are likely to release photooxidative products, and thus photogenotoxicity should be also addressed. Photoxicity has been a biological indicator of nitrosamine activity (Nishie *et al*., 1972). Studies in Ames bacteria (*Salmonella typhimurium*) have indicated that UVA-irradiation of N-dialkylnitrosamines releases nitric oxide, with subsequent production of alkyl radical cations and active oxygen species which cause DNA strand breaks, oxidative and alkylative DNA damage and mutation (Arimoto-Kobayashi *et al*., 2010).

For reproductive toxicity, in the nirosamine group, the data from N-nitrosodimethylamine (62-75-9) indicated that it is a confirmed reprotoxic chemical with main effects being observed as fetotoxicity (Refer appendix H for R sheets). This suggests the need for the methods which are mainly focused upon measuring fetotoxic effects.

6.3.1.1 General considerations of *in vitro* tests

Currently available *in vitro* tests are accepted by regulatory bodies mostly for genotoxicity testing. It has been suggested that they can be used in future to determine the starting dose of *in vivo* studies, assist in evaluation of data from animal studies especially in identification of species differences, or to increase understanding of the toxicological mechanism of action of the substance. They cannot be used to replace testing in animals completely, although this may be possible in the future. *In vitro* data may be useful for predicting acute toxicity in humans and a range of tests have been investigated that permit calculation of an IC50 (inhibitory concentration 50%) value, though none are yet approved by OECD. It has been suggested that the results of *in vitro* cytotoxicity tests may be good predictor of acute oral toxicity in humans, based on rat or mouse data (Kinsner-Ovaskainen et al., 2009). However, this aspect needs to be further investigated.

Main advantages of *in vitro* test methods:

- 3R (Reduce, refine and replace the use of animals for toxicity testing)
- Easy
- Cheap
- Less time consuming
- Good for studying mechanisms of toxicity at molecular and cellular level
- Potentially robust

- High throughput: it is possible to test hundreds of chemicals in a short time, and it is possible to combine them with chip or robotic technology for performing or evaluation of the results
- Specific types of toxicity can be studied such as photogenonotoxicity
- Human primary cells (e.g. lymphocytes) or stable cell lines can be used which might be closer to humans than animal systems
- Some assays can be adopted for fast in situ screening (in exposed environment for biomonitoring)

Major disadvantages of *in vitro* models and test methods:

- Major disadvantage is that toxicokinetic studies can not be perform by using *in vitro* system
- Most of the tests use a prediction model for correlating *in vivo* and *in vitro* data experimental data. For *in vitro* data these prediction models depend upon the concentration curves which are highly dependent upon factor like e.g. the cell density, serum concentration and quality of the media. A slight change in any of the factors may result in a shift in the concentration curves and incorrect use of in vitro data in combinations with *in vivo* experiments
- Correlation of experimental data (*in vitro* and *in vivo*) with human data is very challenging even in cases where human primary cells or cell lines derived from humans are used
- There is a lack of metabolic activation for some *in vitro* systems that can be partly overcome by using external metabolic systems (feeder cells or co-cultivation with potent metabolically active cells, S9 fraction, using metabolically active cells and cell lines).
- Current *in vitro* methods for testing reproduction toxicity either do not include metabolic activation or introduction of metabolic systems is not yet included in the validation program. Presence of metabolic activation system is relevant for testing the toxicity of nitrosamines and nitramines.
- Testing volatile chemicals and airborne particles *in vitro* is a major technical challenge if the test represents inhalation toxicology

6.3.2 Consideration of validated *in vitro* tests for mutagenicity and carcinogenicity

Table 6.9 shows a list of appropriate methods in vitro mutagenicity/genotoxicity and carcinogenicity.

Table 6.9 All appropriate *in vitro* methods

In vitro mutagenicity/genotoxicity tests	
1. OECD 471	Prokaryote assay, reverse gene mutation test: Ames test.
2. OECD 473	Eukaryote assay, Chromosomal damage: In vitro mammalian cytogenetic test (chromosome aberrations).
3. Comet assay (JaCVAM validation)	Eukaryote assay, DNA damage effects/DNA adduct formation/: In Vitro Comet Assay, Single-cell Gel Electrophoresis (SCGE) Technique.
4. OECD 476	In vitro mammalian cell gene mutation assay.
5. OECD 479	In vitro Sister Chromatide Exchange Assay in Mammalian Cells
6. OECD 480	Saccharomyces cerevisiae, Gene Mutation Assay
7. OECD 481	Saccharomyces cerevisiae, Mitotic Recombination Assay
8. OECD 487	In vitro Mammalian Cell Micronucleus test
In vitro carcinogenicity test	
OECD draft review paper (No 31)	Cell transformation assays, combination of different assays: 1. Syrian Hamster Embryo (SHE). 2. BALB/C assay. 3. C3H/10T1/2 assay.

6.3.3 Consideration of validated in vivo tests for mutagenicity and carcinogenicity

Table 6.10 shows a list of appropriate methods for *in vivo* mutagenicity/genotoxicity and carcinogenicity.

Table 6.10 All appropriate *in vivo* methods

In vivo mutagenicity/genotoxicity tests:	
1. OECD 474	Mammalian erythrocyte micronucleus test.
2. OECD 475	Mammalian bone marrow chromosome aberration test.
3. OECD 477	Sex-Linked Recessive Lethal Test in Drosophila melanogaster
4. OECD 478	Rodent Dominant Lethal Test
5. OECD 483	Mammalian Spermatogonial Chromosome Aberration Test
6. OECD 484	Mouse Spot Test
7. OECD 485	Mouse Heritable Translocation Assay
8. In vivo comet assay	Eukaryote assay to detect DNA damage and different DNA lesions in different organs and tissues In Vivo Comet Assay, Single-cell Gel Electrophoresis (SCGE) Technique.
Chronic toxicity / Carcinogenicity tests:	
1. OECD 451	Carcinogenicity study
2. OECD 452	Chronic toxicity study
3. OECD 453	Combined Chronic Toxicity/ Carcinogenicity studies.

6.3.4 Testing strategy considerations for testing carcinogenicity and mutagenicity

For genotoxicity/mutagenicity, three different *in vitro* tests are usually initially chosen for 2-3 different endpoints, usually both prokaryotic and eukaryotic tests. If 2 out of 3 tests are positive, then the compound is considered genotoxic and likely to be carcinogenic. *Mutagenicity* refers to the induction of permanent transmissible changes in the amount or structure of the genetic material of cells or organisms. *Genotoxicity* is a broader term and refers to processes which alter the structure, information content or segregation of DNA and are not necessarily associated with mutagenicity.

In vivo acute toxicity tests are unavoidable even if *in vitro* test data exist. Today there are improved and combined methods for *in vivo* toxicity tests, where more data can be gathered with fewer animals tested. For example the previous OECD 401 method for oral toxicity (LD50) is now improved into the OECD 420, 423 and 425 methods. An option to reduce the number of animal is to include the new OECD draft TG "Using cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests" after being approved by OECD. The acute toxicity results give an estimate of dosages for further endpoint testing.

The initial *in vitro* genotoxicity/mutagenicity tests are generally followed by *in vivo* genotoxicity/mutagenicity tests, before any long term animal testing are initiated. If the outcome

is positive, further long-term carcinogenicity testing might be considered unnecessary to avoid suffering of animals. If the outcome is negative, then the compound might be a non-genotoxic carcinogen, and further long term carcinogenicity testing is initiated.

6.3.5 Consideration of validated *in vitro* tests for reproduction toxicity

Table 6.11 shows a list of validated *in vit*ro methods. OECD has two *in vitro* validated tests which measure the hormonal activity as their endpoint. The ECVAM validated tests assess the embryo and developmental toxicity.

Table 6.11 List of validated *in vitro* methods

OECD	**Endpoints**
455	Esterogen receptor binding and luciferase activity
New TG Draft	Hormone production of 17β-estradiol (E2) and testosterone (T)
ECVAM	
Whole embryo culture	Developmental toxicity: IC50 and ICmax for malformations; ICnoec for total morphological score (TMS) as the sum of scores for all organ anlagen.
Micromass	Developmental toxicity: ID50 (50% inhibition of cells differentiation and no. of foci); IC50 (50% inhibition of cell viability and growth).
Embryonic Stem Cells	Embryotoxicity; ID50 (50% inhibition of cardiac cell differentiation), IC50D3 (50% viability of D3 cells) and IC503T3 (50% viability of 3T3 cells)

6.3.6 Consideration of validated *in vivo* tests for reproduction toxicity

Below is a list of validated *in vivo* methods with their main emphasis of different categories of reproduction toxicity (Table 6.12).

Table 6.12 List of validated *in vivo* OECD methods

OECD	**Method**	**Main emphasis**
415	One generation	Rep / some Feto aspects/ Dev
416	Two generation	Rep / Feto/ Dev
414	Prenatal developmental	Dev
426	Developmental neurotoxicity	Neurotoxicity
New TG Draft	Extended one generation (415+416+414+426)	Rep / Dev / Neuro/ Immuno
407	Repeated dose 28 day oral	Endocrine disruption
421	Rep/dev toxicity	Rep / Feto/ Dev
422	Combined 407+421 (422 is screening reproductive toxicity studies)	Rep / Feto/ Dev
440	Uterotrophic Bioassay in Rodents: A short-term screening test for oestrogenic properties	Endocrine disruption
441	Hershberger Bioassay in Rats: A Short-term Screening Assay for (Anti)Androgenic Properties	Endocrine disruption

Table 6.13 OECD validated methods for measuring fetotoxicity

OECD	Method	Time	Advantages	Disadvantages
415	One generation	11 weeks	Extrapolation to man No-effect levels and permissible human exposure	Only some aspects of fetotoxicity (still births)
416	Two generation	18 weeks	Several aspects of fetotoxicity* Extrapolation to man No-effect levels and permissible human exposure	Time consuming Expensive
421	Rep/dev toxicity	7.7 weeks	Several aspects of fetoxicity* Dose range finding study	Limited means of detecting postnatal effects
422	Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test Combines OECD 407+421	7.7 weeks	Several aspects of fetoxicity* Uses fewer animals when compared to combination of 407 + 421 Dose range finding study	Limited means of detecting postnatal effects

**(no. of implantations, post implantation loss, counting of corpora lutea, duration of gestation, still births, runts etc.)*

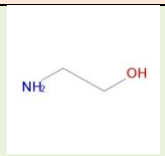
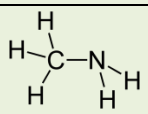
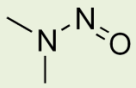
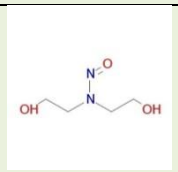
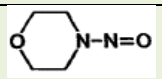
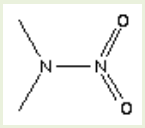
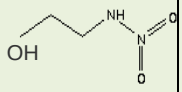
From this list, it can be seen that there are four relevant methods (OECD- 415, 416, 421, 422) which measure the fetotoxicity in addition to reproductive and developmental effects. We therefore, focused on the advantages and disadvantages of these four methods (Table 6.13).

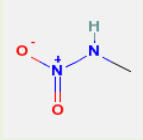
From table 6.13, it can be seen that OECD test method 422 measures several aspects of fetotoxicity and uses fewer animals (10 animals of each sex for 3 treatment groups and control) when compared to combination of OECD 407 (5 animals of each sex for 3 treatment groups and control) and 421 (10 animals of each sex for 3 treatment groups and control).

6.4 Recommendations for Call Off 2 - Hazard assessment

A list of chemicals was selected to represent each chemical group mentioned in the scope of TQPamine3. The reasons for selection of chemicals and testing of single compounds have been described in chapter 5. Hazard assessment of these potential flue gas compounds was performed and is provided in Table 6.4. The hazard assessment of these compounds provided knowledge about where data on the compounds are available, needed or missing. In order to fill the gaps where information is needed or missing, we recommend testing of 8 compounds. Out of these 8 compounds, we recommend 4 compounds for C testing, 4 compounds for M testing and 6 compounds for R testing. A list of compounds recommended for C, M or R testing are summarized in a table shown below (Table 6.14)

Table 6.14 Recommendations for potential flue gas compounds.

Chemical group	Name of the chemical	CAS Number	Structure	Data bases examined	Comment on human health
Amine	Ethanol, 2-amino- (MEA)	141-43-5		RTECS+ IUCLID+ GESAMP+ CPDB- IRIS- TOXNET+	**Need further testing for M and C[A)]**
Alkylamines	Methylamine	74-89-5		RTECS+ IUCLID+ GESAMP+ CPDB- IRIS- TOXNET+	**Need further testing for C**
Nitrosamines	N-nitrosodimethylamine	62-75-9		RTECS+ IUCLID+ GESAMP- CPDB+ IRIS+ TOXNET+	**To be used as a positive standard and a benchmark on dose for C, M and R testing**
	N-nitrosodiethanolamine	1116-54-7		RTECS+ IUCLID+ GESAMP- CPDB+ IRIS+	**Need further testing for R**
	4-nitroso-morpholine	59-89-2		RTECS+ IUCLID- GESAMP- CPDB+ IRIS- TOXNET+	**Need further testing for R**
Nitramines	Dimethylnitramine	4164-28-7		RTECS+ IUCLID- GESAMP- CPDB+ IRIS- TOXNET+ CCRIS+ Scifinder+	**Need further testing for R**
	Ethanolnitramine	74386-82-6		RTECS- IUCLID- GESAMP- CPDB- IRIS-	**Need further testing for C, M and R**

Chemical group	Name of the chemical	CAS Number	Structure	Data bases examined	Comment on human health
	Methylnitramine	598-57-2		RTECS+ IUCLID-GESAMP-CPDB+ IRIS-TOXNET+ CCRIS+	**Need further testing for R and M**

[A)] *It should be stressed that though we suggest MEA for further testing (due to lack of knowledge,) this is temporary decision as further check with ongoing REACH dossiers which are still not known should be done.*

6.5 Recommendations for the protocol- Methods

The methods described below for C, M and R testing are recommended as general test strategies for degradation products of amine technology. Since a tier system has been recommended for testing, some of the described methods may take more than 6 months time period (time frame for Call Off 2) for completion.

6.5.1 Recommendation of methods for testing mutagenicity/genotoxicity and carcinogenicity

The recommendations described below are based on testing of chemicals lacking all necessary data for mutagenicity, genotoxicity and carcinogenicity. When relevant data are available the quality of the data should be judged before decisions of testing are made. Data information should be based on testing by validated methods (OECD) and by GLP-approved test laboratories.

6.5.1.1 Step-by-step testing

A recommendation for a step-by-step procedure for safety evaluation of CO_2 capture flue gas compounds is shown in Table 6.15.

Step 1:

Table 6.15a Strategy for acute toxicity.

Step 1, Acute Single Dose Toxicity		Rational for choice of method(s)
OECD 425	Up-and-Down Procedure for acute oral toxicity testing.	There are a limit test and a main test. The limit test can be used efficiently to identify chemicals that are likely to have low toxicity. LD50 values are calculated and it is possible to compute confidence intervals. This test is more used for environmental chemicals and unknown chemicals.

Step 2:

Perform 3 in vitro genotoxicity tests, addressing 3 different endpoints in this order:
a) OECD 471
b) OECD 487 or 473
c) OECD 476

Knowledge about the metabolic profile of a substance indicates that the standard *in vitro* tests are not sufficiently reassuring and a further *in vitro* test, or an *in vivo* test, may be needed in order to ensure mutagenicity potential is adequately explored. An metabolic activation alternative to rat liver such as S-9 mix, or a metabolically active cell line like HepG2 cells or genetically engineered cell lines should be used to test 'amine 3' compounds.

Table 6.15b Strategy for genetic toxicity *in vitro*.

Step 2, Genetic Toxicity *in vitro*		**Rational for choice of method(s)**
OECD 471	Prokaryote assay, reverse gene mutation test: Ames test.	*Validated method and the most commonly used initial method for genotoxicity screening.*
OECD 487	In vitro Mammalian Cell Micronucleus test	*Validated and commonly used method. Mammalian systems used. Results are easy to screen.*
OECD 473	Eukaryote assay, /Chromosomal damage: In vitro mammalian cytogenetic test (chromosome aberrations).	*Validated and commonly used method. Mammalian systems used. Results are easy to screen. The method has been used with positive result for relevant flue gas compounds.*
OECD 476	In vitro mammalian cell gene mutation assay, the thymidine kinase (TK) locus assay or the hypoxanthine phosphoribosyltransferase (HPRT) assay.	*Validated and commonly used method. Mammalian systems used. Results are easy to screen. The method has been used with positive result for relevant flue gas compounds.*

- ➔ ***If negative test results,*** the substance is likely to be non-genotoxic, and no further testing is required
- ➔ ***If equivocal test results,*** 1 out of 3 positive=> additional tests om different cell models is required
- ➔ ***If positive results,*** 2 out of 3 positive=> **genotoxicity established**, and the compound is determined to be a **non-threshold substance.** *Continue with in vivo genotoxicity tests.*

Table 6.15c Strategy for genetic toxicity *in vivo*.

In vivo genotoxicity tests:		**Rational for choice of method(s)**
OECD 474	Mammalian erythrocyte micronucleus test.	Validated and commonly used method. The method has been used with positive result for relevant flue gas compounds.
OECD 475	Mammalian bone marrow chromosome aberration test.	Validated and commonly used method. The method has been used with positive result for relevant flue gas compounds.

- ➔ ***If positive results,*** *then the substance is considered a* ***genotoxic rodent carcinogen****. No further carcinogenicity testing is necessary. This is a* ***non-threshold substance.***
- ➔ ***If negative results,*** *then the substance is likely to be* ***non-genotoxic****. Go to step 3 and test for non-genotoxic carcinogenicity.*

Step 3:

Table 6.15d Strategy for carcinogenicity tests.

Step 3, Chronic toxicity / Carcinogenicity		Rational for choice of method(s).
OECD 453	Combined Chronic Toxicity/ Carcinogenicity studies. 12-24 months.	Validated method. Alternative fused test of the two common OECD 451 and OECD 452 methods. Reduces number of animals. The OECD 451 method has been used for all Amine3 compounds that currently have a TD50 value. **Results:** carcinogenic properties, tumor incidence in relation to dose (TD50), latency period, tumor multiplicity, potential for metastasis.

➔ ***If positive results,*** *substance is considered* ***a carcinogen.***
➔ ***If negative results,*** *If negative results, substance is considered* ***non-genotoxic***

We recommend R testing for C positive compounds due to the following reasons:

1) *There are certain compounds such as few phthalates which although being C negative have R effects through their hormone mimicking action. The release in question does not contain phthalates, but a range of other compounds in flue gas which might interfere with hormone levels. So there might be some compounds in the flue gas which are C negative but R positive.*
2) *Compounds that are both C and R positive will be of more concern to the public than C positive only compounds. We would recommend the company to prepare a dossier as complete as possible to avoid any criticism in the future.*

6.5.1.2 Rational for choice of methods for testing mutagenicity/genotoxicity and carcinogenicity

In vitro cytotoxicity tests for determination of IC50 can in some cases be used to estimate LD50 or LC50. They are not proposed in here as they are not yet OECD validated. However, the OECD draft guidance document on using cytotoxicity tests to estimate starting doses for acute oral systemic toxicity tests should be taken into consideration once OECD validated.

Acute toxicity tests. The OECD 425 tests for oral exposure were chosen by us for determination of median lethal dose (LD50). Concentrations obtained are used for setting dosages for further toxicity tests.

In vitro genotoxicity tests. Apart from being a validated commonly used gene mutation method for prokaryotes, the OECD 471 Ames method has been used often for relevant flue gas compounds with positive outcome, like alkylamines, nitrosamines and nitramines, therefore we chose OECD 471. The OECD 476 method is the only validated gene mutation method for mammalian cells, and it has been used to prove mutagenicity of compounds relevant for flue gas emissions (e.g. alkylamines and nitrosamines). The OECD 473 assay has been proposed for chromosomal damage analysis as it has been used with positive outcome for nitrosamine (N-nitrosomorpholine).

The *In vitro* Comet assay is very promising, quick, inexpensive, easy to perform, well established in many laboratories and is under validation by ECVAM. Final validation study is expected in autumn 2010, and submission of validation results and draft TG is expected early 2011 (https://www.oecd.org/dataoecd/22/61/41339719.pdf). Therefore we propose to wait for finalization of the JaCVAM validation study before including it to the strategy. The OECD 487 micronucleus method was chosen as it is widely used and pick up true positives. The OECD 479 (SCE) and 482 (UDS) methods are either not specific, error prone and/or complicated to perform, so we do not recommend these methods. The OECD 480 and 481 methods of gene mutations in *Saccharomyces,* were not chosen to be of primary interest due to the possibility of species specific differences.

In vivo genotoxicity tests. The OECD 474 *In vivo* micronucleus test and 475 *in vivo* chromosome aberration test are both validated and commonly used method. They have also both been used with positive result for the compounds of relevance for flue gas emissions (nitrosamines). The *in vivo* comet assay is in final stage of validation by ECVAM/JaCVAM but was not chosen as it is not yet OECD validated. It is very promising method that can detect DNA damage in different organs and tissues (liver, blood, lung, etc.) and. The OECD 477 method with gene mutations in *Drosophila*, was not chosen to be of primary interest due to the possibility of species specific differences. The OECD 478, 483, 484 and 485 methods have not been previously documented to be particularly useful for validation of relevant flue gas compounds and were therefore not chosen. The OECD 486 UDS in vivo method is an old, rough assay, not specific for DNA damage or repair, and the outcome of this test is uninformative. We therefore do not recommend this method.

In vitro carcinogenicity test. The Cell Transformation assays are the only currently available *in vitro* alternative methods to *in vivo* carcinogenicity tests. They are very promising and should be taken into consideration if OECD approves the draft guideline.

In vivo carcinogenicity test. The OECD 453 method is recommended in our strategy. It is an alternative method where the OECD 451 and 452 methods are combined. We selected OECD 453 method as it is time and cost effective, and uses fewer animals. In addition, it addresses both chronic toxicity and carcinogenicity in the same assay.

6.5.1.3 Relevance for using the recommended methods for testing mutagenicity/genotoxicity and carcinogenicity

The methods chosen address specific endpoints that are highly relevant for the type of damage we expect from these compound, either gene mutations (OECD 471 and 476) or chromosomal damage (OECD 473 and 487). The step-by-step strategy is relevant for avoiding unnecessary experiments by estimating the dosages and the toxicity with simpler assays *in vitro* (e.g. OECD 471, 476 or 487 and 473), and thereby reducing the costs and the number of animals in the *in vivo* tests (e.g. OECD 474 and OECD 475). Finally the long term toxicity tests (Step 3) are recommended to be performed only after judging the outcome from the genotoxicity tests (Step 1 and 2), which also contributes to reducing time, cost and animals.

6.5.2 Recommendation of methods for testing reproduction toxicity

We recommend a two tier study for testing the reproduction toxicity (Table 6.16). Some of the recommended flue gas compounds for reproduction toxicity testing (see Table 6.14) have shown to be fetotoxic (refer R sheets in appendix H) and have a high oral toxicity and inhalation toxicity, e.g. the nitrosamine N-nitrosodimethylamine. This chemical may therefore be included as a positive standard and a benchmark on dose. The tier 1st includes a standard OECD test 425 for determining the LD50 of the chemical where no previous LD50 data is available. This should be followed by OECD test 422 for testing fetotoxicity. The outcome of the 1st tier will determine the need for a 2nd tier where a detailed study with any one or two of the OECD test methods 415 and 416 should be performed. Test evaluation forms of the recommended methods are described in Appendix D.

Table 6.16 Recommended reproduction toxicity testing protocol

Step 1: For acute toxicity

Acute Single Dose Toxicity		**Rational for choice of method**
OECD 425	Up-and-Down Procedure for acute oral toxicity testing.	Suggested in cases where no LD50 data is available. It includes a limit test and a main test. It can be used to calculate LD50 values for environmental chemicals and unknown chemicals.

Step 2: For sub-chronic toxicity

Sub-chronic Toxicity		**Rational for choice of method**
OECD 422	Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test	It is a range finding screening test for reproduction toxicity. It measures several aspects of fetotoxicity and provides information on dose concentration. It is a combination of OECD 407 and 421, and uses fewer animals as compared to combined animals being used for 407 and 421.

Step 3: For chronic toxicity

Chronic Toxicity		**Rational for choice of method**
OECD 415	One-Generation Reproduction Toxicity Study	Long term study for several aspects of reproduction toxicity i.e. parental, fetal and developmental toxicity in first generation.
OECD 416	Two-Generation Reproduction Toxicity	Long term study for several aspects of reproduction toxicity i.e. parental, fetal and developmental toxicity in first and second generation.

Combined Protocol for reproduction toxicity testing

Combined Protocol for reproduction toxicity testing		
1st tier	LD50 when no data available – suggest OECD 425	OECD 422
2nd tier		One or two of OECD 415 and 416 depending on outcome of 1st tier

6.5.2.1 Rational for choice of methods for testing reproduction toxicity

Hazard evaluation of reprotox data from nitrosamines group suggests that it has main effects on fetotoxicity. Based on this data, we can presume that the rest of the nitrosamines in the group (1116-54-7 and 59-89-2) are fetotoxic as well.

In vitro methods: OECD and ECVAM validated *in vitro* tests do not measure fetotoxic effects in particular and do not include metabolic activation. However, metabolic activation may be relevant for potential flue gas compounds like nitrosamines and nitramines, and we therefore propose the use of *in vivo* test methods for testing the reproduction toxicity of nitramines and nitrosamines.

In vivo methods: Since very little or no information on acute toxicity or repeated dose toxicity is available for most of the recommended compounds for reproduction toxicity testing, some initial acute toxicity data or repeated dose toxicity testing needs to be done. This can be done with OECD test method 425 for acute oral toxicity or OECD 407 for repeated dose toxicity. Both OECD test methods 421 and 422 measure several aspects of fetotoxicity, however OECD test method 422 is a combination of OECD 407 (5 animals of each sex for 3 treatment groups and control) and 421 (10 animals of each sex for 3 treatment groups and control). It uses fewer animals (10 animals of each sex for 3 treatment groups and control) when compared to combination of OECD 407 (5 animals of each sex for 3 treatment groups and control) and 421 (10 animals of each sex for 3 treatment groups and control). Therefore we recommend OECD test method 422 which would provide information on dose concentration and fetotoxicity and is in accordance with reduction of animals for toxicity testing.

6.5.3 Recommendation of methods for Sensitization

We will not recommend including sensitization tests for the Protocol, based on the following reasons:

- There are no validated methods for measuring sensitization from inhalation, an endpoint relevant for this project.
- The concentrations of flue gas chemicals in the neighbourhood environment are expected to be far below what can be considered to induce sensitization.
- Most of the sensitization data available from reported studies and the classification of chemicals as respiratory allergens are based entirely upon clinical experience because of lack of well validated animal models. This classification is thus highly dependant upon several variables.

7 Ecotoxicity and biodegradation data

7.1 Ecotoxicity data of flue gas compounds

A hazard assessment has been done for the soil and aquatic compartments for the flue gas compounds selected as a test case for hazard assessment (see Table 5.1). Literature data for potential flue gas components were searched by the following databases:

IUCLID (http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=dat)
ECOTOX (http://cfpub.epa.gov/ecotox/)

In addition ecological data were collected from Material Safety Data sheets (MSDS), if available. Scientific literature was investigated by the search engine **SciFinder search**. A review of the findings is shown in Table 7.1, while a list of the data and the literature list are shown in Appendix I and Appendix J.

We used the database IUCLID and SciFinder as a basis for the data search. ECOTOX were searched when very few or no data were oberved in IUCLID. MSDS were consulted for all substances.

The search showed that data for direct air exposure exist for some plant species, but only for formaldehyde. The effects are obviously dependent on the time of exposure, although the endpoints used may differ somewhat. For aquatic tests the ecotoxicity test results were found for all compound groups except nitrosamines and nitramines. Most literature data were found for formaldehyde and ammonia, including several trophic levels. For ammonia the toxicity differed significantly, and this was mainly the results of ionic form of ammonia. If present as cation (NH_4^+) the ecotoxicity is lower than for the non-ionic form.

Table 7.1 Summary of ecotoxicity results from databases and from search on SciFinder. The results are shown as number of tests (No), median values and ranges from each source of data. The database ECOTOX were not investigated (NI) when data were found in the IUCLID database or by SciFinder.

Chemical	Trophic level	IUCLID			ECOTOX			MSDS			SciFinder		
		No	Median	Range	No	Median	Range	No	Median	Range	No	Median	Range
Ethanolamine (MEA)	Algae	1	15	15	NI			0			3	80	24.7-100
	Crustaceans	1	65	65	NI			0			0		
	Molluscs	0			NI			0			4	43	27.6-100
	Fish	9	329	150-3864	NI			0			1	5000	5000
Ammonia, NH3	Algae	0			NI			0			0		
	Crustaceans	12	14.2	0.96-189	NI			0			13	126	5.5-189
	Rotifers	0			NI			0			2	11.1	3.2-20.4
	Molluscs	0			NI			0			2	0.025	0.013-0.037
	Insects	0			NI			0			7	1.605	0.69-2.11
	Fish	33	0.75	0.16-9.14	NI			0			19	0.44	0.11-1.55
Formaldehyde	Algae	1	0.3	0.3	NI			0			2	7.8	0.9-14.7
	[A)]Plants	0			NI			0			3	840	78-1060
	Crustaceans	1	2	2	NI			1	2	2	27	16.9	1.9-178.6
	Worms	0			NI			0			3	0.48	0.39-0.79
	Insects	0			NI			0			3	340	287-450
	Amphibians	0			NI			0			12	11.25	6.0-100
	Fish	28	67.5	6.7-440	NI			3	94	26-100	65	40.3	0.4-69.8
Acetaldehyde	Algae	1	237	237	NI			0			0		
	Crustaceans	1	48.3	48.3	NI			1	2		3	9114	5807-12427
	Fish	8	88.5	30.8-153	NI			3	41	24-100	0		
Acetamide	Algae	0			0			0			1	10000	
	Protozoa	0			0			0			1	99	
	Crustaceans	0			0			0			1	10	
	Fish	0			2	11650	10000-13300	1	13	13	0		
Methylamine	Algae	0			NI			0			0		
	Crustaceans	2	432.5	163-732	NI			0			0		
	Fish	3	710	150-1000	NI			0			0		
Dimethylamine	Algae	2	19.5	9-30	NI			0			0		
	Crustaceans	1	286	286	NI			0			1	49.4	
	Fish	6	205	118-396	NI			0			0		

[A)] Result for plant species from air exposure – values as $\mu g/m^3$ air

Table 7.1 Continued

Chemical	Trophic level	IUCLID			ECOTOX			MSDS			SciFinder		
		No	Median	Range	No	Median	Range	No	Median	Range	No	Median	Range
N-nitrosodimethylamine	Algae	0			1	4	4	0			0		
	Crustaceans	0			1	10	10	0			0		
	Fish	0			5	3300	3300	0			0		
N-nitrosodiethanolamine		0			0			0			0		
N-nitrosomorpholine		0			0			0			0		
Dimethylnitramine		0			0			0			0		
Ethanolnitramine		0			0			0			0		
Methylnitramine		0			0			0			0		

7.2 Biodegradability

Biodegradation is not part of a hazard evaluation, but is one of the parameters included in risk assessment. The reason for that is that biodegradation is one of the process factors important for the fate of a compound in the environment (together with abiotic processes like hydrolysis and photolytic degradation). Indirectly, the biodegradation processes are also important for toxicity, since transformation of compounds by biodegradation (and other degradation processes) may alter the toxicity related to the original mother compound.

Biodegradation data are important as part of the fate estimation of flue gas emission compounds in biotic compartments (soil, water, sediments). Biodegradation can be grouped in a number of ways, including screening and simulation tests, tests related to the different compartments. The screening tests are normally separated between ready and inherent biodegradability tests.

The sources for evaluation of flues gas component biodegradation data included the following databases:
IUCLID (http://ecb.jrc.ec.europa.eu/esis/index.php?PGM=dat)
BIODEG database from the Syracuse Research Corporation (http://www.syrres.com/what-we-do/databaseforms.aspx?id=382)

In addition data from MSDS were used, if available. The scientific literature was searched on the ISI Web of Science search engine (http://apps.isiknowledge.com/). For the IUCLID and BIODEG databases the searches were conducted with CAS number for the individual chemicals, while the Web of Science was searched. The search results for the individual compounds are shown in Table 7.2, while the references from the Web of Science searches are placed in Appendix K.

Evaluations of the biodegradation characteristics of the flue gas compounds are individually described below. Results from screening and simulated tests are shown in Table 7.3 below for the individual compounds. Only compounds with available data (see Table 7.2) are presented.

Table 7.2 Literature search of biodegradation data for individual flue gas compounds.

Chemical Group	Name of the chemical	CAS Numbers	IUCLID	BIODEG	Other literature[B)]
Amine	MEA	141-43-5	**X**	**X**	**X (4)**
NH3	NH3	7664-41-7	**X**[A)]	No data	**X**
Aldehydes	Formaldehyde	50-00-0	**X**	**X**	**X (16)**
	Acetaldehyde	75-07-0	**X**	**X**	**X (4)**
Amides	Acetamide	60-35-5	No data	**X**	**X (1)**
Alkylamines	Methylamine	74-89-5	**X**	**X**	**X (1)**
	Dimethylamine	124-40-3	**X**	**X**	No data
Nitrosamines	N-nitrosodimethylamine	62-75-9	No data	**X**	**X (10)**
	N-nitrosodiethanolamine	1116-54-7	No data	**X**	No data
	N-nitrosomorpholine	59-89-2	No data	No data	**X (1)**
Nitramines	Dimethylanitramine	4164-28-7	No data	No data	No data
	Ethanolnitramine	74386-82-6	No data	No data	No data
	Methylnitramine	598-57-2	No data	No data	No data

A) Transformation to NO_2/NO_3 which take part in eutrophication processes
B) Numbers of relevant papers in brackets

Table 7.3 Biodegradation information collected from IUCLID, BIODEG and MSDS. The results are shown as number of tests (No. Tests), and as median values and ranges for each substance.

Chemical	Test	No. Tests	Test results (% biodegradability at the end of test)			
			Median	Highest	Lowest	References
MEA	Ready biodegradability	23	80	100	30	BIODEG, IUCLID
	Simulated – Sewage	1	65	65	65	BIODEG
Formaldehyde	Ready biodegradability	11	78	100	0	BIODEG, MSDS (Acros), IUCLID
	Simulated – Sewage	3	99	99	82	BIODEG, IUCLID
Acetaldehyde	Ready biodegradability	6	60.2	69.8	27.5	BIODEG, MSDS (Acros), IUCLID
	Simulated – Sewage	2	66.5	97	36	BIODEG
	Other	1	15	15	15	Anaerobic (IUCLID)
Acetamide	Ready biodegradability	2	22.5	45	0.05	BIODEG
Methylamine	Ready biodegradability	10	79.3	100	0	BIODEG, IUCLID
Dimethylamine	Ready biodegradability	16	61.4	100	30	BIODEG, IUCLID
	Simulated – Sewage	3	99	100	93	BIODEG
	Simulated – Aquatic					
	Simulated – Soil	1	68-84	68	84	IUCLID
	Simulated – Sediment	1	90	90	90	IUCLID (beach experiment)
N-nitrosodimethylamine	Ready biodegradability	3	72	100	0	BIODEG
	Simulated – Sewage	1	100	100	100	BIODEG
	Simulated – Soil	9	30	60	0	BIODEG
N-nitrosodiethanolamine	Ready biodegradability	1	94	94	94	BIODEG
	Simulated – Soil	6	98	100	0	BIODEG

The searches showed that available biodegradation information was limited for most of the compounds, as shown in Table 7.3.

For MEA a total of 23 screening tests were reported in the IUCLID or BIODEG databases, with a range of biodegradability from 30-100 %). The median result of the degradation was 80% biodegradability, i.e. MEA may be defined as a ready biodegradable compound. Results from inherent biodegradability tests were not found. One simulated sewage test showed that MEA removal was 65 % with a retention time of 23 hours and a use of a semi-continuous aerated system (SCAS). No standard biodegradation studies in soil were reported.

Ammonia will be an essential degradation product released by flue gas emissions. Contrary to the other flue gas components degradation of ammonia may result in unwanted consequences. Ammonia may be transformed to nitrite or nitrate (NO_2/NO_3) microbial nitrification, and elevated concentrations may result in eutrophication. At a local scale, the deposition of nitrogen in the form of ammonia can result in eutrophication of sensitive ecosystems and the acidification of the soil. Eutrophication is briefly described in a separate section of this report under future research requirements (see cpt. 11).

Aldehydes like formaldehyde and acetaldehyde are ready biodegradable by standard methods. A number of screening tests as described in the IUCLID and BIODEG databases showed that formaldehyde is a ready biodegradable chemical. No inherent biodegradability results were recorded for these chemicals. Simulated sewage studies showed nearly complete removal of formaldehyde, but higher variability for acetaldehyde in SCAS systems.

For acetamide only two screening biodegradability tests were reported for this substance, both showing low biodegradability. This compound can therefore not be defined as ready biodegradable.

Methylamine was shown in screening tests to be ready biodegradable. Although some biodegradation studies showed 0 % degradability this was the result of high concentration and toxic effects on the microbes. No inherent or soil biodegradability tests were reported. For dimethylamine results from several screening tests indicated that this chemical was ready biodegradable, although median results were close to limit for ready biodegradability. Simulated sewage testing showed nearly complete removal of dimethylamine within a retention time of 120 hours in a SCAS system, while a soil study showed 68-84 % biodegradability of ^{14}C-labeled compound after 7 days of degradation, and with a concentration-dependent degradation rate (increasing concentrations resulted in higher degradation rates). The IUCLID database also reported a simulated sediment study (beach study) for dimethylamine with > 90 % TOC removal 10 days after application.

Screening biodegradability tests were reported for two of the nitrosamines, N-nitrosodimethylamine and N-nitrosodiethanolamine. Median results indicated that both were ready biodegradable, although results for 3 tests with N-nitrosodimethylamine ranged s from 0-100 % biodegradability. Lack of biodegradability was associated with high concentration and toxic effects on the microbial populations. A simulated sewage test of N-nitrosodimethylamine showed 100 % removal during a 24-hour incubation period with daily feeding during a 7-week test period. A number of soil biodegradation experiments with ^{14}C-labelled or non-labelled test substance showed degradability between 0 and 100 % for the two nitrosamines.

No screening data were available for the nitrosamine N-nitrosomorpholine or for the nitramines.

The scientific literature included some valuable information on biodegradation of flue gas +-compounds. A study of the biodegradability of **MEA** in a soil-groundwater bioreactor with indigenous bacteria showed MEA degradation within 20-25 days (Mrklas et al., 2004). Another study indicated that MEA was successfully biodegraded under both aerobic and anaerobic conditions, with ammonium, acetate, and nitrogen gas as the dominant by-products. Cold temperatures (5 ◦C) reduced the biodegradation rates significantly compared to rates at room temperature (Ndegwa *et al.,* 2004). Small aldehydes are rapidly biodegraded by soil bacteria. In a study with acclimated soil bacteria formaldehyde was biodegraded by 70 % within 24 hours (Mirdanadi *et al.,* 2005). Formaldehyde is also biodegraded at anaerobic processes in wastewater systems under denitrification and methanogenic conditions (Eiroa *et al.*, 2007). Although no scientific studies were identified on the biodegradation of **acetamide**, this substance is a degradation product from biodegradation of acetonitrile and acrylonitrile, with subsequent generation of acetic acid and ammonia for acetonitrile or acrylamide followed by acrylic acid and ammonia for acrylonitrile (Li *et al.,* 2007). Research studies on biodegradation of **nitrosamines** indicated slow biodegradability of N-nitrosodimethylamine and other nitrosoamines in soil and lake water, with lag phases of nearly 30 days occurred before slow disappearance (Tate and Alexander, 1975). Both N-nitrosodimethylamine and N-nitrosomorpholine were been reported with a half-life in anaerobic aquifers of > 100 days (Pattersen *et al.*, 2010). However, studies of microbial communities from groundwater systems showed that there was a potential for nitrosamine biodegradation (Gunnison *et al.*, 2000). It was reported elevated removal in groundwater by incidental and active recycled water recharge (Zhou *et al.*, 2009). N-nitrosodimethylaminane was effectively biodegraded (96%) in a biofilm reactor based on hydrogen, resulting in generation of methylamine and ammonia as degradation products (Chung *et al.*, 2008).

7.3 Methods for ecotoxicity testing

Ecotoxicity involves the identification of chemical hazardous to the environment. Ecotoxicity studies are used to measure the effects of substances on fish, wildlife, plants and other wild organisms. Ecotoxicity studies are conducted both for submission of information to regulatory authorities (e.g. Klif) and for studies and predictions of the impacts in specific environments.

Ideally, the whole ecosystem should be considered, but for practical and scientific reasons, one is restricted to the analysis of the effects of subsystems (individual organisms). Ecotoxicity tests are widely used, because i) most of the pollutants eventually are found in water, and ii) of practical reasons, such as ease of testing.

The outcome of such ecotoxicity testing will be *hazard* evaluations, i.e. the inherent effects of chemicals or mixtures of chemicals, without considering the impacts related to the exposure concentrations.

Ecotoxicity refers to the hazards of both aquatic and terrestrial animals and plants. In general the ecotoxicity tests concepts include the testing of different interdependent trophic levels in the various environments. Typically three levels of the food chain are represented:

- First level: Primary producers – organisms obtaining their metabolism and biomass from inorganic sources (autotrophic organisms)

- Second level: Herbivores – plant eaters, i.e. the organisms consumes primarily autotrophs
- Third level: Carnivores – organisms preying on herbivores

In addition, the omnivores prey on both plants and animals, and may therefore be represented in both second and third levels.

This system is better established for aquatic systems than for other environments, and aquatic organisms are often used as a first indication of environmental effects of chemicals. For aquatic organisms validated tests are described for all three levels:

- Phytoplankton tests representing the first level
- Invertebrate tests with organisms like *Daphnia* (freshwater) or *Acartia* (seawater), representing the second level
- Fish tests representing the third level

In addition to the ecotoxicity tests degradation and bioaccumulation tests are often included in the ecotoxicity-concept as part of describing the environmental characteristics of chemicals. Degradability includes biological or abiotic degradation of chemicals to characterize their persistence in the environment. Bioaccumulation includes the accumulation or concentrations of chemicals in living organisms, which may or may not lead to toxic effects. In aquatic systems bioconcentration is determined by comparing the concentrations in the organism and the water surrounding the organism. An important aspect of bioaccumulation is the biomagnification, in which chemicals increase in concentrations upward in the food chain as the result of preying activity.

7.3.1 Ecotoxicity endpoints and methods

The effect/endpoint, which is established before testing, is evaluated by comparing the chemically exposed organisms (treated) with the untreated organisms (untreated controls). If the test is being performed on a chemical which is not readily solvable in water, a solvent (carrier) may be used to prepare stock solutions, and then a solvent control, organisms only exposed to the solvent, have to be included. A positive (reference) control is also often included in the test, using a reference toxicant which has a known effect range on the test organism based on previous experience. It is used to determine the health and sensitivity of the organisms, to compare the relative toxicities of substances by using the control as an internal standard, to perform inter-laboratory calibrations, and to evaluate the reproducibility (precision) of the test data with time. Whenever possible, chemical analysis should be performed to measure the concentrations to which the test organisms are being exposed. In addition, it is valuable to measure chemical residues in tissues of exposed organisms (body burden). Internal concentrations of chemicals are seldom the same as the external concentrations, and some substances which are readily absorbed but only slowly lost or released can build up to very high concentrations. In addition, chemicals in the environment may also reach organisms through the food chain/web, an exposure route usually not covered in standard toxicity tests.

By far the most commonly used endpoint in aquatic ecotoxicological testing is mortality/immobilization. Other endpoints may be effects on birth, maturation and reproduction.

These endpoints may be used assessing ecological significance of chemical exposure through calculations of biomass loss. Other effects, which may be indirectly influence ecologically important parameters (secondary effects) are for instance impairment of respiration, increase in detoxification enzymes, altered hormone metabolism (endocrine disruption), changes in pigmentation, behavioural changes etc. If a dose/concentration-response relationship is determined for a chemical, the threshold for an effect is often given as lethal concentrations (e.g. LC50), no observed effect concentration (NOEC), and lowest observed effect concentration (LOEC).

The general experimental design requires careful control over test conditions (e.g. pH, temperature, dissolved oxygen concentrations, photoperiod). Aquatic test organisms are exposed in test chambers (e.g. glass tanks) to various concentrations of test chemical in water solutions, while soil and sediment organisms are exposed in soil/sediment microcosms. For aquatic tests there are generally three types of test systems being used for testing of chemicals. In static tests the chemical is added once to the system, no flow occurs, and the test medium is not changed during the experiment. In semi-static tests the test medium and test compound are periodically replaced, while flow-through tests are conducted with test medium and test compound supplied at a constant rate and concentration to the organism. Test types can also be divided into short-term tests with single species, long-term tests with single species, tests with multispecies systems and finally tests using in vitro systems. Also for soil and sediment tests both static and flow-through test systems have been developed.

Biodegradation is commonly determined as primary biodegradation or ultimate biodegradation. Primary biodegradation refers to the use of chemical analyses to determine the depletion of specific chemicals as the results of biological transformation, i.e. the biochemically mediated change. Ultimate biodegradation also includes the further degradation from organic to inorganic carbon, often called mineralisation (CO_2 evolution). Determination of ultimate biodegradation is normally performed by the use of respirometric analyses, in which oxygen consumption or CO_2 evolution is determined. The source of organisms for biodegradation are microorganisms from water (freshwater or seawater), soil, sediments (freshwater or marine), or domestic or industrial sewage. Test systems are normally static systems, but also flow-through test systems have been developed.

Biodegradability tests are normally separated in ready biodegradability tests and inherent biodegradability tests. Ready biodegradability tests are screening tests, conducted under aerobic conditions, in which a high concentration of the test substance (in the range of 2 to 100 mg/L) is used and biodegradation is measured by non-specific parameters like dissolved organic carbon (DOC), biochemical oxygen demand (BOD) and CO_2 production. Domestic sewage, activated sludge or secondary effluent is the typical source of microorganisms in the tests for ready biodegradability. The inoculum should not be pre-adapted to degradation of the test substance by previous exposure to the test substance or structurally related chemicals. A positive result in a test for ready biodegradability can be considered as indicative of rapid and ultimate degradation in most environments, including biological sewage treatment plants (STPs). A chemical attaining the pass level in these tests at a certain rate after termination of the lag phase may be classified as "ready biodegradable". The pass level depends on the analytical parameter measured. Inherent biodegradability tests are aerobic tests that possess a high capacity for degradation to take place, and in which biodegradation rate or extent is measured. The test procedures allow prolonged exposure of the test substance to microorganisms and a low ratio of test substance to biomass, which offers a better chance to obtain a positive result compared to tests for ready biodegradability. Some of these tests may be conducted using microorganisms that have previously been exposed to the test substance, which frequently results in adaptation leading to a

significant increase of the degradation rate (OECD Guideline for Testing of Chemicals - Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3, 2006).

Bioaccumulation tests are performed to determine the distribution of a chemical between biological tissue (or a phase representing this) and the surrounding environment (e.g. water). The simplest way of describing bioaccumulation is to determine the distribution of a chemical between two immiscible phases, like octanol and water, to determine the potential for accumulation in fatty tissues. In these tests the octanol-water coefficients are determined (Pow). These tests are conducted either by determination of the ratio between octanol and water in a shake-flask method (e.g. OECD 107), or by determination of the retention time by high-performance liquid chromatography (HPLC), where the stationary phase represent the octanol and the mobile phase the water phases (OECD 117). However, more elaborate studies include the bioaccumulation or bioconcentration of a chemical in a specific organism, which include accumulation and excretion mechanisms. In these tests the bioconcentration (BCF) is determined as the ratios between concentrations of a chemical in animal tissues and in the medium outside the organisms.

7.3.2 Summary of methods

Validated test methods are described in a number of databases. Some of the most common of these are:

- OECD Guidelines for Testing of Chemicals
- International Organisation for Standardisation
- American Society for Testing and Materials (ASTM)
- United States Environmental Pollution Agency (US-EPA)

7.3.2.1 Ecotoxicity methods

Standard toxicity tests are in general aimed at establishing the relationship between a dose/concentration of a chemical and a response (endpoint). In aquatic ecotoxicological testing the test substance is administered with water (saltwater or freshwater) or sediment, and concentration-dependent effects are then determined. Most tests are developed for testing the impact of chemicals in the water column (freshwater or saltwater), but a few are also for aquatic sediment test (OECD Tests No. 218, 219 and 225, ASTM Tests No. E1706-05e1, E1367-03(2008), E1611-00(2007) and E2591-07, US-EPA Tests No. 8501735, 8501740, 8501790, 8501800, and ISO Tests No. ISO 16712:2005, ISO 10872:2010). Testing of the effects of chemicals in sediments is mostly used for chemicals with low water solubility. A few tests also employ activated sludge to assess the impact of a chemical on microbial/bacterial processes (US-EPA – 8506800, ISO 9509:2006).

Full guidelines for ecotoxicity tests may be found at these websites:
OECD: http://www.oecd.org/department/0,3355,en_2649_34377_1_1_1_1_1,00.html
ASTM: http://www.astm.org
US EPA: http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm
ISO: http://www.iso.org/

The standard tests describe tests regimes and protocols using specific organisms for assessing their sensitivity to chemicals. Other describes tests which may be used for different species from

both freshwater and saltwater. A wide range of species may be tested, and the most commonly used freshwater ecotoxicity tests used are with microalgae (*Pseudokirchneriella subcapitata*), daphnids (*Daphnia* sp, Crustacea) and fish (Zebrafish - *Danio rerio*). Test guidelines for these species are available from different suppliers with identical or similar protocols, e.g. the acute toxicity testing of *Daphnia* sp (OECD - Test No. 202, US-EPA – 8501010 and ISO 6341:1996). There are also standard ecotoxicity tests for bacteria and other microorganisms, plants, macroalgae, rotifers, nematodes, insects, oligochaetes, crustaceans and amphibians. Thereby organisms at different trophic levels are represented. Table 7.4 shows the different available test guidelines from OECD, ISO, ASTM and US-EPA sorted on organism group. Different developmental stages are also represented for some of the organisms to determine toxicity to the most sensitive developmental stage (e.g. egg, larval stages), or to assess the impact of a chemical on the development *per se*. The US-EPA have developed tests using microcosms, where several species are used together to simulate the effects of chemicals on a model ecosystem (US-EPA – 8501900 and US-EPA - 8501925). Because different species will have different threshold for chemical toxicity, several tests may be used. The standard test species may not be resident in the area where chemical discharges are expected, and testing toxicity of chemicals on local species may therefore sometimes be preferable.

Test evaluations of ecotoxicity methods for various compartments are presented in Appendix E.

Chemicals may differ in their terms of type of effects they cause to a biological system. The most ecologically relevant endpoint is mortality, and it is also the most commonly used endpoint in standard ecotoxicity testing. However, some chemicals may have specific effects on biological systems which are not reflected in such tests. Acute toxicity tests only reveal the concentration of chemical exposure to which 50 % of a test population dies (LC_{50}) after a limited time of exposure (usually 96 hrs), and although this is a relatively easy and cost-effective endpoint to measure, it does not give direct information regarding other effects which may be of importance reflecting organism health, and they do also not cover potential long-term effects or effects of chronic exposure. Chemicals may display negative effects on respiration, ventilation, hormone systems, neurological systems, offspring, growth, maturation, immune system and more. These different "modes of toxicity" are actually poorly understood for most chemicals, and as far as standard ecotoxicity testing is concerned, limited information to these effects can be provided. The most commonly used endpoints assessed in the standard toxicity tests are acute toxicity (mortality/immobilization), impairment of growth and reproductive effects.

Some test guidelines have also been proposed for assessing chemicals in terms of their ability to cause alterations in an organisms' hormone/endocrine system, so-called endocrine disrupting chemicals (EDCs). One EDC test has been validated and accepted and accepted as OECD test (OECD – Test No. 230), while five tests have been proposed and are currently under review. One of these is for testing (anti)androgenic activity of chemicals in sticklebacks (*Gasterosteus aculeatus*), while the others are used to assess other effects on endocrinology in fathead minnow (*Pimephales promelas*), the Japanese medaka (*Oryzias latipes*) and the zebrafish (*Danio rerio*). The accepted test is a 21-day fish assay involving screening for estrogenic and androgenic effects and aromatase activity in fathead minnow (*Pimephales promelas*), the Japanese medaka (*Oryzias latipes*) or zebrafish (*Danio rerio*).

Table 7.4 Different organism groups used in standard aquatic ecotoxicity testing. FW= fresh water. SW= salt water. S=sediment. E=estuarine. AS=activated sludge.

Organism group	OECD	ISO	ASTM	US-EPA
Plant/algae	Test No. 201 (FW) and 221 (FW)		E1415-91(2004)e1 (FW), E1218-04e1 (FW/SW), E1924-97(2004) (FW/SW), E1841-04 (FW), D3978-04 (FW), E1498-92(2004) (SW)	8504400 (FW), 8504450 (FW), 8505400 (FW)
Insects	Test No. 218 (S/FW) and 219 (S/FW)		E1706-05e1 (S/FW)	8501735 (FW), 8501790 (FW)
Nematode		10872:2010 (FW)		
Annelids	Test No. 225 (S/FW)		E1611-00(2007) (S/SW), E1562-002(2006) (SW)	
Rotifer		20666:2008 (FW)	E1440-91(2004) (FW/SW/E)	
Molluscs			E724-98(2004) (SW), E2122-02(2007) (SW/FW), E1022-94(2007) (SW)	8501025 (SW/E), 8501055 (SW/E), 8501710 (SW)
Echinoids			E1563-98(2004)e1 (SW)	
Crustaceans	Test No. 202 (FW) and 211 (FW)	20665:2008 (FW), 16712:2005 SW/E), 6341:1996 (FW), 10706:2000 (FW), 14669:1999 (SW)	E1706-05e1 (FW/S), E1367-03(2008) (SW/E/S), E1295-01(2006) (FW), E1463-92(2004) (SW/E), E2317-04 (SW), E1191-03a(2008) (SW), E1193-97(2004) (FW)	8501010 (FW), 8501020 (FW), 8501035 (SW/E), 8501045 (SW/E), 8501300 (FW), 8501350 (SW/E), 8501735 (FW), 8501740 (SW),
Amphibians	Test No. 231 (FW)		E2591-07 (FW/S), E1439-98(2004) (FW)	8501800 (FW)
Fish	Test No. 203 (FW), 204 (FW), 210 (FW), 212 (FW), 215v, 229 (FW) and 230 (FW)	7346-1:1996 (FW), 7346-2:1996 (FW), 7346-3:1996 (FW), 12890:1999 (FW), 10229:1994 (FW), 15088:2007 (FW),	E1768-95(2008) (FW), E1241-05 (FW)	8501075 (FW/SW), 8501085 (FW), 8501400 (FW/SW), 8501500 FW/SW), 8501730 (FW),
Microorganisms	Test No. 224 (AS)	9509:2006 (AS)		8506800 (AS)

7.3.2.2 Biodegradability tests

Standard biodegradability tests are normally used to predict the fate of chemicals in biotic systems. Regulatory these tests are important to avoid discharges of persistent chemicals in the environment. Chemicals defined as ready biodegradable are expected to be rapidly removed from aerobic systems by bacterial metabolism.

Guidelines for validated biodegradability testing can be found on the same websites as the ecotoxicity tests shown above:

OECD: http://www.oecd.org/department/0,3355,en_2649_34377_1_1_1_1_1,00.html
ASTM: http://www.astm.org
US EPA: http://www.epa.gov/ocspp/pubs/frs/home/guidelin.htm

ISO: http://www.iso.org/

A summary of the available methods for standardised biodegradability tests are shown in Table 7.5. In this table the test methods from the different sources are compared, and several similar test methods are available from different sources. A similar comparison between OECD and ISO guidelines has is presented been by ISO (ISO/TR 15462).

A test protocol evaluation of the OECD tests recommended for the protocol are shown in Appendix F

In Table 7.5 the methods have been separated in various test systems. It is important to differentiate the tests, since the various systems have different aims. The separation has been made with respect to what kind of biodegradability (ready, inherent or simulation) and compartment (surface freshwater, sediment, soil, or seawater). All ready biodegradability tests are normally conducted under aerobic conditions, while simulated tests often include both aerobic and anaerobic systems. In addition, specific tests have been designed for measurements of biodegradation under strictly anoxic conditions, as gas evolution tests (production of CO_2, CH_4 or H_2S).

Ready biodegradability tests:
Ready biodegradability is determined based on pass levels reached during a 10-day window, normally within a 28-day test period. The 10-day window begins after biodegradation has reached 10 % removal as measured by dissolved organic carbon (DOC), theoretical oxygen demand (ThOD) or theoretical CO_2 evolution ($ThCO_2$). Within the test period the pass level should reach 60 % ThOD/$ThCO_2$ or 70 % DOC removal (OECD Guideline for Testing of Chemicals - Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3, 2006). These pass levels represent practically complete ultimate biodegradation of the test substance as the remaining fraction of 30-40 % of the chemicals then are assumed to be assimilated by the biomass as products for biosynthesis.

Typical measurement principles in ultimate ready biodegradability tests are decrease in DOC (OECD 301A, ISO 7827, ASTM E1279, EPA 835.3170), CO_2 measurements by respirometric analyses (OECD 301B), ISO 9439, EPA835.3140) or by determination of inorganic carbon (OECD 310, ISO14593), biological oxygen demand (BOD) in closed systems (OECD 301D, ISO 10707), or changes in gas pressure by manometry (OECD391F, ISO9408). Most of these tests are all commonly used for determination of ready biodegradability.

Most ready biodegradation tests are described for typical fresh water conditions with microbial sources from activated sludge, sewage or surface water. Marine tests have also been described (OECD 306, ISO, 16221, EPA835.3169) as a variant of the closed bottle tests, and with seawater as source for bacteria.

Inherent biodegradability tests:
Inherent biodegradability has been designed to evaluate if potentials for biodegradation of a chemical exist under aerobic conditions. Inherent biodegradability may be measured as primary biodegradation by specific analyses for the test chemical or as ultimate biodegradation by DOC or respirometric analyses. Biodegradation above 20 % may be regarded as proof of inherent primary biodegradability, while biodegradability above 70 % is regarded as proof of inherent ultimate biodegradability (OECD Guideline for Testing of Chemicals - Revised Introduction to the OECD Guidelines for Testing of Chemicals, Section 3, 2006). Positive inherent biodegradability indicates that a chemical has potentials for biodegradability under favourable conditions (e.g. i a

well-working sewage treatment plant), while negative inherent biodegradability indicates persistence in nature. Further testing of such persistent chemicals can be performed in simulation tests by realistically low concentrations of chemicals.

Inherent biodegradability tests are usually performed by bacterial inocula from activated sludge, and with determination of DOC or COD. Test periods may be 28 days or up to several months.

The test methods principles include semi-continuous aeration system (SCAS) (OECD302A, ISO9887, ASTME1625, EPA835.3210/5045), Zahn-Wellen/EMPA test with aerated vessels and rather high concentrations of chemicals (OECD302B, ISO9888, EPA835.3200), a modified MITI system with automated closed BOD measurements (OECD 302C). In addition inherent biodegradability tests have been developed for soil systems (OECD304, EPA835.3300).

Table 7.5 Comparison of validated biodegradability tests from different sources

Test systems	Test methods	OECD	ISO	ASTM	US-EPA
Ready biodegradability	DOC die-away test	301A	7827	E1279	835.3170
	CO_2 evolution test	301B	9439		835.3140
	Modified MITI test	301C		E1720	
	Closed bottle test	301D	10707		
	Modified screening test	301E	7827		
	Manometric test	301F	9408		
	CO_2 in sealed vessels	310	14593		
	Biodegradability in seawater	306	16221		835.3160
Inherent biodegradability	Modified SCAS test	302A	9887	E1625	835.3210
	Modified SCAS test insoluble and volatile chemicals				835.5045
	Zahn-Wellens/EMPA test	302B	9888		835.3200
	Modified MITI test (II)	302C			
	Concawe test	302D (draft)			835.3215
	Soil test	304			835.3300
Simulation Tests	Aerobic sewage treatment: activated sludge units	303A	11733		835.3240
	Porous pot test				835.3220
	Aerobic sewage treatment: biofilms	303B			835.3260
	Aaerobic and anaerobic transformation - soil	307			835.4100/4200
	Aaerobic and anaerobic transformation – aquatic sediment systems	308			835.4300/4400835.3190
	Aerobic mineralisation in surface water	309	14592		835.3280
	Biodegradability in wastewater	314			
Soil	Inherent biodegradability	304			835.3300
	Aerobic and anaerobic transformation	307			835.4100/4200
Sediment	Aerobic and anaerobic transformation	308		E1624	835.4300/44008
Surface water	Aerobic mineralisation	309			835.3190
Subsurface	Anaerobic biodegradation in the subsurface				835.5154
Seawater	Biodegradability in seawater	306	16221		835.3160
Anaerobic	Anaerobic biodegradability				835.3400
	Anaerobic biodegradability in digested sludge	311	11734	E2170	835.3420
	Anaerobic biodegradability in the subsurface				835.5154

Simulation tests:
Simulation tests have been developed for various compartments, including sewage treatment plants (STP), soil, sediments and water. No specific pass levels have been determined for these simulation tests.

STP tests are performed with activated sludge units (OECD303A), ISO 11733, EPA 835.3249/835.32220), or biofilm units (OECD 303B, EPA835.3260). Although no pass levels have been set for tests, recommendations for test substance removal of 10-20 mg/l DOC has been recommended in guidelines (OECD303A, OECD303B).

Soil tests may be performed with aerated soil (aerobic conditions) or water-logged soil (anaerobic conditions). In simulation tests for soil, water or sediments, low concentrations of test substance are recommended, i.e. low enough to ensure that biodegradation kinetics obtained reflect what is expected in the environment, e.g. 1-100 µg/L in surface water test (OECD309). Optionally, the use of radioactively labelled chemicals (^{14}C-labelled) is recommended (OECD307). Use of radiolabelled chemicals enables the determination on mineralisation by measurements of $^{14}CO_2$-evolution.

In ready and inherent biodegradability tests the test conditions like temperature are often high (20-25°C), and this may not be realistic conditions for biodegradability in Nordic countries. In addition, bacterial inocula may be higher than in relevant discharge environments. However, the conditions in the simulation tests may be adapted to local conditions dependent on season (e.g. 4-20°C), while microbial inocula may be realistically low (e.g. 10^3 to 10^6 cells/L).

Abiotic biodegradation:
Chemicals in the nature may also be degraded by abiotic processes as hydrolysis, oxidation and photolysis, and these processes may appear in combination with biological degradation. In aquatic, soil and sediment systems, biodegradation and hydrolysis are estimated to be more important processes than phototransformation, unless the chemicals are exposed directly to sunlight. The abiotic processes may also result in biodegradable transformation products.

A standard method for hydrolysis is described in the OECD Guideline 111, in which abiotic hydrolysis at three different pH-levels is determined at 50°C, while a method for phototransformation is described in the OECD guideline 316, with the use of a xenon lamp as solar simulator (wavelength 290-800 nm) (OECD111; OECD316).

7.4 Test methods relevant for ecotoxicity and biodegradation

No validated ecotoxiciy tests have been documented for direct exposure to gas samples. All tests described in the various Guidelines shown in Table 7.4 have exposure routes through water phase. Thus, if flue gas or flue gas components are to be tested by validated ecotoxicity methods the gas phase compounds should be transferred to a liquid phase. Aquatic toxicity methods may therefore be recommended for testing.

Ecotoxicity tests represent different uptake and toxicity mechanisms. It is generally recommended to include at least three different trophic levels, using aquatic species, These are recommendations both included in the REACH Directive for environmental testing of new chemicals, and in the Harmonized Offshore Notification Format (HOCNF) for offshore chemicals. Both systems include mandatory testing with three trophic levels of species –

- Autotrophic species (phytoplankton)

- Herbivore species ("plankton"-eaters)
- Fish species

The REACH Directive recommends the use of acute tests with freshwater tests, while HOCNF recommends marine species.

7.4.1 Relevant ecotoxicity methods

Based on the assumptions that validated tests from three trophic levels should be included, we recommend to use three acute aquatic tests as a first step in testing of flue gas compounds. Since CO_2 capture plants are onshore facilities we recommend the following validated aquatic tests:

- OECD 201 Freshwater Algae and Cyanobacteria, Growth Inhibition Test
- OECD 202 *Daphnia* sp., Acute Immobilisation Test
- OECD 203 Fish, Acute Toxicity Test

Emissions to air are considered important since the emissions can be dispersed over long distances. At present, there are no standard ecotoxicity tests describing air toxicity directly, but because of the high water solubility of the amines, it is reasonable to believe that the effect of air emissions will mainly be transferred to water and terrestrial environment; hence, the following Guidelines could then be considered:

- Earthworm
    a. OECD 207/222: Earthworm acute/reproduction test
- Insects
    a. OECD 226: Predatory mite reproduction in soil
    b. OECD 213/214: Honeybees: oral toxicity/contact toxicity
- Birds
    a. OECD 205/206: Avian Tests: dietary toxicity/reproduction.
- Plants
    a. OECD 208: Terrestrial plant test
    b. OECD 221: Lemna growth inhibition;
    c. OECD 227: Vegetative vigour test

We regard the soil test (earthworm) and a plant test more relevant than tests for insects and birds, except for specific research projects. The exposure to insects and bird may differ significantly due to the mobility of these organisms.

Three different levels of relevance should be considered; i) relevant test organism, ii) relevant exposure routes and iii) relevant end points. The relevance of the organisms used in standard aquatic toxicity tests (e.g. OECD TG 201, 202, 203, 204, 210, 211, 212, 215, and 221) is poor for the Norwegian environment. Several of the species most widely used in acute tests (e.g. the phytoplankton *Pseudokirchneriella subcapitata/Desmodesmus subspicatus*, the grazer *Daphnia*

magna and the fish *Danio rerio*, *Pimephales promelas*, *Cyprinus carpio*) are not native to Norwegian environments. Finally, since the mixtures in the flue gas from are expected to be complex, and given the fact that little information is generally available for most of these with respect to modes of toxicity, it is difficult to predict which effects they will have on a test organism.

Since all methods described above are acute tests, we may also include chronic tests, with exposure over more than one generation. Typical chronic methods include the effects on reproduction, and a number of reproduction inhibition methods are described in the OECD Guideline:

- OECD 207 Avian Reproduction Test
- OECD 211 *Daphnia magna* Reproduction Test
- OECD 220 Enchytraeid Reproduction Test (soil)
- OECD 222 Earthworm Reproduction Test
- OECD 229 Fish Short Term Reproduction Assay

7.4.2 Relevant biodegradation methods

Biodegradation will only be relevant for water and soil compartments. No biological degradation is expected to appear in the atmosphere compartment.

OECD recommends the performance of biodegradability tests preferably by simulation tests (OECD, 2006). However, we realize the lack of realism in testing a significant number of flue gas components by complex simulation tests. We therefore only recommend testing of simulation tests for substances which are described as toxic (see cpt. 7.4.3) and are persistent by screening tests. The most convenient screening biodegradability methods are ready biodegradability methods:

- OECD 301A DOC Die-Away Test
- OECD 301B CO_2 Evolution Test
- OECD 301C Modified MITI Test (I)
- OECD 301D Closed Bottle Test
- OECD 301E Modified OECD Screening Test
- OECD 301F Manometric Respirometry Test

If substance is ready biodegradable (biodegradability > 60 % for oxygen consumption or CO_2 evolution or > 70 % for DOC removal) there should be no needs for further testing. If not ready biodegradable we suggest to investigate the potential for inherent biodegradability by one of the following methods:

- OECD 302A Inherent Biodegradability: Modified SCAS Test

- OECD 302B Inherent Biodegradability: Zahn-Wellens/EMPA Test
- OECD 302C Inherent Biodegradability: Modified MITI Test (II)

Based on the inherent biodegradability test the substance may be defined as inherent primary biodegradable (> 20 % biodegradable) or inherent ultimate biodegradable (> 70 % biodegradable).

We also recommend the performance of an abiotic degradation test for substances not ready biodegradable:

- OECD 111 Hydrolysis

If substances are not ready or inherent biodegradable and considered ecotoxic (cpt. 7.4.1) we suggest to perform a simulation test. Validated simulation tests are described for sewage, water, sediment and soil compartments. We expect the soil compartment as the most relevant receiver of persistent flue gas substances, and the following simulation test is therefore recommended:

- OECD 307 Aerobic and Anaerobic Transformation in Soil

7.4.3 Recommendations ecotoxicity and degradation methods for the Protocol

A summary of a suggested tiered ecotoxicity testing is described in Table 7.6.

Table 7.6 Tiered ecotoxicity test system for flue gas components

	Test methods	Decisions
1st tier	OECD 201 OECD 202 OECD 203	Proceed to 2nd tier if substance is toxic and persistent and or bioaccumulative[A)]
2nd tier	One or several of OECD 207, 208, 211, depending on outcome of 1st tier	

[A)] Persistence is determined by biodegradability testing. It is assumed that no potentially bioaccumulative substances are present in flue gas

Threshold levels for defining substances as toxic to the environment may be considered by expert judgment or by established ecotoxicity threshold levels. In the OSPAR ecotoxicity threshold is defined as EC50 or LC50 ≤ 10 mg/L. Threshold level could also be related to expected emissions (e.g. as a suggested PEC/PNEC value).

If further tests are recommended we suggest that the choice(s) should be made from expert judgment, including discussions on possible physical-chemical characteristics relevant for the fate in biotic environments (e.g. volatility, bioaccumulation potential, soil adsorption characteristics) and probable concentrations in the flue gas emission (see cpt. 5.4).

Recommended degradation methods will combine biodegradation processes with (aerobic) and without (anaerobic) oxygen.

A summary of a suggested tiered biodegradation testing is described in Table 7.7.

Table 7.7 Tiered biodegradation test system for flue gas components

	Test methods	Decisions
1st tier	OECD 301	Proceed to 2nd tier if substance is not ready biodegradable
2nd tier	OECD 302 OECD111	A 3rd tier should be considered if substance is not inherent biodegradable and is ecotoxic[A)]
3rd tier	OECD 307	

[A)] Ecotoxic – if substance is considered toxic by tests described in Table 7.6

The third tier should be considered by expert judgement if a substance is not ready or inherent biodegradable and is ecotoxic (see above). As described for the ecotoxicity testing an expert judgement should include discussion of the possible fate in biotic environments and probable concentrations in the flue gas emission (see cpt. 5.4).

7.5 Recommendations for Call Off 2

Since a single-compound evaluation is recommended for ecotoxicity evaluation the main objective for call off 2 recommendations will be to achieve missing ecotoxicity data for relevant flue gas compounds. However, flue gas compounds and degradation products will not be known until CCM technology is chosen.

In that respect we may prepare preliminary recommendations for the chemicals selected as test case. Table 7.8 shows the type of ecotoxicity and biodegradation data lacking for hazard evaluations of the selected test case compounds in order to determine PNEC. Several of the chemicals lacking essential environmental data are pre-registered in REACH with a registration date of 30.11.2010. However, we do not know if all gaps will be filled by this date, or if all data will be publically available.

However, for some of the compounds relevant data will be available from other TQP Amine projects. For instance, acute data for nitramines will be available from the TQPAmine 5 project.

Regarding the requirements for establishment or development of new methods we do recommend this for Call Off 2, due to a) only validated methods have been recommended for ecotoxicity and biodegradability, and b) the short time period covered by Call Off 2.

Table 7.8 Data lacking from ecotoxicity and biodegradation tests for test case substances Requirements for Tier 3 biodegradability (simulated test) are not included in the table.

Chemical Group	Name of the chemical	CAS Numbers	Ecotox tests	[a)]Biodegradability
Amine	MEA	141-43-5	Chronic	None
NH3	NH3	7664-41-7	Acute algae Chronic	Not relevant
Aldehydes	Formaldehyde	50-00-0	Chronic	None
	Acetaldehyde	75-07-0	Chronic	None
Amides	Acetamide	60-35-5	Acute alga Acute invertebrate Acute fish Chronic	Ready biodeg Inherent biodeg Hydrolysis
Alkylamines	Methylamine	74-89-5	Acute algae Chronic	None
	Dimethylamine	124-40-3	Chronic	Ready biodeg Inherent biodeg Hydrolysis
Nitrosamines	N-nitrosodimethylamine	62-75-9	Acute alga Acute invertebrate Acute fish Chronic	Ready biodeg Inherent biodeg Hydrolysis
	N-nitrosodiethanolamine	1116-54-7	Acute alga Acute invertebrate Acute fish Chronic	Ready biodeg Inherent biodeg Hydrolysis
	N-nitrosomorpholine	59-89-2	Acute alga Acute invertebrate Acute fish Chronic	Ready biodeg Inherent biodeg Hydrolysis
Nitramines	Dimethylnitramine	4164-28-7	Acute alga Acute invertebrate Acute fish Chronic	Ready biodeg Inherent biodeg Hydrolysis
	Ethanolnitramine	74386-82-6	Acute alga Acute invertebrate Acute fish Chronic	Ready biodeg Inherent biodeg Hydrolysis
	Methylnitramine	598-57-2	Acute alga Acute invertebrate Acute fish Chronic	Ready biodeg Inherent biodeg Hydrolysis

[a)] For compounds with previous data close to limit for ready biodegradability we recommend that new ready biodegradability tests are performed before decisions on inherent biodegradability and hydrolysis tests are performed.

8 Hazard and risk assessment for health and environmental exposure

8.1 Definitions

The term "Risk" has been defined by OECD and by REACH (e.g. http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm). Risk is used in everyday language as "the chance of damage / disaster". When used in the process of risk assessment it has specific definitions, the most commonly accepted being "The combination of the probability, or frequency, of occurrence of a defined hazard and the magnitude of the consequences of the occurrence" (EEA, 1998). Therefore, risk can be characterized as **the likelihood of harm.**

If all other factors are equal, especially the exposures and the organisms subject to them, then the risk is proportional to the hazard. However, all other factors are rarely equal. Today risk-based assessment approaches are preferred prior to hazard-based approaches. This is partly due to the recognition that for many environmental issues a level of zero risk is not obtainable or not necessary for human and environmental protection and that a certain level of risk can be accepted.

Risk is a combination of hazard and exposure as indicated in Figure 8.1. This implies that there is no direct relation between hazard and risk; a chemical with a high potential hazard may have a small risk if the (probability of) exposure is very small. Accordingly a chemical with a low potential hazard may have a high risk if the exposure is high.

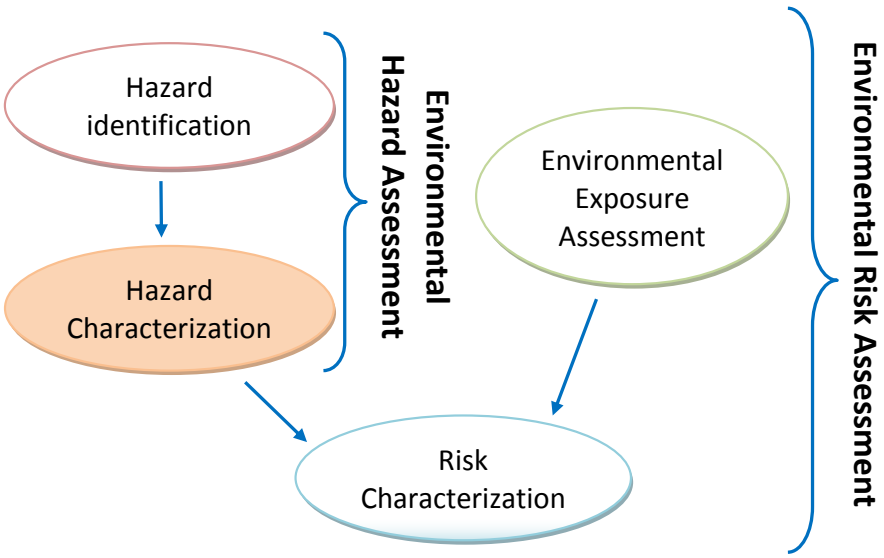


Figure 8.1. The main steps in risk assessment (adopted from OECD)

8.2 Regulatory issues

Emissions of pollutants to the environment in Europe are regulated by authorities on a local, national and international level. In Norway national authorities are represented by the Climate and

Pollution Directorate (Klif), which will be responsible for accepting discharge permits submitted by the pollution owner.

On a European level potential health, safety and environmental (HSE) impacts of emissions are regulated through REACH (European Union regulation for Registration, Evaluation, Authorization and Restriction of CHemicals), the Oslo Paris Convention (OSPAR) and the European Water Frame Directive (WFD). REACH regulates the European production and import of chemicals based on HSE criteria, OSPAR and GESAMP advices on the offshore use and discharges of chemicals and effluents, while WFD seeks to improve the quality of the European waterways and estuaries by reductions of potentially harmful chemicals and effluents.

8.3 Exposure concentrations

8.3.1 Risk assessment and exposure concentrations

Risk assessments vary widely in scope and application. Some look at single risks in a range of exposure scenarios whereas others are site-specific and look at the range of risks posed by an installation. In broad terms risk assessments are carried out to examine the effects of an agent on humans (Health Risk Assessment) and ecosystems (Ecological Risk Assessment). Risk assessment is carried out to enable a risk management decision to be made. Risk management is the decision-making process through which choices can be made between a range of options which achieve the "required outcome", and should result in risks being reduced to an "acceptable" level (EEA, 1998).

The aim of a risk assessment is to combine characteristics of chemical hazards with expected exposure scenarios. The product is to establish a guideline for safe use of the chemical. The guideline can be a regulation as to its safe use in products or exposure limits in food, air and water. Risk assessment is a combination of the likelihood of an occurrence and the severity of the consequences. Thus, it is based on the hazard profile of a chemical as well as a set potential exposure scenarios. A risk assessment is therefore connected to what is considered as critical effects of the chemical as well as the intended use. For a given hazardous chemical the guideline will differ considerably depending on e.g. the population for which it is intended. As an example; we would not recommend the use of a carcinogen in children's toy but we accept it as an intermediary product in industry where the workers are properly educated and protected. In conclusion, the risk assessment is not a uniform exercise but rather directed to specific situations or populations. Therefore, knowledge of exposure concentrations is very important for assessing single or site-specific risks.

8.3.2 Screening methods for exposure concentrations

If relevant data can not be provided for exposure concentrations, a screening method has been described in the Technical Guidance Documents (TGD), see Appendix G. Exposure Assessment involves the calculation of a Predicted Environmental Concentration (PEC). In the TGD PECs are determined for various compartments, a) air, b) aquatic, c) sediment, d) soil, and e) groundwater. The calculations include both a PEClocal for the area close to the emission source and a PECregional where environmental concentrations are estimated on a larger scale.

For the derivation of PECs at a local and regional scale, a standardized generic environment, with default values is used as specified in the EU TGD. The characteristics of the real environment

will, obviously, vary in time and space. When more specific information is available on the location of the emission sources, this information can be applied in refinement of the PEC by deviating from the default values (see Appendix G).

8.3.3 Scenario-based exposure concentrations

Since the TQP Amine projects are related to emissions from CCM and the local conditions at Mongstad, scenario-based emissions and exposure concentrations are likely to be predicted. The exposure concentrations to the population and to vegetation and fauna should therefore rely on conditions relevant for the Mongstad area. However, it is outside the scope of this project to predict any exposure conditions.

For the current project, company has not provided us with exposure concentrations; therefore we have performed a preliminary description of various risk assessment options. Predictions of CCM exposure concentrations are outside the scope of this project, but relevant for the TQP Amine 2 and TQP Amine 4 projects.

8.4 Challenges in health and environmental hazard and risk assessment

Health risk assessment is concerned with morbidity and mortality of individuals, while environmental risk assessment is concerned with effects on populations and ecosystems. Environmental risk assessment must take into account a high number of organisms; all with varying sensitivities to chemicals and various groups have different exposure scenarios. Because of the difficulty in obtaining toxicity data on all organisms in an ecosystem, the usual practice is to use data on selected organisms as representatives for the whole system (EEA, 1998).

There are a number of challenges involved when performing a risk assessment, i.e.:

- Determining the effects at population and community level;
- Selection of end-points;
- Selection of species representative for the system;

For environmental risk assessment specific challenges include:

- The selection of field, laboratory, mesocosm and microcosm tests;
- The incorporation of resilience and recovery factors of the ecosystem

8.4.1 Health-related non-threshold approach

Emissions from CCM involve chemicals areleased to the atmosphere. The plant is part of a large petrochemical based industrial area situated in a rural area. The population of potential exposure will reflect any population as to gender, age and health status. We should therefore consider exposure as air contamination to the general public, and any guideline should have the general population rather than the occupationally exposed as its “target” population.
Some of the flue gas emission substances may precipitate and find their way through water or the food chain to people. Thus, exposure may go beyond direct inhalation. However, in the present (first attempt) risk assessment we have addressed this as an inhalation problem only.
Whenever we have had to do an “expert opinion” on matters that cannot be readily converted into some classification we have chosen to be very conservative and make potential errors on the safe

side. Thus, the reader should assume that any later revisions resulting from more data should increase rather than decrease the ambient air standard that we suggest in this chapter.
The chemicals that have been identified as potential releases from the plant represent a variety of health effects:

- Irritant to skin and airways
- Sensitizers to skin and airways
- Systemic toxicity after oral, percutaneous and inhalation exposure
- Carcinogenic
- Mutagenic
- Reproductive effects

Some of these effects can only be observed after exposure to relatively high concentrations during acute exposure.

Exposure to the general population around an industrial facility like Mongstad is characterized as low-level continuous exposure and it is not expected that acute effects should be observed. We therefore need to focus on compounds causing effects that may occur after long-term and low level exposure. Such effects include carcinogenesis, mutagenesis and changes in fertility and reproduction and inhalation sensitization.
The aim of risk assessment in the current project is:

- determine the critical effect
- determine a no effect dose for the most critical effect
- suggest an ambient air standard
- compare the ambient air standard with the expected flue gas concentration

8.4.1.1 The critical effect

This project specifies a total of 13 substances. The toxic effects noted after a database and literature search includes all endpoints presented in the introduction above. However, there are two chemical groups that deserve special attention:

- nitrosamines which include both acute and serious long term effects.
- nitramines where data is very sparse, but serious long term effects can be expected.

We suggest that genetic effects (mutagenic and carcinogenic) and effects on reproduction are considered as the critical effects for the risk assessment.

8.4.1.2 Risk assessment for mutagenic/carcinogenic compounds following ECHA and REACH

8.4.1.2.1 Guidelines

There are several Guidelines for Risk assessment (RA) of chemicals and substances. Generally, the recommended risk assessment techniques take into account the inherent toxicity of a substance as well as the type and degree of exposure. Risk models incorporate assumptions that will nearly always predict health risks, to assure that the outcome is protective of health. REACH (Annex I, 1.0.1) defines the Derived No-Effect Level (DNEL), i.e. the level of exposure above which humans should not be exposed. In the risk characterization, the exposure of each human population likely or known to be exposed is compared with the appropriate DNEL. The risk to

humans is considered to be adequately controlled if the exposure levels estimated do not exceed the appropriate DNEL (REACH Annex I, 6.4).

Whereas the former legislation on new and existing substances required a comprehensive RA and a risk characterization (RC) for all relevant toxicological effects, REACH requires a RC for the leading health effect (i.e., the toxicological effect that results in the most critical DNEL) for a given exposure pattern (such as duration, frequency, route of exposure and exposed human population) which is associated with an exposure scenario. In case of flue gas compounds both mutagenicity/carcinogenicity as well as reproductive toxicity are considered as relevant endpoints.

8.4.1.2.2 Derivation of the dose-descriptor

The data for calculating dose-descriptors come preferentially from lifetime oral or inhalation studies according to Annex V of Directive 67/548/EEC or other accepted guidelines (e.g. OECD guidelines). For a substance considered genotoxic any tumour type observed in an animal bioassay is taken as relevant to humans and as starting point for a dose-descriptor determination, unless evidence to deviate from this approach is considered sufficiently convincing. This also concerns non-genotoxic carcinogens with tumor promoting mode of action.

The specific dose descriptors:

LD50: Median lethal dose. The dose causing 50 % lethality

LOAEL: Lowest Observed Adverse Effect Level

NOAEL: No Observed Adverse Effect Level (NOAEL) - the highest dose with no toxic effects

BMD: The Benchmark dose; BMD concept involves fitting a mathematical model to dose-response data. The BMD is defined as the dose causing a predetermined change in response.

BMD10: The Benchmark-dose associated with a 10% response (for tumours upon lifetime exposure after correction for spontaneous incidence, for other effects in a specified study)

BMDL10: Defined as the lower 95% confidence dose of a Benchmark-dose representing a 10% tumor response upon lifetime exposure, i.e. the lower 95% confidence dose of a BMD10.

ED10: Effective dose 10 %; a dose representing an increased incidence of 10 % due to a specific exposure (e.g., to a chemical).

TD50: The median toxic dose of a drug or toxin is the dose at which toxicity occurs in 50% of cases

DNEL: Derived no effect-level (DNEL)

DMEL: Derived Minimal-Effect Level; For non-threshold effects, the underlying assumption is that a no-effect-level cannot be established and therefore expresses an exposure level corresponding to a low, possibly theoretical, effect, which should be seen as a tolerable exposure risk.

T25: The dose-descriptor value T25 is defined as the chronic dose rate that will give 25% of the animals' tumors at a specific tissue site and is calculated from the tumor incidence at the selected tumorigenic using linear intrapolation or extrapolation (Dybing et al. 1997).

In the European Guidelines the T25 dose-descriptor is in use for concentration limits of carcinogens, for non-threshold carcinogens and for risk characterization of chemicals in general (Annex I of Directive 67/548/EEC, EC, 1998; EC Regulation 1488/94; EC, 1994; SCCNFP/00690/03). The BMD10 has regulatory use as its lowest confidence value BMDL10 for

the assessment of risks of food ingredients (EFSA, 2005) and comparable dose descriptor, ED10 (LED10), in cancer risk assessment practice by Environmental Protection Agency (US EPA, 2005). RA is usually derived, if possible, from long-term animal studies but more recently in vitro studies and more adequate human epidemiological studies data are recommended to be used to determine the NOAEL and DNEL. A large safety factor is then added — to arrive at a safe level for humans

8.4.1.2.3 The safety (assessment) factor

The safety (assessment) factor (AF) is built in partly to account for the differences between animals and humans, and also to allow for the variability between different populations, and individual variations among people, such as age, genetic background, health and how well nourished they are. The safety factor also accounts for many uncertainty factors, such as the variability in the experimental information and or inter and intra-species variation (including individual susceptibility); the nature and severity of the effect; the sensitivity of the human (sub-) population to which the quantitative and/or qualitative information on exposure applies, etc. DNELs must consider populations (workers, consumers, and general population), exposure routes (inhalation, dermal/eye, oral) and duration of exposure.

Assessment factors for interspecies differences: Interspecies differences result from variation in the sensitivity of species due to differences in toxicokinetics and toxicodynamics. If human data exists no extrapolation is needed. Where data from animal studies are the taken for RA the default assumption in general is that humans are more sensitive than experimental animals. Traditional default suggested for interspecies extrapolation is 10, which sometimes is subdivided in a default of 4 for toxicokinetic differences and a default of 2.5 for toxicodynamic differences.

Assessment factors for intraspecies differences: Humans differ in sensitivity due to a number of biological factors (such as age, gender, genetic predisposition and nutritional status). The intraspecies variation in humans is greater than in the more homogeneous experimental animal population. Defaults typically suggested for the general population (representing all age groups, including children and elderly) are a factor of 10. A lower default factor is generally suggested for the worker population, because it is more homogenous (excluding the very young, very old, not healthy, etc.).

Assessment factors for differences in duration of exposure: The experimental NOAEL will decrease with increasing exposure times and more serious adverse effects may appear with increasing exposure times, a factor allowing for differences in the experimental exposure duration and the duration of exposure for the population and scenario under consideration is normally applied in risk assessment.

Assessment factor for uncertainty in route-to-route extrapolation: This AF is used in cases where no adequate data is available on the relevant route of exposure for the population and exposure scenario under consideration.

There are two basic concepts for hazard and risk estimation - **threshold vs non-threshold.**

For a non-threshold carcinogen, with adequate animal cancer data, DMEL approach is taken. This implies the use of endpoint-specific large assessment factor, i.e. 10 000 to ensure that the exposure causes a minimal risk. The specific dose descriptor BMDL10 is divided by that AF. When it is not possible to set a DMEL, a qualitative approach in the assessment has to be taken.

The 'Large Assessment Factor' approach involves the application of several assessment factors rather than linear extrapolation of dose descriptors and uses BDML 10 (lower confidence limit of BMD) as preferential dose descriptor but this could be modified if necessary. The corrected dose descriptor is then divided by the total assessment factor 10,000 (for general population) and 5000 (for occupational exposure). The use of the BMDL10 is recommended if one wants to reflect the uncertainties and statistical errors in the available cancer dose-response data.
When establishing the DNEL, the uncertainties in the assessment shall be taken into account (e.g., involving species differences, differences in sensitivity among humans, and quality of the database). The DNEL can be considered as an 'overall' No-Effect-Level for a given exposure (route, duration, frequency), accounting for uncertainties/variability in these data and the human population exposed. For workplace exposure, the occupational exposure limits (OELs) may already exist. Under certain circumstances OELs and/or the underlying information used for setting the OELs can be used to derive DNELs.

For derivation of the DNEL (or equivalent dose descriptor T25, ED10, BMD10) the leading health effect for a given exposure pattern (exposure route, population and duration) needs to be selected. For some compounds DNEL cannot be derived either because there is no available data, or data for one or several endpoints are missing or because of threshold effects. The threshold concept assumes the existence of nontoxic dose (NOAEL) no effect unless threshold has been reached. Our review shows that most of TQPAmine3 compounds are mutagens or suspected mutagens and carcinogens. These compounds exhibit both threshold and non-threshold mode of action. Since NOAEL approach is valid only for threshold compounds, for non-threshold mutagens/carcinogens an exposure level without potential effects cannot be established. Generally, in case for the endpoints mutagenicity and carcinogenicity, a non-threshold mode of action is suggested (REACH Annex I, 1.4.1). However, due to the high uncertainties in establishing safe exposure levels, for the threshold compounds, a substantially different approach is needed in relation to assessing risk. Although, there is considerable debate on the use of non-threshold approach for threshold chemicals, it is currently being used as no suitable approach is available. This may result in either over- or under-estimation of risk assessment for certain chemicals.

8.4.1.3 The qualitative approach when no dose descriptor is available for an endpoint

When no reliable dose descriptor can be set for an endpoint, a more qualitative approach has to be chosen. This may apply for acute toxicity, irritation/corrosion, sensitization, and mutagenicity/carcinogenicity. In this case qualitative indications of the potency of the substance are used for developing exposure scenarios with risk management measures and operational conditions for controlling risk.

Several of the potential flue gas compounds are considered or suspected mutagens and possibly carcinogens. If data is available on these compounds, then non-threshold approach with large assessment factor 10,000 will be the most appropriate approach. However, the limitation of this approach is that only single compounds are considered. In most cases there is no available or reliable data and thus it is difficult to derive appropriate dose descriptor such as DMEL or BMDL. In some cases we would be able to use animal TD50 data and derive BMD10 by including additional assessment factor 10. Thus overall assessment factor will be 100,000.

8.4.2 Health-related worst case approach

In some cases we do not have appropriate data for each compound in order to develop DNEL or DMEL values as described by REACH. Alternatives are then:

- Read-across from similar substances
- Use of established standards, e.g. OEL

Both these procedures have been discussed and accepted by REACH as valid for preliminary risk assessments (Guideline document chapter R4, section R.4.3.2.2 for grouping of substances and chapter R8, appendix R8-13 for the use of OEL)
When there is an urgent need to develop a preliminary risk assessment a hazard profile for a group of substances can be developed on the basis of worst case criteria. For a given group of chemicals which have the same functional group of toxicity we may combine the data from individual members of that group into a hazard profile. We may then use whatever exposure standard is available (occupational or general public) for individual members of that group to develop an interim exposure standard which applies to all members of that chemical group. Applying such a procedure may cause some chemicals to receive a stricter regulation than needed – it is thus an approach where you will err on the safe side. In the present case such an approach can be applied to nitrosamines and nitramines. It should be reiterated that this is part of a temporary risk assessment until such time that appropriate data is available.

8.4.2.1 Nitrosamines

As data is very limited for the nitrosamines we suggest combining the data available and giving it the name:

Nitrosamine (NOS) where NOS means “not otherwise specified”

The toxicology profile for nitrosamine (NOS) is the worst case entry for each endpoint of toxicology testing that we have been able to obtain.
For the nitrosamines examined such an approach will give the following result as shown in Table 8.1.

As indicated by the table one out of three is confirmed to have also reproductive effects. The other two are candidates for testing for these aspects. However, it is suggested that the genetic toxicity is the prime critical effect for extended exposure.

8.4.2.2 Nitramines

As data is very limited for the nitramines we suggest combining the data available and giving it the name:

Nitramines (NOS) where NOS means “not otherwise specified”

The toxicology profile for nitramine (NOS) is the worst case entry for each endpoint of toxicology testing that we have been able to obtain.
For the nitramines examined such an approach will give the following result (Table 8.2)

Table 8.1 Suggested assessment for nitrosamines based on the NOS approach.

Name	CAS	Oral	Percutaneous (Dermal)	Inhalation	Long term	Comment
N-nitrosodimethylamine	62-75-9	3	-	4	C M R	Very high acute toxicity OEL-TWA: 0.001 mg/m3
4-nitrosomorpholine	59-89-2	2	-	-	C M	Very high acute toxicity OEL-TWA: 0.001 mg/m3
N-nitrosodiethanolamine	1116-54-7	0	(0)	-	C M	OEL: 0.001 mg/m3 Non-toxic by oral or dermal route.
Nitrosamines (NOS)	***-----***	***3***	***-***	***4***	***C M R***	***Very high acute toxicity OEL-TWA: 0.001 mg/m3***

Table 8.2 Suggested assessment for nitramines based on the NOS approach.

Name	CAS	Oral	Percutaneous (Dermal)	Inhalation	Long term	Comment
1-nitro-piperazine	42499-41-2	-	-	-	-	May produce nitrosamine in a nitrate rich environment
N,N'-dinitropiperazine	4164-37-8	-	-	-	-	
Dimethylnitramine	4164-28-7	1	-	-	C M	
Methylnitramine	598-57-2	-	-	-	*C M?*	
Nitramines (NOS)	***----***	***1***	***-***	***-***	***C M***	***May produce nitrosamine in a nitrate rich environment***

The data we have been able to find is far from satisfactory as input to a risk assessment. We therefore suggest that nitramines should be considered to have the same profile as nitrosamines until further data is provided.

We have also summarized our investigation on if Non-threshold Large assessment factor approach, threshold approach, or Occupational exposure Limit approach would be the most convenient in the form of a table (Table 8.3).

Table 8.3 **Justification of contribution to RA assessment approach for mutagens and carcinogens. NT LAF: Non-threshold 'Large Assessment Factor' approach AF 100000, OEL: Occupational exposure limit approach AF 100. TD 50 value is the dose where 50% of animals have had toxic effect.**

Chemical group	Name of the chemical	CAS Number	Long Term	Dose descriptor (TD50 [mg/kg/day], carcinogenicity as endpoint)	RA contribution
Amine	Ethanol, 2-amino- (MEA)	141-43-5	M?		OEL
NH3	Ammonia	7664-41-7	C M?		OEL NT LAF
Aldehydes	Formaldehyde	50-00-0	C M R S	1.35 (rat, oral)	Threshold approach (Danish RA)* NT LAF OEL
	Acetaldehyde	75-07-0	C M R S	153 (rat, oral)	OEL NT LAF
Amides	Acetamide	60-35-5	C M? R	180 (rat, oral) 3010 (mouse, oral)	OEL NT LAF
Alkylamines	Methylamine	74-89-5	M		OEL
	Dimethylamine	124-40-3	M S		OEL
Nitrosamines	N-nitrosodimethylamine	62-75-9	C M R	0,0959 (rat, oral)	OEL NT LAF
	N-nitrosodiethanolamine	1116-54-7	C M	3,17 (rat, oral)	OEL NT LAF
	4-nitroso-morpholine	59-89-2	C M	0,109 (rat, oral)	OEL NTLAF
Nitramines	Dimethylnitramine	4164-28-7	C M	0.547 (male and female rats, oral)	OEL NT LAF
	Ethanolnitramine	74386-82-6	NA	NA	OEL
	Methylnitramine	598-57-2	C M?	17.4 (male and female rats, oral)	OEL NT LAF

8.4.2.3 Safety factor while using occupational exposure limits

Based on the data available through the database searches included in this project we do not have appropriate data for nitrosamines or nitramines to assume that it could be possible to generate no-effect dose level – and thus a DMEL. However, we do have occupational exposure limits (OEL) for nitrosamines of 0.001 mg/m3 (OEL obtained from RTECS with reference to Austria and Switzerland). As a provisional approach we suggest to use safety factor while using OEL as a standard for the combined summary exposure of nitrosamines and nitramines. A safety factor needs to be included due to the following main issues:

- age
- gender
- health status
- medical surveillance

8.4.2.3.1 Age

OELs are developed for an adult population and do not take into account age dependent changes which for long term exposure is of special importance for children.
Children have a higher uptake from both oral and airway exposure per kg body weight – it is a result of the higher metabolic rate per body unit for children compared to adult. Through the same rational the elderly may have a somewhat lower uptake.

The blood brain barrier continues to develop to the age of at least 16 years of age. Particularly in early childhood children may have a significant higher brain uptake of contaminants compared to adult. Also the toxicodynamic properties is different as the young is under development and permanent damage may be inflicted based on a higher sensitivity. There is no indication that the nitrosamines and nitramines are selectively accumulated or cause organ specific damage in nervous tissue

Many compounds undergo metabolic changes which may increase the toxicity (metabolic activation) or enhance their excretion. The respective metabolism pattern of an individual will reflect both the genetic basis as well as other exposure to compounds which are capable of such metabolic transformation. Children have a different metabolism profile towards contaminants compared to adults – a major reason being that they during their lifetime have encountered a smaller array of chemicals. For the nitrosamines and nitramines, the overall toxicology certainly suggests that these compounds act through metabolites and that contaminant metabolism may play an important part in causing the effects.

8.4.2.3.2 Gender

Nitrosamines are reprotoxic and they may act both on the parental side as well as being fetotoxic and cause developmental effects. As they are mutagenic the parenteral effects might be both on the female and male side. Parenteral effects are often associated only with the maternal effects but there is ample documentation from occupational toxicology that mutagenic compounds in the workplace may cause reproductive effects through paternal exposure only. For the nitrosamines we suggest that both adult (both genders) at reproductive age and the fetus should be considered as critical members of the exposed population.

8.4.2.3.3 Health status

The work force is thought to be healthy – either the absence of illness or have a well controlled disease (e.g. diabetes or blood pressure problems). The general population will include a wide range of health status. Some of the illnesses may have characteristics which are important for consideration of safe levels of exposure. Examples are exposure to irritant for people with permanent lung dysfunction. Another example is exposure to compounds which require xenobiotic metabolism for its detoxification and excretion – and the patient has a reduced liver or kidney functional capacity. The nitrosamines falls in the category of both being irritant as well as metabolized xenobiotics – and thus the liver/kidney status of a person may modify the toxic effects. However, it is unlikely that airway exposure will introduce similar problems.

8.4.2.3.4 Medical surveillance

In industry where hazardous chemicals are used there shall be a health and safety surveillance adjusted to the character of the activities. Similar health and medical surveillance cannot be done for the general population

8.4.2.3.5 Correction factor

There are many occasions where people are exposed for complex mixtures and where data is not available for each component or the mixture. Sometimes this calls for temporary air quality guidelines based on OELs. OELs have been used to generate air quality standards for special situations with continuous exposure for more than 10 days, e.g.:

- saturation diving
- submarines
- space stations

Correction factors of 2-10 have been introduced for a range of effects from temporary discomfort to carcinogenesis. These situations are occupational exposures to adult males of (extremely) good health.

We would suggest a correction factor of 10 to include continuous life long exposure for an adult healthy population. Then multiply this factor by 10 to adopt the OEL to the general population. Therefore the combined correction factor would be 100, which means that the lowest OEL available should be divided by 100.

8.5 Data for environmental hazard assessment

8.5.1 PNEC determination

The EU Technical Guidance Documents (TGD) recommends the determination of the ratio between Predicted Environmental Concentration (PEC) and the Predicted No-Effect Concentration (PNEC) for individual compounds as a method for risk assessment.

The calculations of PEC can be made either by scenario-based approach for the individual emission sources (recommended) or be provisionally estimated by a screening method. The latter approach is described in Appendix G and is referred to in cpt. 8.3.2. If PEC is higher than PNEC this represents a risk and efforts should be taken to reduce the risk. Essential for environmental risk assessment is that hazard can be predicted by the determination of a concentration of a chemical which do not represent harm to organisms in the environment. One such way of effect assessment is the preparation of the predicted no-effect concentration – PNEC.

The methods to derive the PNEC are described in the EU Technical Guidance Documents (TGD). The methods for obtaining a PNEC value are summarised below, but more extensively described in Appendix G.

A PNEC is regarded as a concentration below which an unacceptable effect will most likely not occur. In principle, the PNEC is calculated by dividing the lowest short-term L(E)C50 or long-term NOEC (no effect concentration) value by an appropriate assessment factor.

Ideally PNEC values should be derived for all environmental compartments where a compound may end up. For flue gas emissions this will include air, vegetation, soil and groundwater (see Appendix G). Often, few or no ecotoxicity data will be available for other than aquatic organisms. If the concentration of a substance in the aquatic environment does not exceed its PNEC, this ensures an overall protection of the environment. Certain assumptions are made concerning the aquatic environment, which allow an extrapolation to be made from single-species short-term toxicity data to ecosystem effects, although these assumptions may be uncertain. It is assumed that ecosystem sensitivity depends on the most sensitive species and, protecting ecosystem structure protects community function. It is generally accepted that protection of the most sensitive species should protect structure, and hence function.

When the pool of data from which to predict ecosystem effects is very limited (e.g. only short-term toxicity data are available), assessment factors must be used. In this case, the effect assessment performed with assessment factors can be supported by a statistical extrapolation method is the database on Species Sensitivity Distributions (SSDs) is sufficient for its application. If a large data set from long-term tests for different taxonomic groups is available, statistical extrapolation methods may be used to derive a PNEC. The method should be applied to all reliable available NOECs from chronic/long-term studies. The NOEC is defined as “the highest concentration tested at which the measured parameter shows no significant inhibition”. Ecotoxicity data for a broad range of taxonomic groups (described in the EU TGD) is required.

For most substances, the pool of data from which to predict ecosystem effects is very limited as, typically, only short-term toxicity data are available. In these circumstances, it is recognised that, while not having a strong scientific validity, empirically derived assessment factors (AFs) must be used. The AFs reflect the degree of uncertainty in extrapolation from laboratory toxicity test data for a limited number of species to the 'real' environment. AFs applied for long-term tests are smaller as the uncertainty of the extrapolation from laboratory data to the natural environment is reduced. For this reason long-term data are preferred to short-term data. Thus, data from **chronic tests** may be favoured instead of short-term **acute tests**. However, data from chronic tests are usually not available, or available only to a limited extent, for most compounds. The complexity of chronic ecotoxicity tests may also reduce the reproducibility of these tests. However, the chronic tests are more relevant for long-term ecotoxicity. We therefore regard it as important to include both acute and chronic tests for regulatory purposes.

AFs for deriving PNECs in the aquatic environment are shown in Table 8.4. Specific assessment factors for sediment, soil and marine environments have also been addressed (Appendix G). The size of the AF depends on the confidence with which a PNEC value can be derived from the available data. This confidence increases if data are available on the toxicity to organisms at a number of trophic levels, taxonomic groups and with lifestyles representing various feeding strategies. Thus lower AFs can be used with larger and more relevant datasets than the base-set data. Essentially, the more limited the existing data set is the higher the assessment factor.

Table 8.4 Assessment factors used to derive a $PNEC_{aquatic}$ (EU TGD, 2003)

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels of the base-set (fish, Daphnia and algae)	1000
One long-term NOEC (either fish or Daphnia)	100
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10
Species sensitivity distribution (SSD) method	5-1 (to be fully justified case by case)
Field data or model ecosystems	Reviewed on a case by case basis

8.5.2 PNEC determinations of flue gas compounds

Provisional ecotoxicity data for the selected flue gas compounds are shown in cpt. 7 (Table 7.1). Based on these data PNECs were estimated for the compounds (Table 8.5). Only data from acute tests were available for the compounds, and assessment factors of 1000 (see Table 8.4) were used for the most sensitive trophic level. When several species were tested within the most sensitive trophic level, we used median values as the for the PNEC determinations.

Table 8.5. Provisional PNECs for potential flue gas components, based on available data (see Table 8.2).

Chemical	Trophic level	Species	PNEC (µg/l)
Ethanolamine (MEA)	Phytoplankton	*Scenedesmus subspicatus*	15
Ammonia, NH3	Fish	No specific species	0.44
Formaldehyde	Crustacean	*Daphnia magna*	2.0
Acetaldehyde	Crustacean	*Daphnia magna*	48.3
Acetamide	Crustacean	*Cladocera*	10
Methylamine	Crustacean	*Daphnia magna*	433
Dimethylamine	Phytoplankton	*Selenastrum capricornutum*	19.5
N-nitrosodimethylamine	Phytoplankton	*Pseudokirchneriella subcapitata*	4
N-nitrosodiethanolamine	No data		No data
N-nitrosomorpholine	No data		No data
Dimethylnitramine	No data		No data
Ethanolnitramine	No data		No data
Methylnitramine	No data		No data

Crustaceans and phytoplankton were the most sensitive organisms for most of the flue gas compounds. Data for most 2 of 3 nitrosamines and for all nitramines were not available for PNEC determination.

8.6 Recommendations for Call Off 2

8.6.1 Potential health hazard and risk

For the current project, Company did not provided us with exposure concentrations and a full risk assessment is not in the scope of the TQP Amine 3 project. We have therefore focused on preparations of hazard recommendations as part of a risk assessment in the current project:

1. If exposure and toxicity data are available, non-threshold approach (see cpt. 8.4.1.2) with large assessment factor 10,000 will be the most appropriate approach. In that case a very good dataset of DNEL/DMEL is required.For a non-threshold carcinogen, with adequate animal cancer data, we recommend a DMEL approach.

2. If limited or no data are available and read across between compounds belonging to the same group, a worst case and OEL approach will be an acceptable approach of risk determination of health effects from flue gas emissions, with a recommended assessment factor of 100.

8.6.2 Environmental hazard and risk

As shown above (Table 8.5) data are missing for PNEC determinations for some compounds. Some of these data may be provided by other TPQ amine projects. For instance TQP Amine 5 will provide ecotoxicity data for the nitramines which may be used for PNEC determinations.

If environmental concentrations may be provided by other TQP Amine projects PEC/PNEC calculations for the flue gas compounds may be determined through Call Off 2.

9 Sampling methods for toxicity studies

Results from sampling of flue gas emissions are crucial for comparison of emissions and toxicity data to be used in a risk assessment approach.

As mentioned in the ITT it was requested to propose methods on "*how to sample and handle representative extracts of emissions to air from post combustion amine based CO_2-capture* ": The proposed methods should ensure that a) representative extracts are sampled and b) that the extracts are still representative at start and during toxicity testing.

9.1 The basis for sampling

The basis for the sampling strategy is to collect flue gas removed from the absorber and use these samples for selected toxicity tests. This will require manual sampling combined with one or more methods for sample preservation before toxicity testing.

The strategy for isokinetic sampling has been described in a separate report in TQP Amine 1 (Wittgens, 2010), and only a brief background will be given here.

As stated in the ITT the sampling method should be able to collect representative emission extracts containing gaseous, liquid phases and aerosols/particulates. The complete emission (including entrained droplets and evaporated substances) and compound groups of interest include primarily amines, ammonia, aldehydes, amides, alkylamines, nitrosamines and nitramines, with specific focus on alkylamines, nitrosamines and nitramines. Due to low concentrations of these compounds, large sampling volumes may be needed for toxicity testing.

9.2 Standard methods for manual sampling

Methods for manual sampling have been described in a separate report submitted in TQP Amine 1 (Wittgens, 2010). In brief, this report describes the background for sampling from stationary sources, the design of a representative measurement location, and a generic design for measurement site. As a background for the current project some essential issues in the TQP Amine 1 project, which are of importance for toxicity testing of flue gas samples, are summarised here, while a more extensive summary is shown in Appendix B.

Flue gases in a stack may be inhomogeneous due to stratification or swirling caused by duct design and geometry, as shown in Figure 9.1. Therefore, concentrations need to be measured both at several points across the same plane, as well as in several planes. It is also essential that the samples are collected isokinetically, i.e. with the same velocity as the main stream in the duct. This is further explained in Appendix B.

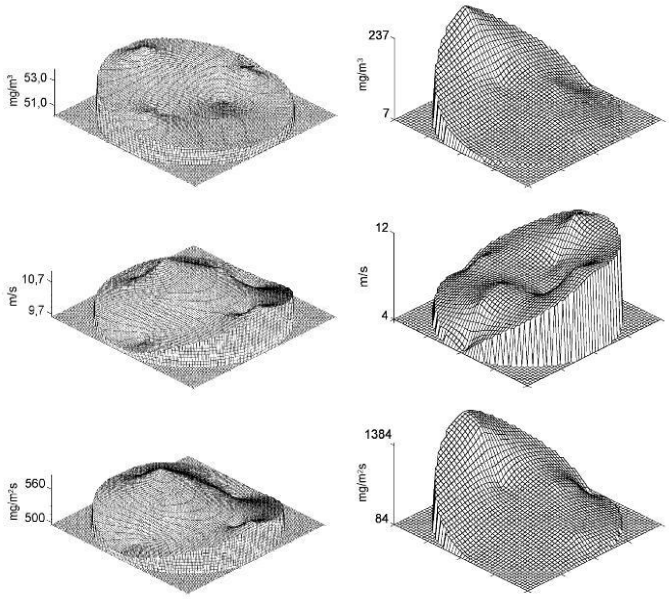


Figure 9.1 Example of homogeneous (left) and inhomogeneous (right) profiles of mass concentration, velocity and mass flow density (reference DIN EN 15259.)

9.3 Standard methods for recovery and analyses

A number of recovery methods for sampled flue gas have been described. The objective of these methods are to concentrate and preserve target analytes for chemical analyses. Some of these recovery methods are described in Table 9.1.

Recovery methods for flue gas compounds include filtration, liquid absorbers, solid adsorbers and chemical conversion. All these recovery methods require some destructive actions for trapping of target analytes. Particulate compounds and aerosols trapped on filters are subsequently destructed with acid for metal analyses. Filter materials and solid sorbents used for sampling organic components are extracted in solvents for concentration and analyses of organic components (e.g. diethylether/n-hexane or other solvents). For condensation, cooling of flue gas below 20°C is required. Condensates are then extracted with n-hexane. Samples are concentrated before analyses (e.g. ISO11338-2:2003). Alternatively components in flue gas samples may be specifically converted to target analytes which can be directly analysed to high specificity (e.g. VDI 3862).

Table 9.1 Recovery methods for analyses of flue gas compounds. Some relevant references for validated methods are described.

Recovery method	Target analytes[a)]	Analytical methods[a)]	Relevant references
Filtration - PTFE, quartz fibre	Dust, particles, specific analytes	Gravimetric methods	EN 13284-1: 2002)
Absorbers – HNO_3/H_2O_2	Heavy metals	AAS, ICP-OES, ICP-MS	EN 14385:2004
Cooled condenser	PCDD/PCDF, PAH	GC-MS	EN 1948-1 to 3 2006, ISO 11338-1:2003
Solid adsorbers	PCDD/PCDF, PAH	GC-MS	EN 1948-1 to 3 2006, ISO 11338-1:2003
Chemical conversion-DNPH	Aldehydes, ketones	HPLC	VD 3862-2:2000

[a)] Abbreviations: DNPH, 2,4-nitrophenylhydrazine; PCDD, polychlorinated dibenzodioxins; PCDF, polychlorinated dibenzofurans; PAH, polycyclic aromatic hydrocarbons; AAS, atomic absorption spectroscopy; ICP-OEC, inductively coupled plasma atomic emission spectroscopy; ICP-MS, inductively coupled plasma mass spectrometry; HPLC, high-performance liquid chromatography

Recovery methods post combustion amine based CO_2-capture compounds (see cpt. 2.2) are described in Table 9.2. As shown in this table the relevant recovery methods for flue gas compounds include the same recovery principles as described in Table 9.1. All these compound groups require the use of absorbents or solid adsorbents with subsequent solvent desorption.

In general, the stability of flue gas components for analytical characterization is not well characterized. The information in current literature is insufficient and partly contradictive with respect to the thermal stability of some components (Wittgens et al., 2010).

Methods for characterization and identification of target analytes in flue samples are show in Table 9.1. Typical analytical methods include gravimetric analyses of dust particles, AAS, and ICP-methods for metals, and GC-MS and HPLC methods for organic compounds. Analytical methods specific for CO_2-capture compounds are shown in Table 9.2.

Table 9.2 Methods for collection of relevant compound groups in flue gas samples

Flue gas compounds	Particulate phase/aerosols	Gaseous phase	Analyses	Reference
Amines (MEA)	Filter[a)]	XAD-2 adsorber coated with NITC	HPLC	http://www.osha.gov
Aldehydes/ketons	Glass fibre/quartz wool filters	DNPH-conversion to hydrazones	HPLC and UV-detection	VDI 3862-2
Ammonia	Filter [a)]	H_2SO_4 absorber	IC	VDI 2461-2
Amides	Filter [a)]	Silica gel adsorber	GC-NDP	OSHA CSI
Alkylamines (methylamine)	Filter [a)]	XAD-7 adsorber coated with NBD chloride in tetrahydrofuran	HPLC	http://www.osha.gov
Nitrosamines	Filter [a)]	Thermosorb-N/ascorbic acid impregnated filters	GC-MS / GS-NP	http://www.osha.gov

[a)] Filter quality not described

9.4 Specific sampling requirements for toxicity testing

A number of considerations are important to be aware of with respect to toxicity testing of flue gas samples:

- Non-destructed samples should be used. As shown in Table 9.1 and Table 9.2 all relevant sampling methods described as validated methods include some steps to destruct the samples. However, if toxicity tests are to be performed, non-destructed samples are required. Therefore liquid acid absorbers and immobilizing solid adsorbers are not fit for this type of sampling.

- Validated toxicity methods are almost exclusively recommended for use with water-based samples. The use of condensers provides an option for obtaining flue gas samples in one water-based sample. As long as the condensors are used for trapping the components in a neutral non-destructive environment this approach may be considered for further development.

- The high content of ammonia in the flue gas may pose a problem. Ammonia may raise the pH beyond the acceptance criteria of many toxicity tests. Methods for removing or reducing ammonia may therefore be of interest to develop to avoid pH-changes which may influence on toxicity tests.

- Several of the compounds of toxicological importance are present in flue gas in very low concentrations, as described from various confidential studies. This may require the need for sampling of large volumes of flue gas.

9.5 A concept study for a sampling method with potential for toxicity testing

9.5.1 Condensation

One trapping method which is mentioned in several of the standard methods above is **condensation**. However, condensation methods described above are mainly performed at ambient or cooled down temperatures. Since the boiling points of several of the volatile compounds in the flue gas are low they may escape the condensate trapping at conditions described in the standard methods.

A condensation approach will result in trapping of the flue gas compounds in a liquid phase. Therefore the route of administration for toxicity testing, both *in vivo* and *in vitro*, will be liquid. As stated in the ITT flue gases contain compounds both in gaseous phase, in aerosols and on particles. By using a condensation methods all compounds will be present in one liquid phase, which is beneficial for toxicity testing. This will also be of importance for storage and transportation of samples.

9.5.2 Cold trap concept study

In order to further investigate the possibilities for using cold trap condensation as a non-destructive method for sampling of volatile flue gas components, initial experimental studies were performed in this project.

A cold trap concept could theoretically be a potential common and generic principle for non-destructive trapping of a broader range of water-soluble compounds in flue gas from a CO_2-plant. It is essential that the selected condensate temperature should be well below boiling points of all relevant flue gas compounds in order to trap flue gas compounds in representative concentrations.

The most important feature with the cold trap concept is that it will eliminate the need for absorption solutions or solid adsorbents / desorption solutions that will interfere with toxicity studies. Another important feature is elimination of the classic problem of sampling a gas stream with high water content, making the use of filter and adsorbent tubes very difficult.

To verify that it was possible to trap also the amines with the highest volatility, it was suggested to perform a concept study as parts of TQP Amine 3 (Activity 01 – Sampling of extracts of emissions to air for toxicity testing) and TQP Amine 1 (Subtask 2 – Manual sampling). The study "Analytical and preparative sampling of volatile amines in a cold trap - Concept study" was performed in agreement with Company.

Due to the fact that a possible problem with co-sampling of ammonia with other component was identified, the question was raised whether selective sampling of ammonia with exclusion of the less volatile compounds was possible with cold trap. To obtain data on this, ammonia was included in the study together with methylamine, ethylamine and dimethylamine, and a strategy for how to generate controlled and stable cold trap temperatures (within the range of 0 to -196 ºC) was established.

A system suitability test (SST) was performed in advance of the sampling main experiment. This study was made to verify equipment and control of temperatures.
The main conclusions from the SST were:

1) A 0.5 L gas washing bottle with open tube would serve as a model system
2) Water is trapped as ice between -21 and -196 ºC

3) At -196 ºC air is trapped as liquefied O_2/N_2. This shows that even the most volatile gases can be trapped by the cold trap
4) Selection of the cold trap temperature is essential
5) It is possible to select and control trapping temperatures over several hours within the range of 0 to -196 ºC by use of different water/solvent baths

The main experiments in the concept study "Analytical and Preparative Sampling of Volatile Amines in a Cold Trap" were conducted as follows (more details are given in Appendix B): The test mixture consisted of 1 mg/mL of ammonia and 10 ug/mL of each of the alkylamines methylamine, ethylamine and dimethylamine. Cold trapping was performed in an empty gas washing bottle at -20 and -75 ºC. A midget impinger with 20 mL 0.1 N H_2SO_4 absorption solution was mounted behind the cold trap in the sampling train to collect the compounds not trapped in the condensate. The concentrations of amines were analyzed in the water trapped in the cold trap and in the absorption solution by GC-MS after derivatisation. The total amounts of amines were calculated in the trapped water and the absorption solution. The results are shown in Table 9.3.

Table 9.3a Experiment performed at -20 ºC

Analyte	Amount in cold trap (percent)	Amount in adsorption solution (percent)
Ammonia	16	84
Methylamine	60	40
Ethylamine	54	46
Dimethylamine	64	36

Table 9.3b Experiment performed at -75 ºC

Analyte	Amount in cold trap (percent)	Amount in adsorption solution (percent)
Ammonia	71	29
Methylamine	92	8
Ethylamine	85	15
Dimethylamine	92	8

The main conclusions from the main experiment are that:

1) The most volatile amines (ammonia and alkylamines) can be trapped by the cold trap
2) Alkylamines are trapped more efficiently than ammonia
3) The trapping ratio of compounds in cold trap and backup impinger showed that the more volatile compounds are increased with lowered temperature.

The results and conclusions from the SST and the main experiment are satisfying compared with the scope of the study, and the concept study must be considered as successful. Given the fact that there is no existing methodology available for preparative sampling of flue gas, the cold trap concept represent a promising alternative to fill this gap. Improved ammonia removal may be achieved with a "fine tuning" of the condensation temperature(s). In addition, ammonia may be removed selectively.

The preservation of a flue gas extract after sampling is crucial, due to the likely presence of degradation products and reaction products with unknown stability in the flue gas. The cold trap

concept offers the possibility of a very fast and easy transfer of the sample to storage at -196 ºC in liquid nitrogen, simply by direct transfer of the complete trap to a nitrogen tank. To our knowledge, storing at -196 ºC is the ultimate way of preservation when no chemical stabilization is possible.

9.6 Recommendations for Call Off 2

Non-destructed samples should be used for flue gas sampling. The concept study described above indicated that condensation could be a relevant strategy for such method development. However, such a method is not yet fully developed, and a number of questions still need to be answered:

- Although a condensation cold-trap concept may be developed within a relatively short period of time, it is evident that it may be difficult to establish the complete method within the time frame of Call Off 2. If concentration steps are required, this will increase the time of test method development further.
- There are no standard validated methods for toxicity testing of flue gas samples. It is also clear that methods for toxicity testing of emission samples must be established and validated. Since this will be a time-consuming process, we do not see that this will be realistic to ascertain within the Call Off 2 period.
- The problem of high pH caused by ammonia may be partly solved by the cold trap approach, with selective removal of ammonia. However, we are not sure that this problem will be solved within the Call Off 2 period.

Before these questions are answered we do not regard it as possible to develop sampling methods and flue gas toxicity methods within the time frame of Call Off 2.

However, it is essential that reliable methods for chemical analyses are available for hazard and risk assessment. However, these methods are outside the scope of this project and are handled in TQP Amine 1.

10 Suggested approach for the Test Protocol - emission compound toxicity

Based on the available tools, methods and data for emissions of flue gas compounds from CO_2 capture plants we suggest a number of options for an emission toxicity test protocol. A draft Test Protocol is enclosed in Appendix A of this report.

10.1 Basic principles

The basic principles behind the test protocol are:

1. The main part of the protocol should rely on the testing of single compounds expected to be part of emissions.
2. Data from mammalian toxicity and ecotoxicity studies should be related to data from flue gas sampling for risk assessment

3. Health and environmental data should be collected from various information sources and the quality of the data evaluated, if possible

4. Where limited data are available, or data quality is questionable, testing by validated methods should be performed by acknowledged laboratories, and according to accepted quality assurance (QA) criteria (e.g. GLP)

5. Health-related data and test requirements are more important for some endpoints than others. In that respect data related to DNA damage/carcinogenicity and reproduction toxicity should be ranked high, representing long-term health effects

6. Ecotoxicity evaluations are based on standard approaches for determinations of predicted no-effect concentrations with aquatic test organisms. Aquatic data may be supplemented with data from tests for soil and plant species, if judged to necessary by expert opinion.

7. Biodegradation evaluations are based the determination of ready biodegradability, and with options for inherent biodegradability and for simulation testing. Abiotic degradability should also be determined for biologically recalcitrant chemicals

8. We do not recommend testing of mixtures of chemicals at the moment since appropriate methods are not available at the moment. Further efforts are needed to -

    a. Establish sampling methods

    b. Establish toxicity methods to be used for emission compound and mixture toxicity

    c. Determine if toxicity of individual compounds is additive

    d. Establish test system(s) for comparison of the toxicity of flue gas compounds

9. Full risk assessments should not be part of the Protocol until reliable dispersion and fate models are available for CCM.

10. Data from mammalian tests included for hazard assessment as part of a risk evaluation should be obtained from two approaches

    a. Determination of DNEL\DMEL (DMEL recommended) if sufficient data are available

    b. Worst case approach if not sufficient data are available for DNEL\DMEL determination

11. Data from ecotoxicity tests included for hazard assessment should be obtained from the determination of a PNEC

12. Data from biodegradability tests may be used for determination of biodegradation rates in the environment, as part of the determination of environmental concentrations

10.2 Protocol organisation

The Protocol is currently separated into two parts:

- Part 1 – Test methods

  This part describes the proposed methods and flow-sheets for performances. Only validated OECD methods are included as toxicit0y and biodegradation methods. Methods for hazard analyses are described both for health and environmental data. Methods for flue gas sampling are not included in the protocol since these methods are not yet developed, or are the subject of the project TQP Amine 1. Methods for chemical analyses of flue gas compounds are not described, since these are the subject of the project TQP Amine 1.

- Part 2 – Protocol for testing of single flue gas substances

  This part describes the principles for testing of substances determined or expected to be present in flue gas emission mixtures. The sources of information required for obtaining health and environmental information and for selection of test methods (if required) are described (with reference to the methods described in Part 1). Included in this part is a flow-sheet for the toxicity testing.

Additional parts may be added to the Protocol in the future, if methods are developed. These may include:

1. Protocol for full risk assessment
2. Protocol for testing mixtures of flue gas substances
3. Protocol for testing of flue gas emission samples

A protocol for full risk assessment should combine the data from hazard assessment included in the current protocol with methods for estimating exposure concentrations. For CCM we suggest that exposure concentrations should be determined from relevant emission data, in combination with data obtained from atmospheric dispersion studies of flue gas compounds, and from possible estimations of the fate of flue gas compound processes in the atmosphere and in other environmental compartments. Some of these data may be provided by collaboration between scientific groups involved in several of the TQP Amine projects.

If a future protocol for toxicity testing of mixtures of flue gas substances is prepared, toxicity methods should be simple *in vitro* mammalian or ectoxicity methods. We suggest that the initial objective of such a testing should be to decide if toxicity of flue gas can be caused by additive mechanisms, or if different compounds show interactions. It is essensial that possible toxicological interactions between flue gas compounds are characterised in a research project before further recommendations are included for the Protocol.

A protocol for the testing of flue gas emission samples should describe the possible information received from the toxicity testing of flue gas emission samples by toxicity methods (mammalian or ecotoxicity test methods). For this purpose we suggest to use simple *in vitro* test methods. The data from these tests may be used for comparison of the overall toxicity of gas samples originating from different solvent technologies. If additive toxic responses are proven (see above) the results from such tests may be used for verification purposes, by comparison of emission sample toxicity and expected toxicity calculated from the data of individual substances. If sample toxicity is

significantly higher that expected from calculations this may indicate potentials for some "uncharacterized" compounds in the flue gas.

11 Future research requirements

We suggest that future requirements for studies relevant to this project should be separated in two major issues:

a) Provide data for risk assessment
b) Development of methods for toxicological measurements of flue gas emissions

The research requirements described below are those which are not included in Call Off 2, with a longer time frame than suggested for Call Off 2.

11.1 Risk assessment

Risk assessment includes both the determination of toxicity and the environmental exposure concentrations, and the combinations of the two sets of parameters are essential for the determination of the risk to the population and the environment.

In this report we have recommended the use of validated toxicity methods to be used for the toxicity-part of risk assessment, and only accepted OECD procedures have been included for health and environmental toxicity methods. However, our suggestions are based on the currently available OECD methods.

However, as new methods are being accepted as OECD methods these should also be considered for use for risk assessment of flue gas compounds. In Europe there is a pressure towards replacing animal testing by *in vitro* tests. As *new in vitro* tests are accepted as OECD tests some of these may be considered to be supplements to animal testing. The use of *in vitro* tests may even be used to reduce the numbers of *in vivo* tests required. However, *in vitro* tests can never replace *in vivo* tests since several important toxicological mechanisms can only be determined by animal testing, e.g. metabolic activation. The development of alternatives to animal testing is not the topic of the current project, and for risk assessment we suggest that new relevant methods may be considered for a flue gas toxicity protocol as soon as they become validated.

Data for predictions of environmental concentrations are very important in order to predict exposure concentrations. Emission data for flue gas samples are available, and the methods for obtaining these data are the topic of TQP Amine 1. However, the post-emission processes are very important for the determination of exposure concentrations, and these are the topics of TQP Amine 2 (dispersion models) and TQP Amine 4 (atmospheric processes). It is therefore important that all the information from these TQP Amine projects, and other relevant projects with accessible data, are utilised in order to obtain a good risk assessment model for CCM.

11.2 Sampling and toxicity testing of flue gas emissions

Identifying methods for sampling and testing of flue gas emissions was one of the main objectives of TQP Amine 3. The purpose of such sampling and testing would be to compare the overall toxicity of emissions from different solvent technologies, and to identify possible "unknown" compounds of toxicological importance. As described in the preceding parts of the report methods were not currently available, neither for sampling for toxicity analyses nor for performing toxicity

analyses of flue gas samples. The reasons for this are described in other parts of this report. We will here describe some possible research requirements for the sampling and testing of flue gas emissions.

11.2.1 Sampling methods

As discussed in cpt. 9 the use of a condensate method may have potentials for toxicity testing of flue gas samples. By this approach samples will be trapped in one liquid phase instead of being distributed between gaseous, particulate and droplet phases. Since the condensate method has potentials for sampling of flue gas for toxicity testing, we recommend that further studies are conducted for developing this approach for emission toxicity studies. However, the development and testing of such methods will take more time than a Call Off 2 period.

Some of the major research requirements are listed below for a condensation approach:

- Characterizing condensate trapping of most relevant flue gas compounds
- Investigate the impact of high ammonia concentrations on toxicity
- Developing methods for concentration of toxicologically important compounds appearing in low concentrations in flue gas emissions without violating
- Characterisation of sampling method representativeness and reliability
- Characterisation of stability of flue gas compounds from sampling to toxicity
- Identification of toxicity methods relevant for testing of flue gas emission samples

If ammonia becomes a problem for toxicity testing, samples may be prepared with reduced ammonia content. The samples will then not be representative for flue gas. Also, if low concentrations of some compounds require concentration of these, distribution between compounds may be changed when compared to the original samples due to selective concentrations. As mentioned above, improved ammonia removal may be achieved by a “fine tuning” of the condensation temperatures. In addition, methods for selective removal of ammonia may be used. For instance, zeolite has been used for removal of ammonia from wastewater systems (Cooney *et al*., 1999).

The flue gas samples may appear in low concentrations, and concentration steps may be provided. One way of concentrating the flue gas samples as condensate may be the ECOWAT principle. The ECOWAT principle is designed for water purification and describes removal of contaminants from the water. Pure water is crystallised as hydrate, while contaminants are not crystallised (www.ecowat.no). In this way the contaminants are separated from pure water and concentrated. Thus, this principle may therefore also be used for concentrations of contaminants and removal of clean water, to concentrate substances in a condensate, as possible required for trapped flue gas compounds in low concentrations.

11.2.2 Toxicity methods for flue gas samples

11.2.2.1 Testing of complex mixtures

Complex mixtures can be assessed through direct toxicity testing or considering summation of toxicity contributed from individual compounds.

Toxicity testing of complex airborne mixtures is a challenge in whatever test system is employed. Inhalation tests using animals in an exposure chamber is difficult because gas mixtures may condensate and changes due to the physical conditions in the chamber. Some of these problems are can be avoided by using direct nose/mouth exposure of experimental animals. Equipments and techniques have recently been developed for the direct exposure of *in vitro* cell cultures to airborne components – both particulate and volatiles. These systems may become important tools in developing further *in vitro* approaches to toxicity testing of airborne contaminants. The sample preparation will be virtually the same as for standard animal inhalation procedures. These techniques are potentially helpful for comparing relative toxicities of similar compounds or mixtures.

An alternative to toxicity testing of the complex mixture itself is to address the knowledge we have on individual components of the mixture. A variety of summation formulas have been developed based on toxicokinetics and –dynamics. Some of the models can be very helpful for up to 3-5 compounds with similar toxic effects. However, they are less helpful when there are many components that differ in their toxicity endpoint. In such cases regulatory bodies have often opted for a worst-case approach giving the mixture a rating/classification based on severity of effect although it is caused by a minor component.

A future evaluation of complex airborne mixtures like flue gas should include comparing several approaches:

- Testing of individual compounds followed by theoretical considerations
- Testing of real mixtures
- Testing of synthetic mixtures

We recommend the development of such an approach, and this will need the support of industry as well as public research funds. However, this will go far beyond the short-term scope of the present study.

11.2.2.2 Toxicity methods

Due to the complexity of flue gas mixtures we suggest that simple *in vitro* tests should be preferred for these types of studies. We therefore suggest that different *in vitro* tests for several toxicity endpoints are considered for these types of studies. Several *in vitro* tests have been described above, and in addition to these some other methods may also be considered, as described below:

Cytotoxicity–

- Clonogenic assay (plating efficiency, colony forming ability), validated scientifically
- Proliferation (growth activity) assay or simple version relative growth (in ECVAM validation together with genotoxicity *in vitro*)
- *In vitro* cytotoxicity as starting point for determination of LC50, NRU 3T3 or NHK 3T3 (OECD draft 129)

Mutagenicity/genotoxicity

- *In vitro* comet assay both for detection of strand breaks (under ECVAM/JaCVAM validation) or for detection of oxidized DNA lesions (photogeonotxicity assay)
- *In vivo* comet assay (JaCVAM/ECVAM validation) is useful to detect DNA damage in different organs and is already used for in vivo combined endpoints study
- *In vitro* Cell Transformation Assays; either 1.Syrian hamster embryo (SHE) assay 2.Balb/c assay 3.C3H/10T1/2 assay (EU method B.21)

Reproduction toxicity

- The Stably Transfected Human Estrogen Receptor-alpha Transcriptional Activation Assay for Detection of Estrogenic Agonist-Activity of Chemicals (OECD 455)

Ecotoxicity tests

- Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) (ISO 11348).

For chemicals with unknown modes of toxic action, the recent and projected advances in toxicogenomics, bioinformatics, systems biology and computational toxicology represent a paradigm shift for toxicity testing of chemicals. High-throughput screening tools, like the use of microarrays and metabolic profiling of molecular effects caused by chemicals with unknown (and known) modes of toxicity, are expected to revolutionize predictive toxicology and elicit a paradigm shift in regulatory toxicity testing and risk assessment. These approaches are still in their infancy, however, the use of microarrays to elucidate possible modes of toxicity of and establish effect limit have recently been published (Poynton *et al*., 2008).

In order to evaluate such methods it is important to investigate a number of essential factors with respect to flue gas samples:

- Detection limits with respect to compound concentrations in flue gas samples: Several compounds of toxicological importance appear in low concentrations in the samples
- Sensitivity to samples with different compound composition: It is essential that toxicity methods are able to separate between samples with different compositions
- Ammonia toxicity: Since ammonia may be a significant contributor to toxicity even after efforts to reduce concentrations it is important to identify the impact of toxicity (and other effects caused by ammonia) on the test methods
- Interactions between different compounds: If toxicity proves to be additive in an *in vitro* method, this method will be of interest for detecting unknown compounds by comparison between flue gas samples and synthetic flue gas mixtures

11.2.3 Experimental systems for *in vitro* toxicity testing of gas samples

For direct toxicity testing of flue gas samples some exposure systems have been suggested.

A direct exposure system at air/liquid interface was developed *in vitro* toxicity studies, using gaseous exposure of human lung cells (Ritter *et al.,* 2001; Pariselli *et al.*, 2009). A commercialised version of the exposure system is shown in Figure 11.1. In this system gaseous samples are exposed to cell cultures on membranes with underlying culture medium wetting the membranes.

Komori and co-workers described a simple gas exposure system for volatile chemicals or gas flow-through based on the Microtox principle, using luminescent bacteria immobilised to a membrane, as shown in Figure 11.2 (Komori *et al.*, 2009).

Thus, testing of various *in vitro* methods for toxicity measurements of flue gas will be relevant for comparison of samples from various sources, e.g. different solvent technologies.

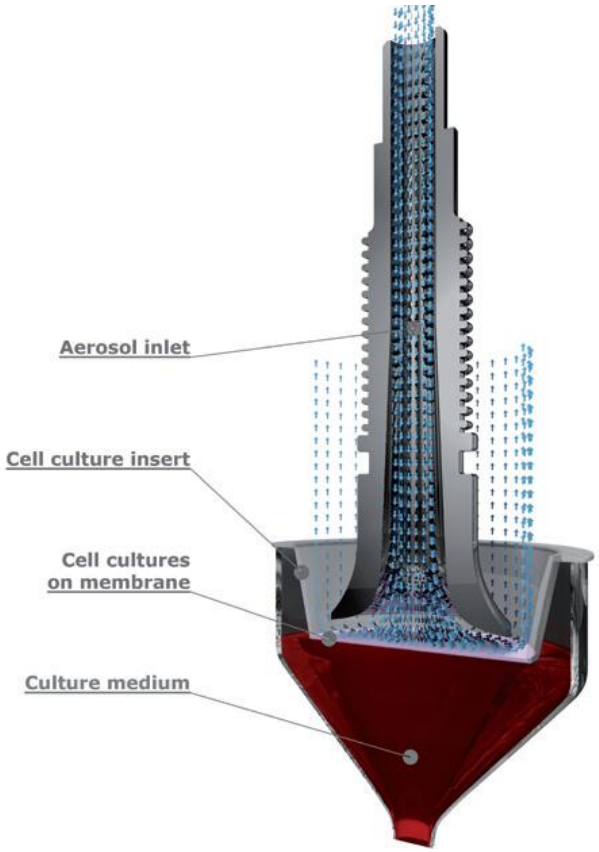


Figure 11.1 Vitrocell system for direct exposure at air/liquid interface (Source: http://www.vitrocell.com/).

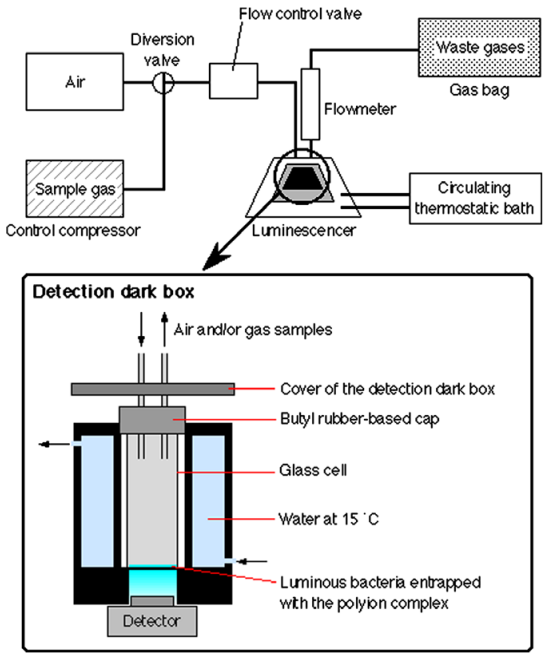


Figure 11.2 Microtox-based system for gas exposure of luminescent bacteria immobilised on a solid matrix (from Komori *et al.*, 2009).

11.3 Degradability

Determination of the degradation of flue gas compounds after emission to the environment is important for the determination of the fate of the emission compounds. Methods for biodegradability and hydrolysis have been suggested in the Protocol. However, photolytic degradation is also of importance, especially for the prediction of the fate of nitrosamines and nitramines (Bråthen *et al.*, 2008). An OECD Guideline for phototransformation in water (OECD316) exists, but no validated methods for phototransformation in air. We suggest that photodegradation methods are investigated and considered to be included in a future revision of the Protocol.

11.4 Eutrophication

Eutrophication is a process that results in a concentration of nutrient content to an extent that increases the primary production. Due to considerable expected emissions of ammonia from CCM eutrophication may become an environmental topic.

After release to the environment ammonia may be oxidized to nitrite and nitrate by aerobic nitrification processes. These nitrogen compounds are well known as fertilizers and may add to the local nitrogen budgets. Eutrophication may impact sensitive ecosystems and attribute to soil acidification.

It has also been suggested that soil fertilisation may lead to nitrosamine generation in the soil (Barabasz *et al.*, 2002).

Due to expected emissions of ammonia from CCM we regard it as essential to investigate the possible impacts of ammonia and nitrification eutrophication around Mongstad. This may be performed as experimental studies, but preferably as monitoring campaigns. Biomonitoring of eutrophication has been suggested by using species sensitive to nitrogen input to the environment.

For instance have lichens been suggested as possible indicators of nitrogen emissions (e.g. Frati *et al*., 2007; Jovan *et al.,* 2006).

12 Conclusions

Based on the recommendations in this report the following conclusions are made:

Emission compounds for toxicity testing: A single compound strategy should be used for risk assessment of flue gas components, including solvents and degradation products. Toxicity testing of flue gas emissions is currently not recommended. The composition of flue gas will change from emission to exposure due to post-emission processes and flue gas samples may change over time. Biological behaviour may be affected by the interactions between emission substances. The predominant components ammonia may affect physiology of test animals, as well as affecting uptake mechanisms of other substances. Single compound approaches are well established for risk assessment of complex mixtures from other environments (e.g. for risk assessment of regular oil emissions). We suggest that all compounds detected in flue gas emissions should be considered for toxicity evaluation, but testing importance ranked according to expected toxicity and emissions.

Hazard and risk assessment: A number of 13 compounds representing different flue gas emission groups were selected as a case study for hazard assessment for provisional risk evaluation. Health and environmental effects of these were surveyed in different databases. Health effects included important endpoints like mutagenicity/genotoxicity, carcinogenicity, reproduction toxicity and sensitization. Hazard classification was made according to the GESAMP system. For environmental tests various databases were investigated to identify ecotoxicity (acute or chronic) to species representing different trophic levels, while biodegradability was investigated to define the substances as ready or inherent biodegradable. The hazard assessment system is described in the Protocol prepared from the project.Toxicity methods for hazard assessment recommended in this project include DNEL/DMEL approach (DMEL preferred) for mammalian toxicity if enough data are provided to determine reliable no-effect or minimal-effect levels. If not sufficient data are available it is recommended to use a worst case approach, based on Scandinavian OEL concentrations. We have not performed any quality assessment of the toxicity data which is used for the basis of OEL. For some of the degradation products no OEL values are available, to our knowledge. Ecotoxicity data are to be used for determination of PNEC values, based on the most sensitive test organism. It is recommended to use data from chronic tests rather than from acute tests, if available. For a full risk assessment toxicity data are relatd to exposure concentrations, obtained from emission data, in combination with estimations based on post-emission process.

Toxicity testing: Toxicity and degradation testing of case study flue gas substances are recommended for a full hazard assessment. Only validated OECD test guidelines are recommended for testing. Tier-based test systems are recommended both for mutagenicity/genotoxicity/carcinogenicity, reproduction toxicity, ecotoxicity and biodegradation testing. The test methods are described in the Protocol prepared for the project.

Sampling of flue gas emissions: A sampling method based on cold trap condensation seems promising for toxicity testing. However, only a system suitability test has so far been performed, and a method is not developed yet for sampling of flue gas for toxicity testing. Therefore, we do not recommend any flue gas toxicity test establishment for Call Off 2, but as part of future research requirements. Further development is expected as part of the TQP Amine 1 project.

Recommendations for Call Off 2: In a Call Off 2 of the ITT a number of actions are to be performed with a short period of time as a result of recommendations made in Call Off 1. The period of time for Call Off 2 has been suggested by Company to last for approximately 6 months. We suggest here that these actions should include testing for toxicity and degradability of substances used for hazard assessment in this project. Some of the recommended mammalian tests will require longer test periods than 6 months.

Future research needs: Future research needs have been identified in this project. For risk assessment should evaluations of new emerging validated methods be of interest, especially *in vitro* methods which can supplement or aid in the reduction of animal testing. Exposure concentrations are essential for risk assessment, and these concentrations should be estimated by combining the outcome of several TQP Amine projects. Toxicity testing of flue gas emissions is an option, but appropriate sampling method(s) should be developed. Toxicity methods for testing of samples should also be established when reliable sampling methods are available. It may also be of future interest to consider the possibilities of using simple exposure systems for *in vitro* testing of gas samples. The potential impacts of ammonia emission on establishment of emission mixture sampling and toxicity methods should be investigated during method establishment. The environmental impacts of ammonia emissions from CCM and the establishment of monitoring methods may also be of interest.

The main objectives of all these efforts should be to characterise the risk associated with the different flue gas emission products and use these data for risk reduction.

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Appendix A TEST PROTOCOL – EMISSION COMPOUND TOXICITY

Test Protocol

Emission Compound Toxicity

Intentions and limitations of the Protocol

The intention of this Protocol is to describe the decisions and methods to be used for evaluation and testing of the toxicity of CO_2-capture flue gas emissions.

The Protocol is the results of the recommendations from the project TQP Amine 3 "*Emission Compound Toxicity*". The objective of this project was to suggest methods for the determination of the toxicity of flue gas emissions from post combustion CO_2-capture. The Protocol is based on current status of knowledge and methods, and future improvements on these matters may result in revisions of the Protocol.

The protocol is based on a "single-substance" approach, i.e. the hazard determination of single flue gas components. Toxicity determination of flue gas or mixtures of flue gas compounds is currently not recommended in the Protocol, a) due to the complex interactions of many chemicals in the flue gas, b) current lack of knowledge about variability and reproducibility of flue gas samples, and c) lack of knowledge about the impact of quantitatively predominant compounds like ammonia on emission toxicity. However, methods for toxicity testing of flue gas may be considered in the future if some of these knowledge gaps are closed, preferable by simple in vitro screening toxicity methods. These methods may also be considered used for the toxicity testing of mixtures of single substances to deduce possible interactions between the substances.

Methods for risk assessment have not currently been included in this Protocol. An important part of risk assessment is the determination of the environmental concentrations of emitted compounds. This is not part of the scope of TQP Amine 3, but part of other TQP Amine Projects. Risk assessment should therefore be included as a result of the information from several TQP Amine projects.

In this Protocol only *validated* methods are recommended. In this respect, only methods recommended by the Organisation for Economic Cooperation and Development (OECD) have been included. Other methods, not currently validated, may be important supplements to OECD methods, but should be further evaluated before included in this Protocol.

Sampling and analyses of flue gas are important in order to relate toxicity of compounds to concentrations in the emissions. Sampling methods are also important for possible future applications for toxicity analyses. Sampling methods and analyses of flue gas samples are part of TQP Amine 1 and therefore not currently included in this Protocol. Methods for sampling of flue gas for toxicity methods have not been developed yet. If reliable sampling methods for toxicity studies of flue gas are developed, these methods will be considered to be included in this Protocol.

Overview of the Protocol

A general overview of different elements in a Protocol for flue gas emissions is shown in Figure 1. This figure includes both elements included in the current Protocol and additional future elements to be included.

Health and environmental (HE) aspects of emissions to various environments should include both toxicity testing and analyses, hazard and risk assessment and actions to reduce emissions of harmful substances to the nature (Figure 1), including descriptions of following elements:

1. Selections of emission substances and available HE information for these substances
2. Hazard analyses from available information
3. Toxicity testing of selected emission substances for full hazard assessment
4. Risk analyses
5. Identification of flue gas compounds representing HE risk
6. Reductions of HE risk

Selection of flue gas test substances must be based on available information related to choice of solvent/solvent technology and to the predictions of degradation processes. Data from experimental studies and emission campaigns should be used for selection of test substances. For compounds of high health/environmental concern (i.e. nitrosamines and nitramines) also theoretical assumptions may be considered.

Possible sources for the collection of health and environmental information will be described in this Protocol.

Validated toxicity testing as described here will include both testing for human health effects by mammalian toxicity tests and for ecotoxicity tests. Biodegradation tests will also be included as part of the ecotoxicity test system.

For human health effects a number of endpoints have been selected that are of high concern. These include genotoxicity, carcinogenicity, effects to reproduction and sensitisation. These are effects related to the concern of the general population. Effects like irritation/corrosion, which are more of occupational concern, have been considered in the summary sheets for chemicals but have not been included for testing here.

Ecotoxicity effects include three trophic levels of aquatic organisms have been included. In addition, acute effects to soil and plant species have been included, since these are relevant for flue gas emissions. A chronic test has also been included to relate effects to long-term exposure. Biodegradability tests have been suggested to define if the test substances are ready or inherent biodegradable, and if recalcitrant substances can be hydrolysed.

Tier-based systems have been suggested for all toxicity tests. Tier-systems will reduce the number of tests to be performed.

Chemical analyses for flue gas samples will be described in another TQP project (TQP Amine 1), and the Protocol will refer to this project for describing analytical methods for qualitative and quantitative characterization of flue gas samples.

This protocol is mainly concerned with the testing of components emitted to the environment in flue gas from post combustion CO_2-capture, and not trapped in the facility by flue gas cleaning. Thus, water wash samples are not included in this protocol, since these will be recycled in the system. It is expected that water wash will be destructed (e.g. by incineration) and should therefore not represent any risk to the environment, unless discharged accidentally. The water wash may be used indirectly for measurements of toxic effects of the emissions. In theory, toxicity measurements of water wash samples can be used for determination of removal of flue gas toxicity prior to emission, since the water wash system is placed in the top of the absorber. However, this is difficult to calculate.

The Protocol is separated in two main parts describing different levels of flue gas emission toxicity testing:

Part1: Description of the methods used in the Protocol
Part 2: Toxicity testing of single flue gas test substances (TSs)

Part 1 focuses on the methods used in the Protocol, including hazard and toxicity/degradation methods. Part 2 describes the flow from selection of test substances, available information, and the test requirements.

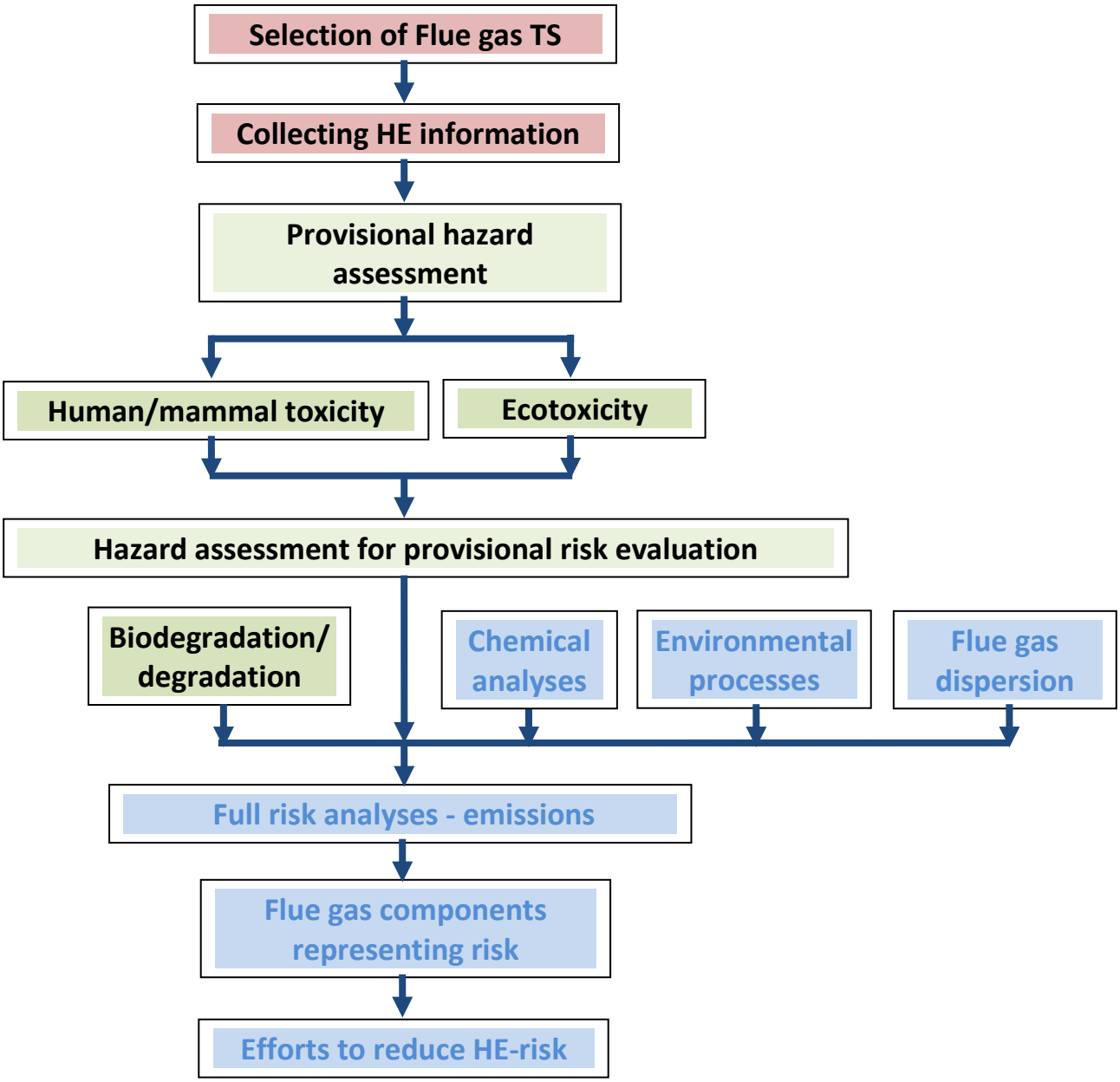


Figure 1 ***Overall outline of hazard and risk assessment of post combustion CO_2-capture flue gas test substances to reduce health and environmental risk. The boxes shown in black letters are part of this protocol, while boxes in blue letters are not. Methods included by the olive green boxes are laboratory methods described in Part 1, while boxes in red accent are described in Part 2.***

PART 1

Laboratory Test Methods
Hazard assessment methods

OVERVIEW OF TEST METHODS

This part describes the test methods included in this protocol. These test methods include methods for:

A. Mammalian toxicity (health effects)
B. Ecotoxicity (environment)
C. Biodegradation and degradation methods (fate in the environment)
D. Hazard method

Performing laboratories should run tests or studies according to approved quality assurance (QA) systems, for instance Good Laboratory Practice (GLP) or similar, or be accredited for specific methods/analyses.

HEALTH, SAFETY AND ENVIRONMENT (HSE)

Several of the chemicals associated with flue gas emissions are of health, safety and environmental concern. Nitrosamines and nitramines are potential carcinogenic chemicals. Amine compounds are in general irritating and corrosive, and some of them may have sensitizing characteristics.

The chemicals must therefore be handled with strict care; all work should be done in fume cupboards. Special care should be taken when storing and handling of nitrosamines and nitramines, following the strictest precautions recommended in the MSDS.

Due to the explosive characters of nitramines specific risk analyses should be performed before handling of these chemicals.

DESCRIPTIONS OF METHODS

PART 1A – Mammalian Toxicity Methods

1A-1: Decisions of test methods for mammalian toxicity

Genotoxicity and carcinogenicity:

Selection of methods for testing of mutagenicity and carcinogenicity should be made in a stepwise way as shown in Figures 2 to 4. The first step includes an acute test for oral exposure for determination of median lethal dose (LD50)(test for acute toxicity, not genotoxicity or carcinogenicity). Concentrations obtained in this test are used for setting dosages for further toxicity tests. The acute oral toxicity test is followed by *in vitro* genotoxicity testing (Figure 2), *in vivo* genotoxicity testing (Figure 3), and by carcinogenicity testing (Figure 4).

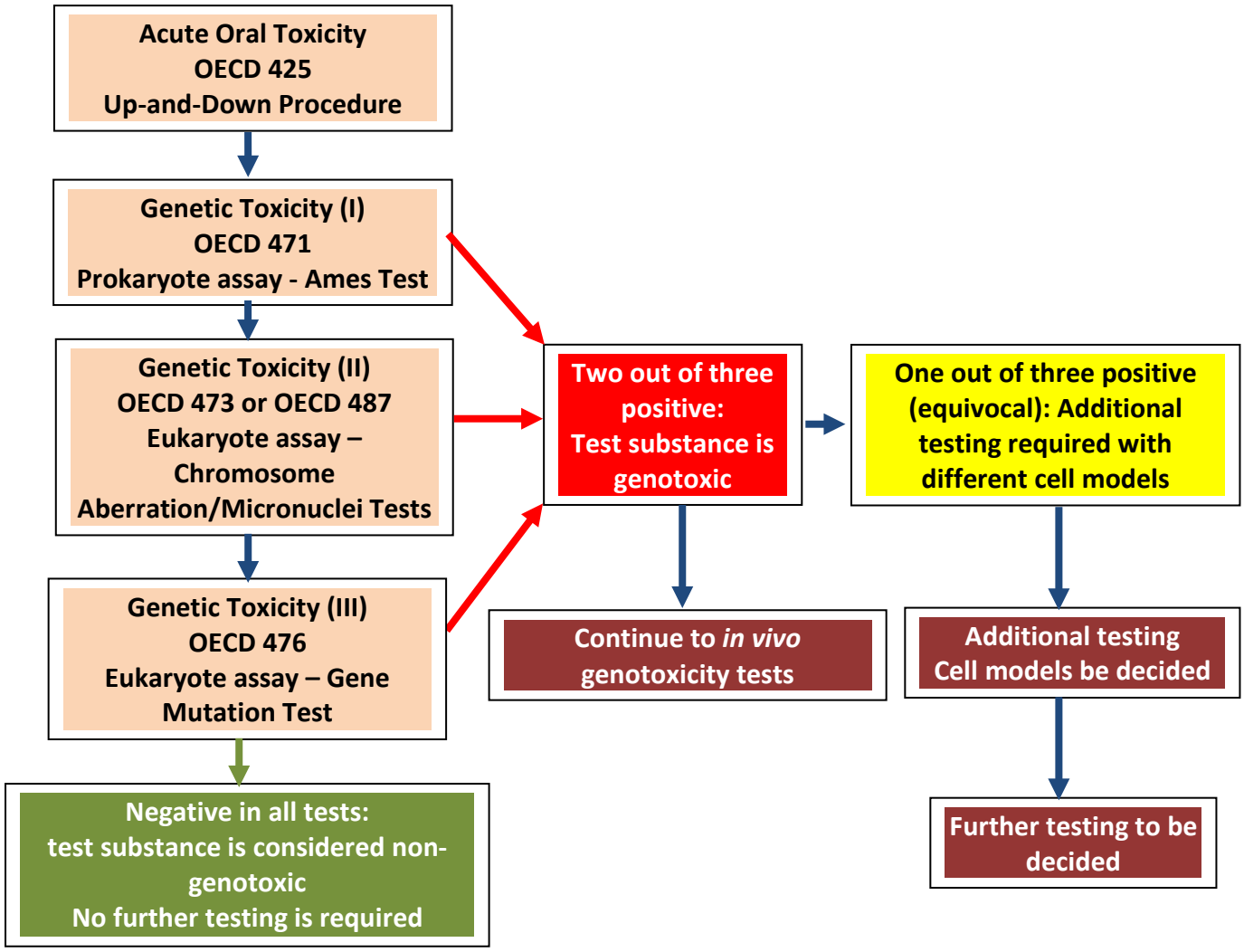


Figure 2 **Flow-sheet for acute oral test and subsequent *in vitro* genotoxicity tests**

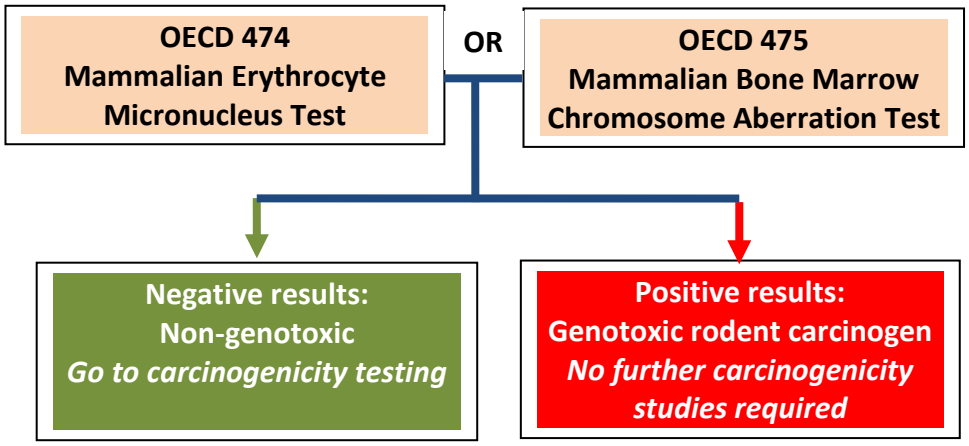


Figure 3 **Flow-sheet for *in vivo* genotoxicity tests. The OECD methods 474 and 475 are choices, and one of them can be used.**

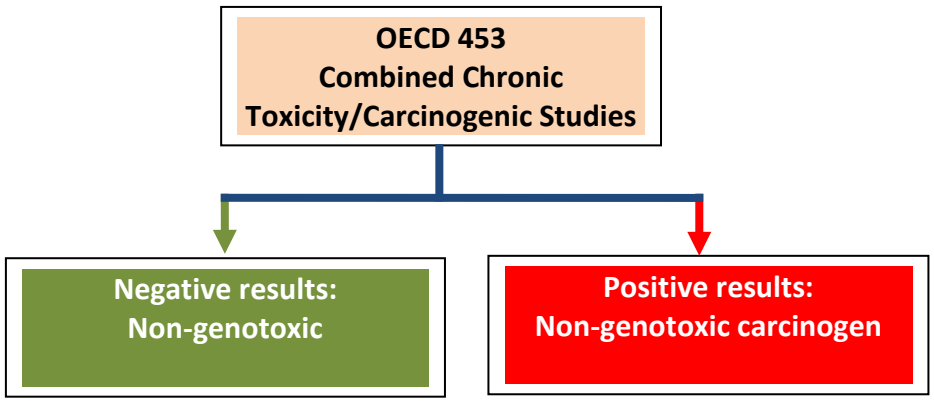


Figure 4 **Flow-sheet for Chronic toxicity / Carcinogenicity.**

Reproduction toxicity:

A number of 9 validated OECD Guidelines and one draft Guideline are available for reproduction toxicity. Of these there are four relevant methods (OECD- 415, 416, 421, 422) which measure the fetotoxicity in addition to reproductive and developmental effects:

The OECD test method 422, which is a combination of OECD 407 and OECD 421, provides information on dose concentration and measures several aspects of fetotoxicity. The OECD method 422 uses fewer animals (10 animals of each sex for 3 treatment groups and control) when compared to individual testing of OECD 407 (5 animals of each sex for 3 treatment groups and control) and 421 (10 animals of each sex for 3 treatment groups and control).

Recommendations for reproduction toxicity testing should be based on a two-tier system (Figure 5): If no data on LD-50 for the chemical is available a test for LD-50 determination (OECD 425) should be conducted. This is the same acute test as recommended before in vitro genotoxicity tests. This test should be followed by determination of fetotoxicity: OECD 422 (subchronic toxicity). If the outcome of the fetotoxicity test shows that the substance is fetotoxic a second tier should be performed, with one of the following tests: OECD 415 or OECD416 (chronic toxicity).

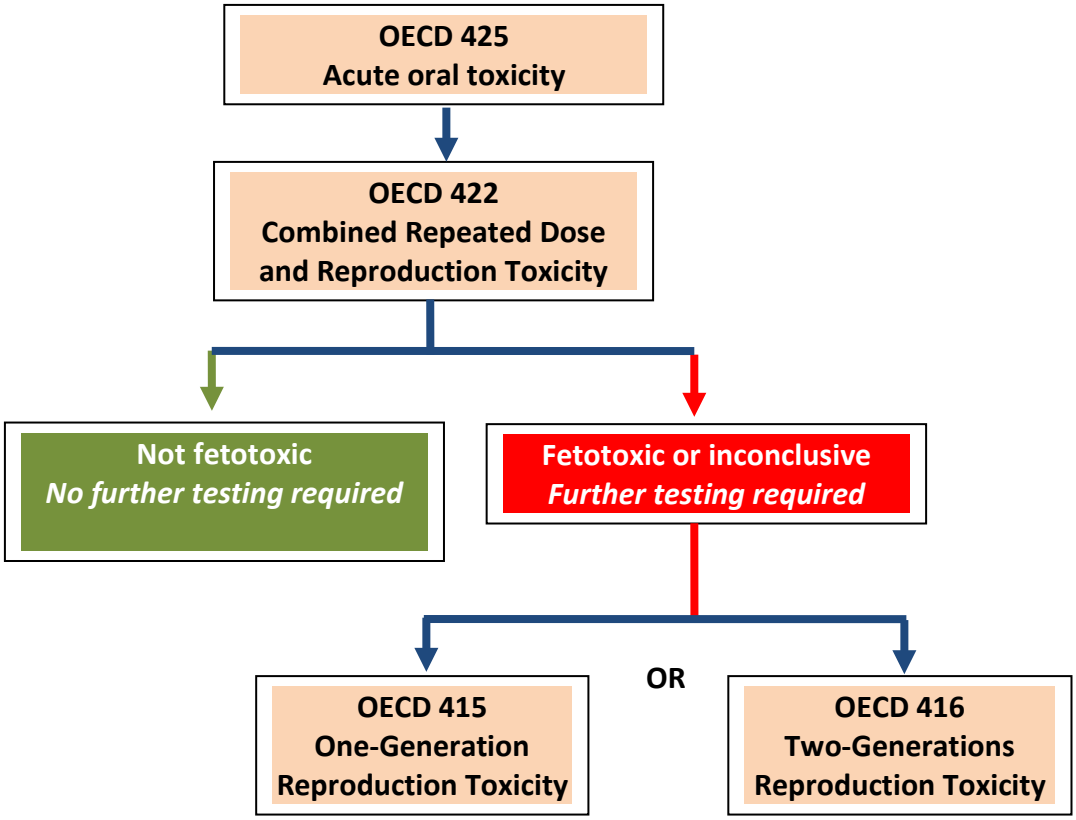


Figure 5 Flow-sheet for reproduction toxicity testing

The OECD methods 415 and 416 are choices, and one of them can be used depending upon the outcome from the first tier with OECD 422.

1A-2: Performance of tests for mammalian toxicity

Procedures for each method are available from OECD:

http://www.oecd.org/document/40/0,3343,en_2649_34377_37051368_1_1_1_1,00.html

Only the principles of each method is described in this protocol

Acute Toxicity

Acute oral toxicity – OECD 425

OECD Test No. 425: Acute Oral Toxicity – Up-and-Down-Procedure (UDP)
Principles: The main test consists of a single ordered dose progression in which animals are dosed, one at a time, at a minimum of 48-hour intervals. The first animal receives a dose a step below the level of the best estimate of the LD50. If the animal survives, the dose for the next animal is increased by a factor of 3.2 times the original dose; if it dies, the dose for the next animal is decreased by a similar dose progression. Each animal should be observed carefully for up to 48 hours before making a decision on whether and how much to dose the next animal. That decision is based on the 48-hour survival pattern of all the animals up to that time. A combination of stopping criteria is used to keep the number of animals low while adjusting the dosing pattern to reduce the effect of a poor starting value or low slope. Dosing is stopped when one of these criteria is satisfied, at which time an estimate of the LD50 and a confidence interval are calculated for the test based on the status of all the animals at termination. For most applications, testing will be completed with only 4 animals after initial reversal in animal outcome. The LD50 is calculated using the method of maximum likelihood. The results of the main test procedure serve as the starting point for a computational procedure to provide a confidence interval estimate where feasible.

In vitro genotoxicity – OECD 471

OECD Test No. 471: Bacterial Reverse Mutation Test

Principles:

The bacterial reverse mutation test uses amino-acid requiring at least five strains of *Salmonella typhimurium* and *Escherichia coli* to detect point mutations by base substitutions or frameshifts. The principle of this bacterial reverse mutation test is that it detects mutations which revert mutations present in the test strains and restore the functional capability of the bacteria to synthesize an essential amino acid.

Suspensions of bacterial cells are exposed to the test substance (liquid or solid) in the presence and in the absence of an exogenous metabolic activation system. At least five different analysable concentrations of the test substance should be used. The recommended maximum test concentration for soluble non-cytotoxic substances is 5 mg/plate or 5 ml/plate. There are two methods: the plate incorporation method and the preincubation method. For both techniques, after two or three days of incubation at 37°C, revertant colonies are counted and compared to the number of spontaneous revertant colonies on solvent control plates.

In vitro genotoxicity – OECD 473

OECD Test No. 473: In vitro Mammalian Chromosome Aberration Test

Principles:

The purpose of the in vitro chromosome aberration test is to identify agents that cause structural chromosome aberrations in cultured mammalian somatic cells. Structural aberrations may be of two types: chromosome or chromatid.
The in vitro chromosome aberration test may employ cultures of established cell lines, cell strains or primary cell cultures. Cell cultures are exposed to the test substance (liquid or solid) both with and without metabolic activation during about 1.5 normal cell cycle lengths. At least three analysable concentrations of the test substance should be used. At each concentration duplicate cultures should normally be used. At predetermined intervals after exposure of cell cultures to the test substance, the cells are treated with a metaphase-arresting substance, harvested, stained. Metaphase cells are analysed microscopically for the presence of chromosome aberrations.

In vitro genotoxicity – OECD 487

OECD Test No. 487: Mammalian Micronucleus Test

Principles:

The mammalian in vitro micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of mammalian cells in culture. Cell cultures are exposed to the test substances both with and without an exogenous source of metabolic activation unless primary cells with metabolizing capability are used. After exposure to the test substance, cell cultures are grown for a period sufficient to allow chromosome or spindle damage to lead to the formation of micronuclei in interphase cells and to trigger the aneuploidy sensitive cell stage (G2/M). Harvested and stained interphase cells are then analysed microscopically for the presence of micronuclei.

Ideally, micronuclei should only be scored in those cells that have completed nuclear division following exposure to the test chemical. In cultures that have been treated with a cytokinesis blocker, this is achieved by scoring only binucleate cells. In the absence of a blocker, it is important to demonstrate that the majority of mononucleate cells are likely to have undergone at least one cell division since exposure to the test substance. For all protocols, it is important that cell proliferation is demonstrated in both control and treated cells, together with aassessment of cytotoxicity in the treated cells scored for micronuclei.

In vitro genotoxicity – OECD 476

OECD Test No. 476: In vitro Mammalian Cell Gene Mutation Test

Principles:

The in vitro mammalian cell gene mutation test can be used to detect gene mutations induced by chemical substances. In the cell lines the most commonly-used genetic endpoints measure mutation at thymidine kinase (TK) and hypoxanthine-guanine phosphoribosyl transferase (HPRT), and a transgene of xanthineguanine phosphoribosyl transferase (XPRT). The TK, HPRT and XPRT mutation tests detect different spectra of genetic events.

Cells in suspension or monolayer culture are exposed to, at least four analysable concentrations of the test substance, both with and without metabolic activation, for a suitable period of time. They are subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection. It is recommended to utilise at least 106cells. Cytotoxicity is usually determined by measuring the relative cloning efficiency (survival) or relative total growth of the cultures after the treatment period. The treated cultures are maintained in growth medium for a sufficient period of time, characteristic of each selected locus and cell type, to allow near-optimal phenotypic expression of induced mutations. Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent to determine the cloning efficiency (viability). After a suitable incubation time, colonies are counted.

In vivo genotoxicity – OECD 474

OECD Test No. 474: Mammalian Erythrocyte Micronucleus Test

Principles:

The mammalian in vivo micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts, by analysis of erythrocytes as sampled in bone marrow and/or peripheral blood cells of animals, usually rodents (mice or rats).
The purpose of the micronucleus test is to identify substances (liquid or solid) that cause cytogenetic damage which results in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes. An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals is an indication of induced chromosome damage. Animals are exposed to the test substance by an appropriate route (usually by gavage using a stomach tube or a suitable intubation cannula, or by intraperitoneal injection). Bone marrow and/or blood cells are collected, prepared and stained. Preparations are analyzed for the presence of micronuclei. Each treated and control group must include at least 5 analysable animals per sex. Administration of the treatments consists of a single dose of test substance or two daily doses (or more). The limit dose is 2000 mg/kg/body weight/day for treatment up to 14 days, and 1000 mg/kg/body weight/day for treatment longer than 14 days.

In vivo genotoxicity – OECD 475

OECD Test No. 475: Mammalian Bone Marrow Chromosome Aberration Test

Principles:

The mammalian in vivo chromosome aberration test is used for the detection of structural chromosome aberrations induced by test compounds in bone marrow cells of animals, usually rodents (rats, mice and Chinese hamsters). Structural chromosome aberrations may be of two types: chromosome or chromatid.
Animals are exposed to the test substance (liquid or solid) by an appropriate route of exposure (usually by gavage using a stomach tube or a suitable intubation cannula, or by intraperitoneal injection) and are sacrificed at appropriate times after treatment. Prior to sacrifice, animals are treated with a metaphase-arresting agent. Chromosome preparations are then made from the bone marrow cells and stained, and metaphase cells are analysed for chromosome aberrations. Each treated and control group must include at least 5 analysable animals per sex. The limit dose is 2000 mg/kg/body weight/day for treatment up to 14 days, and 1000 mg/kg/body weight/day for treatment longer than 14 days.

Chronic Toxicity / Carcinogenocity – OECD 453

OECD Test No. 453: Combined Chronic Toxicity/Carcinogenicity Studies

Principles:

The objective of a combined chronic toxicity/carcinogenicity study is to identify carcinogenic and the majority of chronic effects, and to determine dose-response relationships following prolonged and repeated exposure.

The rat is typically used for this study. For rodents, each dose group and concurrent control group intended for the carcinogenicity phase of the study should contain at least 50 animals of each sex, while for the chronic toxicity phase of the study should contain at least 10 animals of each sex. At least three dose levels should be used, in addition to the concurrent control group for both the chronic toxicity phase and the carcinogenicity phase of the study. The three main routes of administration are oral, dermal, and inhalation. The Test Guideline focuses on the oral route of administration.

The period of dosing and duration of the study is normally 12 months for the chronic phase, and 24 months for the carcinogenicity phase. The study report should include: measurements (weighing) and regular detailed observations (haematological examination, urinalysis, clinical chemistry), as well as necropsy procedures and histopathology. All these observations permit the detection of neoplastic effects and a determination of carcinogenic potential as well as the general toxicity.

Reproduction Toxicity

Reproduction toxicity – OECD 415

OECD Test No. 415: One-Generation Reproduction Toxicity Study

Principles:

This Test Guideline for reproduction testing is designed to provide general information concerning the effects of a test substance (Solid, liquid, gas or vapour) on male and female reproductive performance. The test substance is administered orally in graduated doses to several groups of males and females. Males should be dosed during growth and for at least one complete spermatogenic cycle; females of the Parent generation should be dosed for at least two complete oestrous cycles. The animals are then mated. The test substance is administered to both sexes during the mating period and thereafter only to females during pregnancy and for the duration of the nursing period. This Test Guideline is intended primarily for use with the rat or mouse. Each test and control group should contain a sufficient number of animals to yield about 20 pregnant females at or near term. Three test groups, at least, should be used. It is recommended that the test substance be administered in the diet or drinking water. A limit test may be performed if no effects would be expected at a dose of 1000 mg/kg bw/d. The results of this study include measurements (weighing, food consumption) and daily and detailed observations, each day preferably at the same time, as well as gross necropsy and histopathology. The findings of a reproduction toxicity study should be evaluated in terms of the observed effects, necropsy and microscopic findings. A properly conducted reproduction test should provide a satisfactory estimation of a no-effect level and an understanding of adverse effects on reproduction, parturition, lactation and postnatal growth.

Reproduction toxicity – OECD 416

OECD Test No. 416: Two-Generation Reproduction Toxicity

Principles:

This Test Guideline for two-generation reproduction testing is designed to provide general information concerning the effects of a test substance on the integrity and performance of the male and female reproductive systems, and on the growth and development of the offspring. The test substance is administered daily in graduated doses to several groups of males and females.
Males and females of the Parent generation (5-9 weeks old) should be dosed during growth, during their mating, during the resulting pregnancies, and through the weaning of their first generation offspring. The administration of the substance is continued to first generation offspring during their growth into adulthood, mating and production of a second generation (until the weaning). The rat is the preferred species for testing. Each test and control group should contain a sufficient number of animals to yield preferably not less than 20 pregnant females at or near parturition. At least three dose levels and a concurrent control shall be used. It is recommended that the test substance be administered orally (by diet, drinking water or gavage). A limit test may be performed if no effects would be expected at a dose of 1000 mg/kg bw/d. The results of this study include: measurements (weighing, sperm parameters, oestrus cycle parameters and offspring parameters), clinical daily observations, as well as gross necropsy and histopathology. The findings of this two-generation reproduction toxicity

study should be evaluated in terms of the observed effects including necropsy and microscopic findings. A properly conducted reproductive toxicity test should provide a satisfactory estimation of a no-effect level and an understanding of adverse effects on reproduction, parturition, lactation, postnatal development including growth and sexual development.

Reproduction toxicity – OECD 422

OECD Test No. 422: Combined Repeated Dose Toxicity Study with the Reproduction/Developmental Toxicity Screening Test

Principles:

The test substance is administered in graduated doses to several groups of males and females. Males should be dosed for a minimum of four weeks; females should be dosed throughout the study (approximately 54 days). Normally, matings "one male to one female" should be used in this study.

This Test Guideline is designed for use with the rat. It is recommended that the test substance be administered orally by gavage. This should be done in a single dose daily to the animals using a stomach tube or a suitable intubation cannula. Each group should be started with at least 10 animals of each sex. Generally, at least three test groups and a control group should be used. Dose levels should be selected taking into account any existing toxicity and (toxico-) kinetic data available. The limit test corresponds to one dose level of at least 1000 mg/kg body weight. The results of this study include measurements (weighing, food/water consumption) and daily detailed observations (including sensory reactivity to stimuli), preferably each day at the same time, as well as gross necropsy and histopathology. The findings of this toxicity study should be evaluated in terms of the observed effects, necropsy and microscopic findings. The evaluation will include the relationship between the dose of the test substance and the presence or absence of observations. Because of the short period of treatment of the male, the histopathology of the testis and epididymus must be considered along with the fertility data, when assessing male reproduction effects.

Part 1B – Ecotoxicity Testing

1B-1: Selection of compartments and trophic levels and endpoints for ecotoxicity testing

Acute aquatic tests should always be the first choice to use if no ecotoxicity data exist, and with the use of three trophic levels:

- Primary producers (phytoplankton)
- Herbivors (*Daphnia magna* preferred organism
- Fish (e.g. rainbow trout - *Oncorhynchus mykiss*)

These tests are performed as inhibition or lethality tests.

In addition to acute aquatic tests, the test organisms representing other relevant compartments should be considered.

If a flue gas test substance is considered to resist atmospheric processes and expected to be potentially precipitated on ground by wet or dry precipitation an acute tests for soil and vegetation toxicity test should be considered. When flue gas components are precipitated in soil an acute soil test should be considered. Ecotoxicity tests for vegetation plants may also be relevant for flue gas deposition, performed as acute tests to plants. Insect tests may also be considered, but are currently not included in this Protocol.

Long-term tests may also be considered. This may be of special importance for hazard assessment, as comparison of results between acute and chronic tests.

1B-2: decisions or test methods for ecotoxicity

We suggest to include acute aquatic tests for three trophic levels as mandatory.

Additional tests should be considered if the substance is considered “toxic” to one or more of the aquatic test organisms (mandatory tests). Threshold to toxicity should be set, based on expert opinion[1]. The requirements for further testing is further substantiated if the substance appear to be persistent in the environment, judged from its biodegradability[2]

Ecotoxicity testing should be conducted according to the flow-sheet shown in Figure 6.

[1] A preliminary limit an EC-50 or LC-50 of 10 mg/l may be used for the most sensitive acute ecotoxicity test (Class I or Class II according to classification by Global Harmonisation System)
[2] See 1C-2

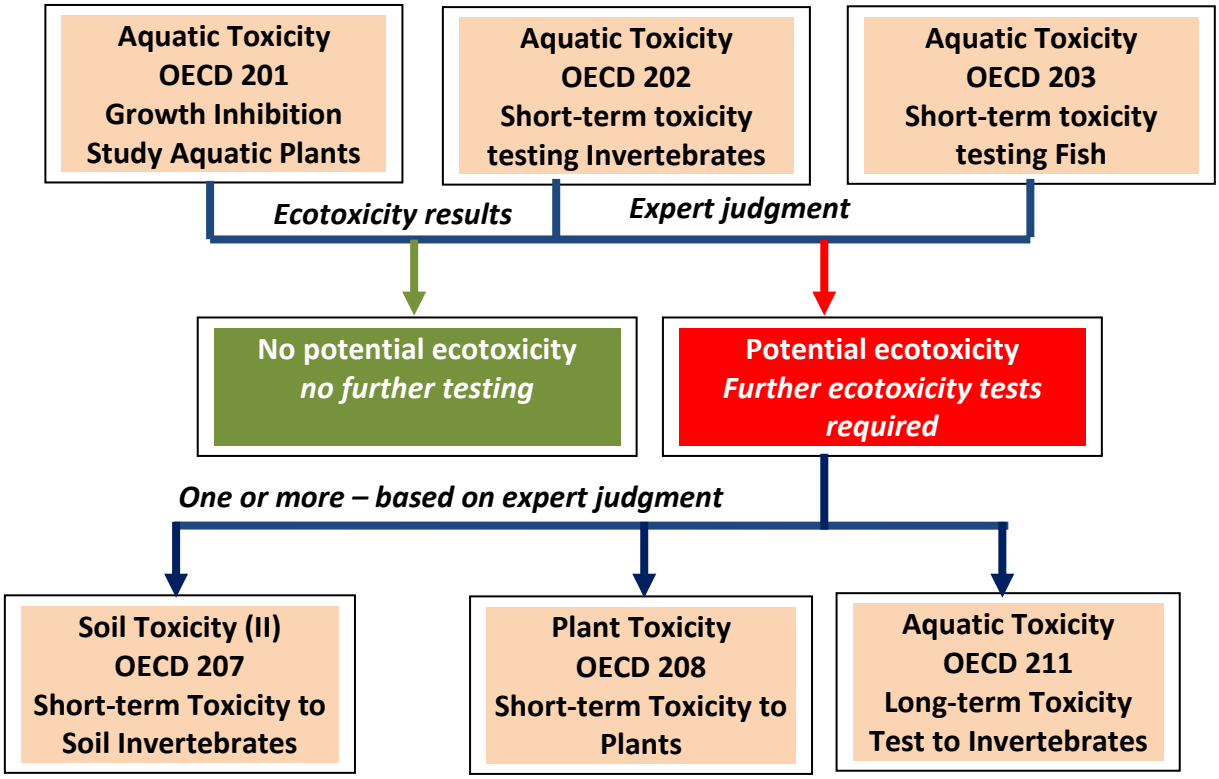


Figure 6 **Flow-sheet for ecotoxicity testing (see Footnotes 1 and 2)**

1B-3: Performance of tests for ecotoxicity

Acute aquatic ecotoxicity – OECD 201

OECD Test No. 201: Alga, Growth Inhibition Test
Principles: The purpose of this test is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria. Exponentially growing test organisms are exposed to the test substance in batch cultures over a period of normally 72 hours. The system response is the reduction of growth in a series of algal cultures exposed to, at least, five concentrations of a test substance. Three replicates at each test concentration should be used. The response is evaluated as a function of the exposure concentration in comparison with the average growth of control cultures. The cultures are allowed unrestricted exponential growth under nutrient sufficient conditions (two alternative growth media: the OECD and the AAP) and continuous fluorescent illumination. Growth and growth inhibition are quantified from measurements of the algal biomass as a function of time. The limit test corresponds to one dose level of 100 mg/L. This study includes: the determination, at least daily, of the algal biomass; the measure of the pH (at the beginning and at the end); microscopic observation. This Test Guideline describes two response variables: average specific growth rate, and yield.

Acute aquatic ecotoxicity – OECD 202

ECD Test No. 202: Daphnia sp. Acute Immobilisation Test

Principles:

This Test Guideline describes an acute toxicity test to assess effects of chemicals towards daphnids (usually Daphnia magna Staus).
Young daphnids, aged less than 24 hours at the start of the test, are exposed to the test substance at a range of concentrations (at least five concentrations) for a period of 48 hours. Immobilisation is recorded at 24 hours and 48 hours and compared with control values. The results are analysed in order to calculate the EC50 at 48h. Determination of the EC50 at 24h is optional. At least 20 animals, preferably divided into four groups of five animals each, should be used at each test concentration and for the controls. At least 2 ml of test solution should be provided for each animal (i.e. a volume of 10 ml for five daphnids per test vessel). The limit test corresponds to one dose level of 100 mg/L. The study report should include the observation for immobilized daphnids at 24 and 48 hours after the beginning of the test and the measures of dissolved oxygen, pH, concentration of the test substance, at the beginning and end of the test.

Acute aquatic ecotoxicity – OECD 203

ECD Test No. 203: Fish, Acute Toxicity Test

Principles:

The fish are exposed to the test substance preferably for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentrations which kill 50 per cent of the fish (LC50) are determined where possible.
One or more species may be used, the choice being at the discretion of the testing laboratory. At least seven fishes must be used at each test concentration and in the controls. The test substance should be administered to, at least, five concentrations in a geometric series with a factor preferably not exceeding 2.2. The limit test corresponds to one dose level of 100 mg/L. This study includes the observations of fish at least after 24, 48, 72 and 96 hours. The cumulative percentage mortality for each exposure period is plotted against concentration on logarithmic probability paper.

Soil ecotoxicity – OECD 207

ECD Test No. 207: Earthworm, Acute Toxicity Tests

Principles:

This Test Guideline includes two methods: a paper contact toxicity test and an artificial soil test. The recommended specie is Eisenia foetida (Michaelsen).
The initial screening test (filter paper contact test) involves exposing earthworms to test substances on moist filter paper in order to identify potentially toxic chemicals to earthworms in soil. Five or more treatment levels in a geometric series and, at least, ten replicates (one worm per vial) for each treatment should be used. Tests are done in the dark and for a period of 48 hours. The artificial soil test gives toxicity data more representative of natural exposure of earthworms to chemicals. It involves keeping earthworms in samples of a precisely defined

artificial soil. Five concentrations, in a geometric series, of the test substance have been applied. One concentration resulting in no mortality and one resulting in total mortality should be used. Four replicates for each treatment are recommended. Mortality is assessed 7 and 14 days after application.

Plant ecotoxicity – OECD 208

ECD Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test

Principles:

This Test Guideline is designed to assess effects on seedling emergence and early growth of higher plants following exposure to the test substance applied to the soil surface or into the soil.

Seeds are placed in contact with soil treated with the test substance and evaluated for effects following usually 14 to 21 days after 50 % emergence of the seedlings in the control group. Endpoints measured are visual assessment of seedling emergence, biomass measurements, shoot height, and the visible detrimental effects on different parts of the plant. The test can be conducted in order to determine the dose-response curve, or at a single concentration/rate as a limit test, according to the aim of the study. An appropriate statistical analysis is used to obtain effective concentration ECx or effective application rate ERx for the most sensitive parameter(s) of interest. Also, the no observed effect concentration (NOEC) and lowest observed effect concentration (LOEC) can be calculated in this test.

Long-term aquatic ecotoxicity – OECD 211

ECD Test No. 211: Daphnia magna Reproduction Test

Principles:

The test method described in this Test Guideline assesses the effect of chemicals on the reproductive output of Daphnia magna Straus. To this end, young female Daphnia are exposed to the test substance added to water at a range of concentrations (at least five). For semi-static tests, at least 10 animals at each test concentration and for flow-through tests, 40 animals divided into four groups of 10 animals at each test concentration, are used. The test duration is 21 days. Reproductive output of the parent animals and the total number of living offspring produced per parent alive at the end of the test should be reported. The study report should also include: the daily counting of the offspring, the daily recording of the parent mortality, the weekly measurement of oxygen concentration, temperature, hardness and pH values and the determination of the concentrations of test substance. Optionally, the sex ratio of the offspring may be recorded. The reproductive output of the animals exposed to the test substance is compared to that of the control in order to determine the lowest observed effect concentration (LOEC) and hence the no observed effect concentration (NOEC). In addition, and as far as possible, the data are analyzed using a regression model in order to estimate the concentration that would cause an x % reduction in reproductive output.

Part 1C – Biodegradability Testing

1C-1: Selection of degradability principles and compartments

Bodegradability testing of flue gas components should only be performed on pure substances. Tests should always be conducted to decide whether each compound is ready biodegradable or not. Test for ready biodegradability should be supplemented with test for inherent biodegradability, if necessary.

Most tests for ready and inherent biodegradability are performed with freshwater-based media and microbial inocula from municipal sewage or activated sludge. If flooding of active soil systems appear oxygen concentrations may be reduced and anaerobic conditions appear. Since aerobic and anaerobic biodegradation differs with respect to degradation mechanisms and electron acceptors, it is advisable to include anaerobic degradation, primarily as a test combining aerobic and anaerobic conditions.

Biodegradation of chemicals is concentration-dependant. Degradation rates may increase with elevated substance concentrations, but are reduced if concentrations reach toxic levels for a chemical. Biodegradation tests require test substance concentrations in ppm-levels, and toxic chemicals may inhibit biodegradation at these levels. Therefore simulation tests may be conducted with low environmentally relevant concentrations. If possible radio-labelled (e.g. ^{14}C-labelled chemicals can be used. If a simulation method is to be performed, a test for biodegradation in soil is preferable.

1C-2: Decisions or test methods for biodegradability

Biodegradability testing should be conducted according to the flow-sheet shown in Figure 7.

We suggest to include a ready biodegradation test as mandatory, if data are not available yet. If test substance is ready biodegradable (i.e. > 60 % BOD of ThOD / CO_2 evolution of $ThCO_2$ or > 70 % DOC removal) no further testing should be needed. When not ready biodegradable, the test substance should be subjected to an inherent biodegradability test and to a test for abiotic degradation (hydrolysis).

If a substance is judged as at least primary inherent biodegradable (i.e. ≥ 20 % of theoretical values) a simulation test should be considered based on “expert opinion”. Expert opinion should include an evaluation of the risk for the substance ending up in terrestrial systems, and if the substance is regarded as potentially toxic to aquatic organisms (see 1B-2). If this is realistic a soil simulated test may be conducted.

If the outcome of an inherent biodegradability test is that the test substance is not judged as at least primary inherent biodegradable (i.e. < 20 % of theoretical values), a soil simulation test should be recommended if “expert opinion” judge the

possibility for the substance to reach the soil compartment as relevant. Photo-transformation test may also be considered, although not yet included in the current Protocol.

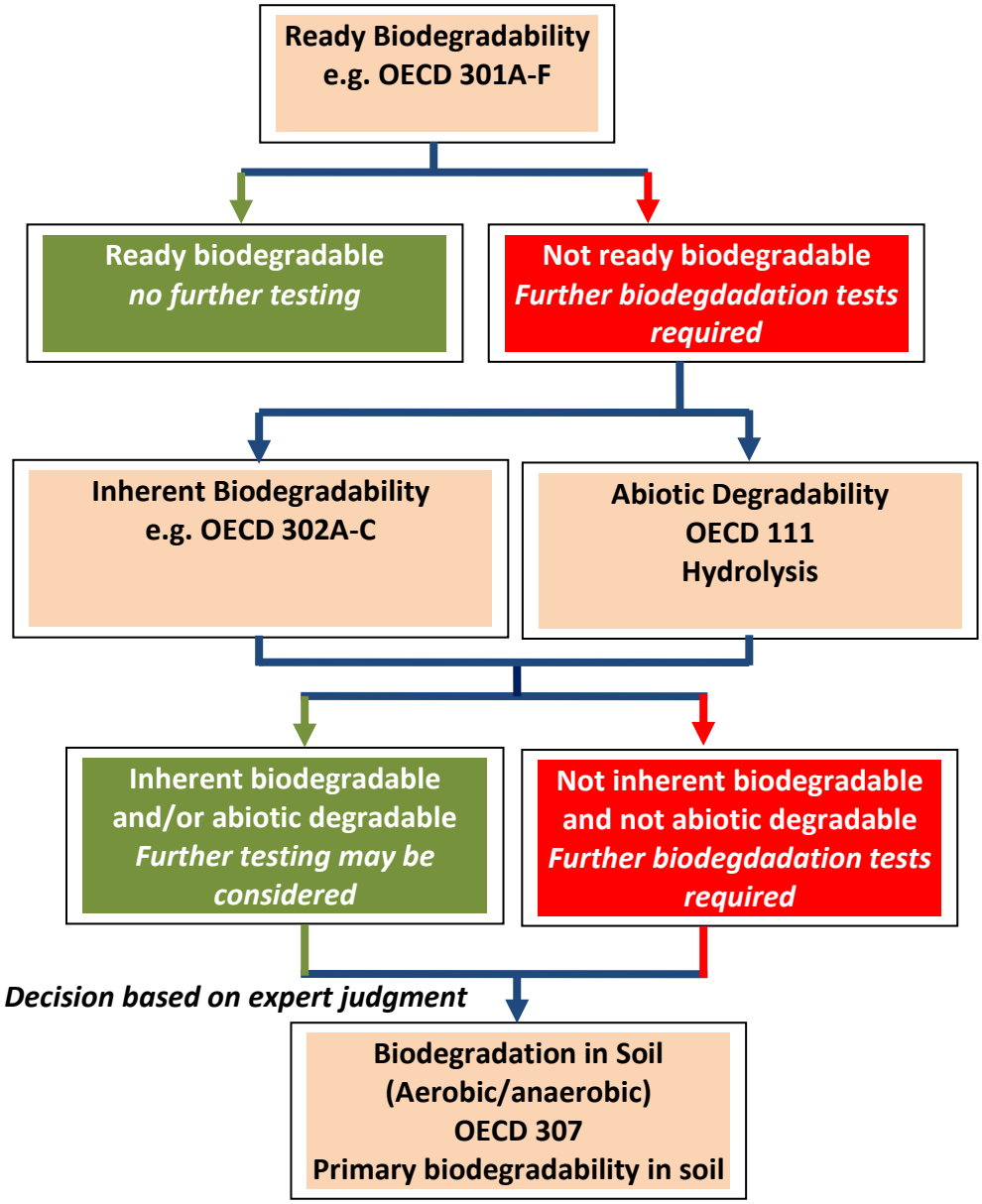


Figure 7 **Flow-sheet for biodegradability testing**

1C-3: Performance of tests for biodegradability

Ready biodegradability – OECD 301

OECD Test No. 301: Ready Biodegradability

Principles:

This Test Guideline describes six methods that permit the screening of chemicals for ready biodegradability in an aerobic aqueous medium. The methods are: the DOC Die-Away, the CO2 Evolution (Modified Sturm Test), the MITI (I) (Ministry of International Trade and Industry, Japan), the Closed Bottle, the Modified OECD Screening and the Manometric Respirometry.

A solution, or suspension, of the test substance, well determined/described, in a mineral medium is inoculated and incubated under aerobic conditions in the dark or in diffuse light. The running parallel blanks with inoculum but without test substance permits to determined the endogenous activity of the inoculum. A reference compound (aniline, sodium acetate or sodium benzoate) is run in parallel to check the operation of the procedures. Normally, the test lasts for 28 days. At least two flasks or vessels containing the test substance plus inoculum, and at least two flasks or vessels containing inoculum only should be used; single vessels are sufficient for the reference compound. In general, degradation is followed by the determination of parameters such as DOC, CO2 production and oxygen uptake. The pass levels for ready biodegradability are 70% removal of DOC and 60% of ThOD or ThCO2 production for respirometric methods. These pass values have to be reached in a 10-d window within the 28-d period of the test.

Inherent biodegradability – OECD 302A

OECD Test No. 302A: Inherent Biodegradability: Modified SCAS Test

Principles:

This Test Guideline describes a method which is an adaptation of the Soap and Detergent Association semi-continuous activated sludge (SCAS) procedure for assessing the primary biodegradation of alkyl benzene sulphonate. The test does not simulate those conditions experienced in a sewage treatment plant.

Activated sludge from a sewage treatment plant is placed in an aeration (SCAS) unit. The test compound (non-volatile, water soluble, organic, non-inhibitoring to bacteria at the test concentration) and settled domestic sewage are added, and the mixture is aerated for 23 hours. The aeration is then stopped, the sludge allowed to settle and the supernatant liquor is removed. The sludge remaining in the aeration chamber is then mixed with a further aliquot of test compound and sewage and the cycle is repeated. The above fill and draw procedure is repeated daily throughout the test. A high concentration of aerobic micro-organisms is used. The length of the test for compounds showing little or no biodegradation is indeterminate, but experience suggests that this should be at least 12 weeks. Biodegradation is established by determination of the dissolved organic carbon content of the supernatant liquor. This value is compared with that found for the liquor obtained from a control tube dosed with settled sewage only.

Inherent biodegradability – OECD 302B

OECD Test No. 302B: Inherent Biodegradability: Zahn-Wellens/ EVPA Test

Principles:

This Test Guideline describes the Zahn-Wellens/EMPA Test. It is used to determine inherent biodegradability.

A mixture containing the non-volatile and water soluble test substance, mineral nutrients and a relatively large amount of activated sludge in aqueous medium is agitated and aerated at 20-25°C in the dark or in diffuse light, for up to 28 days. Blank controls, containing activated sludge and mineral nutrients but no test substance, are run in parallel. The functional capability of the activated sludge is tested using a reference compound (ethylene glycol, diethylene glycol, lauryl sulfonate or aniline). In a typical run 1 or 2 vessels for the test suspension and for the inoculum blank, 1 for procedure control are used. The biodegradation process is monitored by determination of DOC, Dissolved Organic Carbon, (or COD, Chemical Oxygen Demand) in filtered samples, taken at daily or other time intervals. It is mandatory to follow DOC in the test suspension and inoculum blanks in parallel. The ratio of eliminated DOC (or COD), corrected for the blank, after each time interval, to the initial DOC value is expressed as the percentage biodegradation at the sampling time. The percentage biodegradation is plotted against time to give the biodegradation curve. The test is considered valid if the procedural control shows the removal of the reference compound by at least 70% within 14d and if DOC (or COD) in the test suspension is removed relatively gradually over days or weeks, since this indicates biodegradation.

Inherent biodegradability – OECD 302C

OECD Test No. 302C: Inherent Biodegradability: Modified MITI Test (II)

Principles:

This Test Guideline describes the modified MITI test (II). This test permits the measurement of the Biochemical Oxygen Demand (BOD) and the analysis of residual chemicals in order to evaluate the inherent biodegradability of chemical substances which have been found by the Standard MITI Method (I) to be low degradable.

An automated closed-system oxygen consumption measuring apparatus (BOD-meter) is used. Chemicals to be tested are inoculated in the testing vessels (six bottles with different quantities of test chemical) with micro-organisms. In order to check the activity of the inoculum, the use of control substances (aniline, sodium acetate or sodium benzoate) is desirable. During the test period, the BOD is measured continuously. Biodegradability is calculated on the basis of BOD and supplemental chemical analysis, such as measurement of the dissolved organic carbon concentration, concentration of residual chemicals, etc. The BOD curve is obtained continuously and automatically for 14 to 28 days. After the 14 to 28 days of testing, pH, residual chemicals and intermediates in the testing vessels are analysed.

Simulation test in soil – OECD 307

OECD Test No. 307: Aerobic and Anaerobic Transformation in Soil

Principles:

The method described in this Test Guideline is designed for evaluating aerobic and anaerobic transformation of chemicals in soil. The experiments are performed to determine the rate of transformation of the test substance, and the nature and rates of formation and decline of transformation products, to which plants and soil organisms may be exposed.

About 50 to 200 g soil samples (a sandy loam or silty loam or loam or loamy sand) are treated with the test substance and incubated in the dark, in biometer-type flasks or in flow-through systems under controlled laboratory conditions. The treatment rate should correspond to the highest application rate of a crop protection product recommended in the use instructions. Also untreated soil samples are incubated under test conditions. These samples are used for biomass measurements during and at the end of the studies. The rate and pathway studies should normally not exceed 120 days. Duplicate incubation flasks are removed at appropriate time intervals and the soil samples extracted with appropriate solvents, of different polarity, and analysed for the test substance and/or transformation products. Volatile products are also collected for analysis using appropriate adsorption devices. Using 14C-labelled material, the various mineralisation rates of the test substance can be measured by trapping evolved 14CO2 and a mass balance, including the formation of soil bound residues, can be established.

Abiotic degradability - Hydrolysis – OECD 111

OECD Test No. 111: Hydrolysis as a Function of pH

Principles:

This Test Guideline describes a laboratory test method to assess abiotic hydrolytic transformations of chemicals in aquatic systems at pH values normally found in the environment (pH 4 - 9). This Guideline is designed as a tiered approach; each tier is triggered by the results of the previous tier. Sterile aqueous buffer solutions of different pH values (pH 4, 7 and 9) are treated with the non-labelled or labelled test substance (only one concentration, which should not exceed 0.01 M or half of the saturation concentration). They are incubated in the dark under controlled laboratory conditions (at constant temperatures). After appropriate time intervals, buffer solutions are analysed for the test substance and for hydrolysis products. The preliminary test should be carried out for 5 days at 50 ± 0.5°C and pH 4.0, 7.0 and 9.0. The second tier consists of the hydrolysis of unstable substances, and the third tier is the identification of hydrolysis products. The higher Tier tests should be conducted until 90 % hydrolysis of the test substance is observed or for 30 days whichever comes first.

Part 1D – Hazard analyses

The terms "Hazard" has been defined by OECD and by REACH (e.g. http://guidance.echa.europa.eu/docs/guidance_document/information_requirements_en.htm). Hazard is the potential to cause harm.

1D-1: Methods for health hazard

In the present project exposure to humans may occur in an occupational setting or as a result of chemicals being dispersed to the neighbourhood of the production plant.

Exposure to the general population around an industrial facility like Mongstad is characterised by low-level continuous exposure. We therefore need to focus on compounds causing effects that may occur after long-term and low-level exposure.

A hazard classification system based on GESAMP-EHS[3] system based on Hazard analyses will be performed with data generated from mammalian toxicity methods[4].

Ranking will be used for long-term exposure based on the following hazards[5]:

- Carcinogenicity (C)
- Mutagenicity (M)
- Reproductive effects (R)
- Sensitization, primarily by inhalation (S)

The symbols C, M, R and S indicate:

C	Shown to induce or increase cancer in animals or man
M	Shown to cause increased incidence of permanent changes in the amount or structure of the genetic material
R	Shown to cause adverse effects on reproductive ability or capacity, or the development of offspring
S	Shown to be a sensitizer (skin or respiratory)

Hazard of substances should be based on literature search and quality evaluation of available data. Typical sources for health information are:

[3] GESAMP: Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection
[4] Alternatively, a hazard classification system based on the Global Harmonisation System (GHS) may be used. The GESAMP and GHS systems are nearly identical
[5] Acute toxicity (oral and dermal toxicity) will play a minor role in the final overall hazard assessment.

RTECS: http://ccinfoweb.ccohs.ca/rtecs/search.html
IUCLID data sheet: http://ecb.jrc.ec.europa.eu/esis/
GESAMP-list: http://www.imo.org/includes/blastDataOnly.asp/data_id%3D25672/Report-BLGCirc.29annex6doc.pdf
GESAMP background info:
http://www.gesamp.org/publications/publicationdisplaypages/rs64
CPDB: http://potency.berkeley.edu/chemicalsummary.html
EPA-IRIS: http://www.epa.gov/ncea/iris/index.html
Toxnet: http://toxnet.nlm.nih.gov/index.html
CCRIS: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CCRIS
Hazmap: http://hazmap.nlm.nih.gov/
SciFinder: http://www.cas.org/products/scifindr/index.html
PubMed: http://www.ncbi.nlm.nih.gov/pubmed

A GESAMP classification system is shown below (Table 1).

Table 1 Hazard system according to GESAMP

Column Label	Column heading	Explanation
C1	**Oral toxicity LD50 rating codes**	0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw
C2	**Percutaneous toxicity LD50 rating codes**	0: >2000 1: 1000-2000 2: 200-1000 3. 50-200 4: <50 mg/kg bw
C3	**Inhalation toxicity LC50 4 hours exposure rating codes**	0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)
D1	**Skin irritation / Corrosion**	0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive 3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min
D2	**Eye irritation / Corrosion**	0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury
D3	**Long term effects**	Full description of rationale for rating given at bottom of table. Short form rating code: C: Shown to induce or increase cancer in animals or man M: Shown to cause increased incidence of permanent changes in the amount or structure of the genetic material R: Shown to cause adverse effects on reproductive ability or capacity, or the development of offspring S: Shown to be a sensitizer
	GESAMP/EHS rating	GESAMP ratings for each column (C1, C2, C3, D1. D2, D3) and date.
	Expert Judgement	A summary expert opinion on the chemical is given in the comments column. For oral/dermal/inhalation the numbers in respective columns indicate: • Negligible toxicity: 0 • Slight toxicity: 1 • Moderate toxicity: 2 • Moderately high toxicity: 3 • High toxicity: 4 Ratings in brackets: Provisional ratings based on limited or no data. Expert judgment. OEL: Occupational exposure level (Taken from RTECS: Lowest Scandinavian values. If not available then lowest OECD countries values are taken). ***Conclusions and recommendations written in italic bold***

When data are not available from reliable sources testing should be considered (see Part 1A).

1D-2: Hazard assessment methods as part of health risk evaluation

If urgent need to develop a preliminary risk assessment a hazard profile may be developed for a group of substances, based on worst case criteria. We suggest to use "occupational exposure limits" (OELs) taken from the lowest Scandinavian values. If OEL do not exist for a compound a "no otherwise specified" (NOS) approach may be used. We have not performed any quality assessment of the toxicity data which is used for the basis of OEL. For instance if OEL for a nitrosamine has not been specified the toxicology profile for a compound nitrosamine (NOS) is the worst case entry for each endpoint of toxicology testing that we have been able to obtain. We suggest to use assessment factor of 100, i.e. the lowest OEL should be divided by 100.

If a good dataset of Derived No-effect levels (DNEL), or preferably Derived Minimal-effect levels (DMEL) is available, a non-threshold approach may be selected. In that case a large assessment factor of 10,000 is appropriate. A dose descriptor (e.g. carcinogenicity as endpoint) is selected, and a toxic dose (e.g. TD50) is divided on the assessment factor for determination of DNEL/DMEL.

These approaches are used as part of a risk evaluation, and they do not represent a full risk assessment, since exposure concentrations are not available

1D-3: Methods for hazard assessment as part of environmental risk evaluation

For environmental hazard evaluation we suggest that a predicted no-effect concentration (PNEC) is used. This system is a well established system and recommended for environmental hazard and risk analyses by the European Communion, as described in the Technical Guidance Documents (TGD)(http://ecb.jrc.ec.europa.eu/tgd/).
A PNEC requires reliable ecotoxicity data from at least three trophic levels with aquatic organisms (Part 1B). The source of information for ecotoxicity data may be:

IUCLID data sheet: http://ecb.jrc.ec.europa.eu/esis/
ECOTOX: http://cfpub.epa.gov/ecotox/
SciFinder: http://www.cas.org/products/scifindr/index.html

If reliable acute toxicity data from three trophic levels are not available ecotoxicity tests should be performed, preferably with species representing all three trophic levels.

Based on available results from ecotoxicity information a PNEC may be defined according to the following rules:

1. Select the EC-50 from the acute test with the highest toxicity result (most sensitive organism) and define a PNEC by the use of an assessment factor as shown in Table 2 (1000)
2. If reliable ecotoxicity data are available from long-term chronic test(s), use the results from one these tests to establish PNEC (see Table 2), based on no-effect concentrations (NOEC)

Table 2 Assessment factors (AF) for ecotoxicity tests

AVAILABLE DATA	ASSESSMENT FACTOR
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, Daphnia and algae)	**1000**
One long-term NOEC (either fish or Daphnia)	**100**
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae	**50**
Long-term NOECs from at least three species (normally fish, Daphnia and algae)	**50**
Species sensitivity distribution (SSD) method 5-1	**to be fully justified case by case**
Field data or model ecosystems	**Reviewed on a case by case basis**

On the bases of the information in Table 2 PNEC should be determined as follows:

1. If data from only acute aquatic toxicity tests are available an AF of 1000 should be used to determine PNEC from the test with the highest ecotoxicity
2. If PNEC in other soil or sediment compartments is to be determined, ectoxicity data from the relevant compartment may be considered for PNEC determination, even if some of aquatic tests show higher ecotoxicity
3. If data from a long-term ecotoxicity test exist the data from this test should be used for PNEC-determination and with an AF or 100.
4. If data from two or more long-term ecotoxicity test exist and these represent at least two different trophic levels, an AF of 50 should be used for the test showing the highest ecotoxicity.

These approaches are used as part of a risk evaluation, and they do not represent a full risk assessment, since exposure concentrations are not available

Part 1E – Risk assessment

Risk assessment analyses include the combination of toxicity data with the predictions of exposure concentrations. Determination of exposure concentrations combine emission data, modelling of spreading and dispersion in the environment and predictions of fate processes for individual flue gas compounds.

We do not recommend risk evaluation until methods for estimation of exposure concentrations are available.

PART 2

Protocol for testing of single substances

Overview of Test Protocol Part 2

This part of the test protocol describes the decisions for testing of components expected to be part of CO_2-capture emissions. Included in this part are the approaches on how to select flue gas substances for hazard evaluation, data sources for obtaining hazard/provisional risk assessment, selection for further testing, choices of testing methods, and how to use data for hazard assessment. A flow-chart is shown in Figure 8.

The test protocol will consist of several steps (with reference to Figure 8):

Step 2A: Selection of chemicals
Step 2B: Collection of available health and environmental data
Step 2C: Provisional hazard assessment
Step 2D: Selection of test methods for mammalian toxicity
Step 2E: Selection of test methods for ecotoxicity
Step 2F: Selection of test methods for biodegradation testing
Step 2G: Final hazard assessment

Limitations

This outcome of the procedures described in Part 2 of the Protocol will be data for hazard assessment of individual components in a flue gas emission mixture. Chemical or toxicological interactions between chemicals will not be measured, but may be judged by expert opinions.

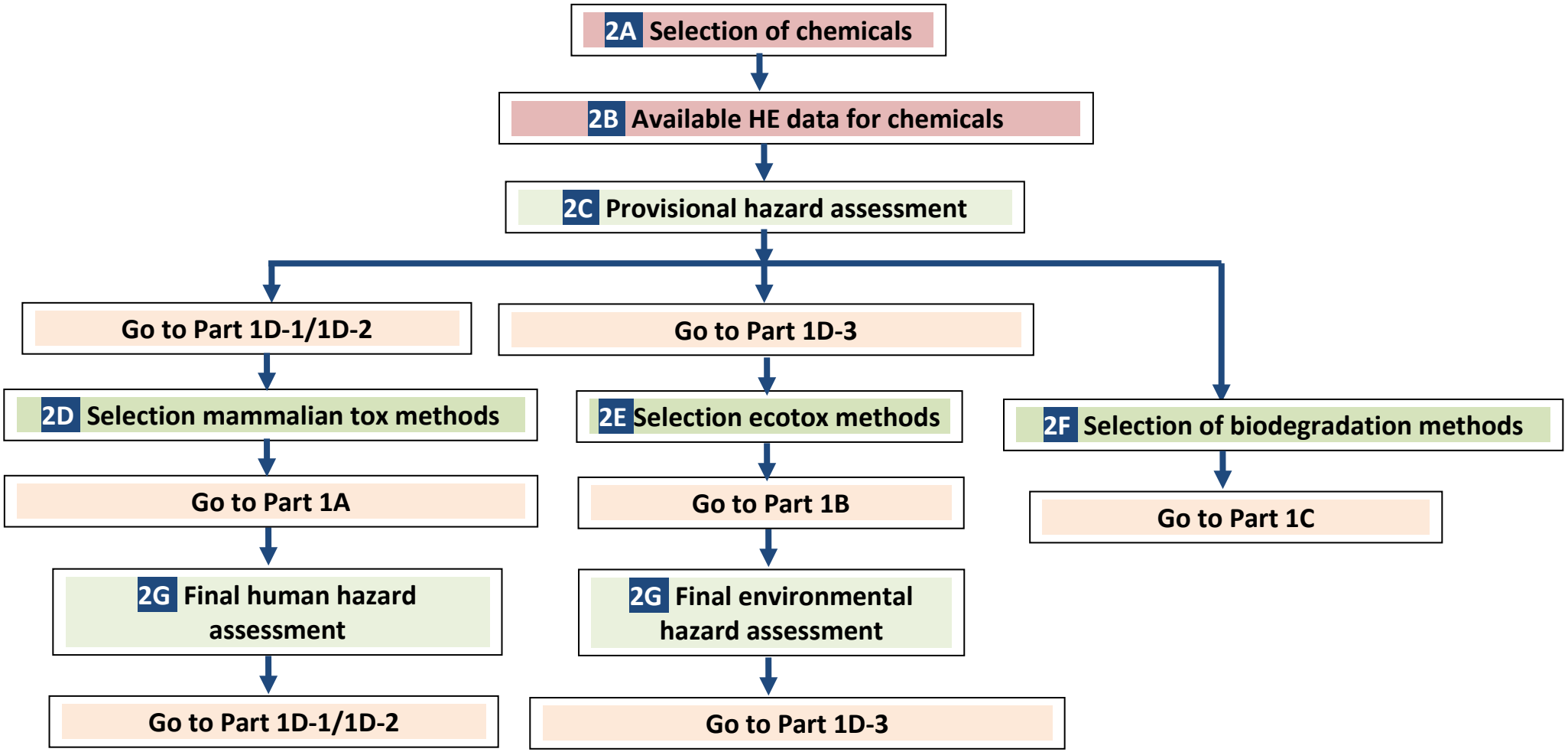


Figure 8 *Overall outline of toxicity testing of single test substances. For numbering of boxes, see text in Part 1 and Part 2.*

Principles for decisions

In part 2 of the Protocol the procedures for toxicological characteristics of individual chemicals expected to appear in CO_2-capture emission samples will be described. This characterisation will depend on selection of appropriate chemicals, based on relevant information from expected emission compounds related to capture technology (solvent and capture technology, flue gas cleaning processes, etc.), information from relevant flue gas emission campaigns, and/or expected emission products based on laboratory data and other experimental information.

Available health and environmental information will be collected for selected test substances, and based on this information a test program shall be planned. Procedures for selection and performances of methods for mammalian toxicity, ecotoxicity and biodegradation are described in Part 1 of this Protocol.

Information from health and environmental tests will be used for evaluations of hazard and risk of individual flue gas substances. Recommended hazard and risk approaches are described in Part 1 of this Protocol. Both for health and environmental risk the outcome from hazard/risk analyses of single compounds may be used indirectly for predictions of the risk(s) associated with different flue gas solvent and capture technologies, as well as gas cleaning processes.

Health, Safety and Environment (HSE)

Several of the chemicals associated with flue gas emissions are of health, safety and environmental concern. Nitrosamines and nitramines are potential carcinogenic chemicals. Amine compounds are in general irritating and corrosive, and some of them may have sensitizing characteristics.

The chemicals must therefore be handled with strict care; all work should be done in fume cupboards. Special care should be taken when storing and handling of nitrosamines and nitramines, following the strictest precautions recommended in the MSDS.

Due to the explosive characters of nitramines specific risk analyses should be performed before handling of these chemicals.

Test Protocol for singe test substances

PART 2A – Selection and characterisation of chemicals

2A-1: Selection of chemicals

Lists of documented and potential emission products must be provided. These lists should be provided from -

1. Available data from emission campaigns
2. Data from published experimental studies
3. Theoretical studies

Expected emission concentrations with relevant CO_2-capture technology should be estimated, based on a) emission campaigns, b) judgments of data from laboratory studies or test facility units, and c) estimated from theoretical studies (if possible). Decisions based on b) and c) should be conducted by "expert opinions"

All potential emission products should be considered for hazard evaluation, but a ranking of substances should be made, as described below (Part 2B).

PART 2B – Collection of available health and environmental data

Available health and environmental (HE) studies should be collected for each chemical. Data should be collected based on CAS numbers, since one chemical may be described by several chemical or generic names. The quality of the data should be judged by "expert opinions". Relevant Sources for HE-data are shown in Part 1 1D-1 and 1D-3.

Chemicals should be grouped according to availability of HE-data as follows –
1. HE information acceptable.
2. HE information inadequate.
3. No HE information available.

In the case of 2 and 3 suppliers of chemicals may be contacted to ask for additional non-published data. REACH may also be consulted (through the European Chemical Agency; ECHA) to check for registration dates and further information. Further, HE-data from chemically related compounds may be considered. Lack of experimental data may be supplemented by estimated toxicity data based on structure-activity relationship (SAR), although SAR data should be treated with great care. Methods for SAR-estimations are described by Joint Research Centre of the European Commission for mammalian toxicology and ecotoxicology (several computational tools) and by US EPA for ecotoxicity and degradation (EPISUITE).

Based on expert opinions the chemicals should be ranked as described below. SAR estimations should be replaced by data from experimental studies.

Based on available HE-information and potential emissions a ranking system should be established for toxicity testing of chemicals, based on the following criteria:

1. High concentrations possible in emissions and potential effects related to mutagenic, carcinogenic and/or reproduction toxicity
2. High concentrations possible in emissions and potential for persistence, bioaccumulation and/or high ecotoxicity
3. Low concentrations probable in emissions and potential effects related to mutagenic, carcinogenic and/or reproduction toxicity
4. Low concentrations probable in emissions and potential for persistence, bioaccumulation and/or high ecotoxicity
5. High concentrations possible in emissions, but no potential effects related to mutagenic, carcinogenic or reproduction toxicity, and no potential for persistence, bioaccumulation or ecotoxicity
6. Low concentrations probable in emissions, but no potential effects related to mutagenic, carcinogenic or reproduction toxicity, and no potential for persistence, bioaccumulation or ecotoxicity

Chemicals ranked within categories 1 to 4 should be tested if relevant HE-data are lacking or judged to be of inadequate quality (expert opinion).

PART 2C– Provisional hazard assessment

Based on the information collected as described above a provisional hazard assessment can be performed.

For hazard assessment based on available data consult section 1-D1 and/1D-2 (human health hazard as part of provisional risk analyses) and 1-D3 (hazard assessment as part of provisional environmental risk analyses).

If decided that essential information for human health or environment are not available a testing program should be planned. By “not essential” information the following are included:

1. Inadequate data for mutagenicity/genotoxicity/carcinogenicity
2. Inadequate data for reproduction toxicity
3. Inadequate data for ecotoxicity
4. Inadequate data for degradability

“Inadequate data” may be interpreted as a) data lacking, b) quality of data poor or uncertain, or c) other insufficient information as judged by expert opinion.

Planning of test programs are shown below (section 2D).

PART 2D– Selection of mammalian toxicity methods

The selection of mammalian toxicity methods will depend on the available information and the judgment of the quality of this information.

Methods for long-term effects should be prioritised, i.e. mutagenicity/genotoxicity/carcinogenicity and reproduction toxicity.

The test requirements, flow-sheets and test methods for these methods are described in Part 1A – Mammalian Toxicity Methods.

Data from mammalian toxicity tests of single chemicals should be used for characterisation of the hazard associated with the chemical, i.e. if the chemical is mutagenic, carcinogenic and/or toxic to reproduction, and for a provisional risk assessment based on non-threshold or worst case approach, depending on the toxicity data.

PART 2E– Selection of ecotoxicity tests

The selection of ecotoxicity methods will depend on the available information and the judgment of the quality of this information.

Chemicals with inadequate data for ecotoxicity should be tested in at least three trophoc levels of acute aquatic tests (phytoplankton, herbivores, fish).

The test requirements, flow-sheets and test methods for these methods are described in Part 1B – Ecotoxicity Methods.

Data from ecotoxicity tests of single chemicals should be used for characterisation of the potential risk associated with the chemical, i.e. determination of PNEC.

PART 2F– Selection of biodegradation tests

The selection of biodegradation methods will depend on the available information and the judgment of the quality of this information.

Chemicals with inadequate data for biodegradation should be tested for at least ready biodegradability.

The test requirements, flow-sheets and test methods for these methods are described in Part 1C – Biodegradation Methods.

For risk assessment biodegradation may be used as input data for PEC-determination in the soil compartment. The procedures for this are described in TGD PEC for SOIL compartment (depending on discharges via air and aquatic compartments.

For bioaccumulation testing, see Footnote[6].

PART 2G– Hazard assessment for provisional risk evaluation

A final hazard assessment will include the combination of the data from provisional hazard assessment (1D-1 - 1D-3) with the data from health and environmental testing (see 1A and 1B).

2G-1: Health hazard and provisional risk evaluation

As part of a risk assessment each flue gas compound should be classified according to the GESAMP-EHS classification system (Table 1). Ranking will be used for long-term exposure based on the following hazards: carcinogenicity (C), mutagenicity (M), reproductive effects (R), sensitization, primarily by inhalation (S)(see 1D-1).

Data should then be used for determination of a non-threshold and/or worst case approach should be a used as part of a provisional risk evaluation (1D-2).

2G-2: Environmental hazard evaluation

Final hazard assessment should be used to determine the PNEC of the compound based on data from the most sensitive test organism. The PNEC should be determined based on acute or chronic toxicity test(s) with the assessment factors described in Table 2 (1D-3).

[6] Experiences have shown that flue gas components all exhibit low bioaccumulation potentials. Testing for bioaccumulation potentials and for bioconcentration in biological tissue is not regarded necessary

SINTEF

Appendix B Test Report – System Suitability Test for cold trap condensation of volatile emission compounds

Appendix B Sampling and analyses of flue gas emissions

B1 Operational conditions

The operational conditions been described in the ITT and further information have been given by the Company (Table B.1).

Table B.1 Nominal conditions and composition in and out of the absorber

Conditions	[A)] From Flue Gas Cooler	[B)] From absorber
Flow (normal)		400 kg/s
Pressure	1.01 bar	1.01 bar
Temperature	20-45°C	25-50°C
Main body velocity		2.0-3.0 m/s
Exit velocity after water wash		20 m/s
Composition		
CO_2	3.4 mol%	0.6 mol%
N_2	76 mol%	81.5 mol%
O_2	13.8 mol%	13.8 mol%
H_2O	6.8 mol%	3 mol%
NOx	3 ppmv	2-20 ppmv
NH_3	2 ppmv	< 50 ppmv
Amines		[B)] < 5 ppmv
[C)] Monoethanolamine		??
[C)] N-nitrosodimethylamine		0.5 ppmv (gas phase)
[C)] N-nitrosodiethanolamine		1 ppmv (solvent concentration)
[C)] Nitrosomorpholine		0.1 ppmv (solvent concentration)
[C)] Dimethylnitramine		0.5 ppmv (gas phase)
[C)] Ethanolnitramine		10 ppmv (solvent concentration)
[C)] Methylnitramine		0.5 ppmv (gas phase)
[C)] Methylamine		0.5 ppmv (gas phase)
[C)] Dimethylamine		0.5 ppmv (gas phase)

[A)] *Information from the ITT TQP Amine 3*
[B)] *Wittgens, 2010*
[C)] *Information provided by Company (kick-off meeting)*

B.2 Basis for sampling

The basis for the sampling strategy is to collect cleaned gas removed from the absorber and use these samples for selected toxicity tests. This will require manual sampling combined with one or more methods for sample conservation before toxicity testing.

The strategy for isokinetic sampling has been described in a separate report in TQP Amine 1 (Wittgens, 2010), and only a brief background will be given here.

As stated in the ITT the sampling method should be able to collect representative emission extracts containing gaseous, liquid phases and aerosols/particulates. The complete emission (including entrained droplets and evaporated substances) and compound groups of interest are primarily amines, ammonia, aldehydes, amides, alkylamines, nitrosamines and nitramines, with specific focus on alkylamines, nitrosamines and nitramines. Due to low concentrations of these compounds, large sampling volumes me be needed.

B3 Standardised methods for manual sampling

Standardised methods for manual sampling have been described in a separate report submitted in TQP Amine 1 (Wittgens, 2010). In brief, this report describes the background for sampling from stationary sources, the design of a representative measurement location, and a generic design for measurement site. As a background for the report, some essential issues in the report are summarised here.

In order to collect representative samples these are taken at ambient temperature (see Table B.1). Sample collections can be performed in a series of generally cooled impingers, and different component groups are collected by appropriate absorbents (acids, solvents) or absorbing tubes for further processes. For toxicity testing we may expect to include large sampling volumes, and absorbents cannot be used since non-destructed samples are needed for toxicity tests. Adsorbents may be used if non-destructive desorption is possible without affecting original toxicity of the flue gas samples.

Flue gases in a stack may be inhomogeneous due to stratification or swirling caused by duct design and geometry (Figure B.1). Therefore average concentrations and velocities at several evenly spaced measurement points across a measurement plane need to be determined, performed as grid measurements over a measurement plane (see Figure B.2) and several measurement planes used (Figure B.3).

Since the flue gas is inhomogeneous it is necessary to remove the sample stream isokinetically, i.e. with the same velocity as the main stream in the duct (Figure B.4). If the main stream velocity is higher than in the sampling probe ($w>v$) sample concentrations may be lower than in the main stream, while a probe velocity higher than the main stream velocity ($w<v$) may result in too high sample concentrations in the probe (Figure B.4).

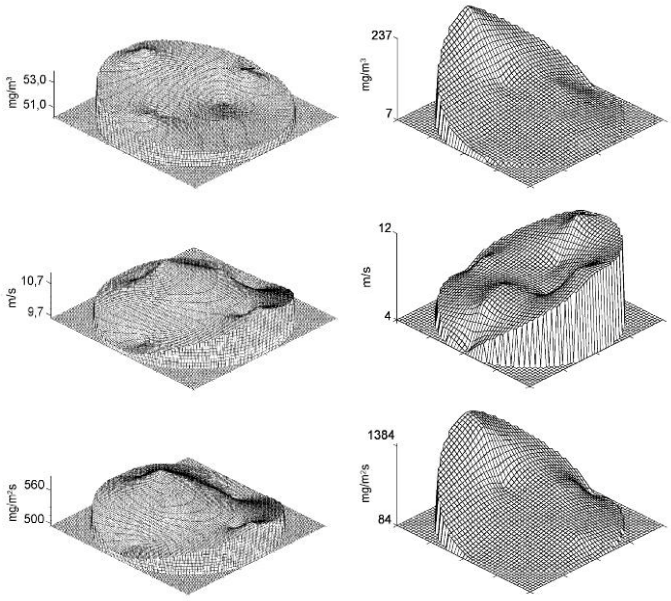
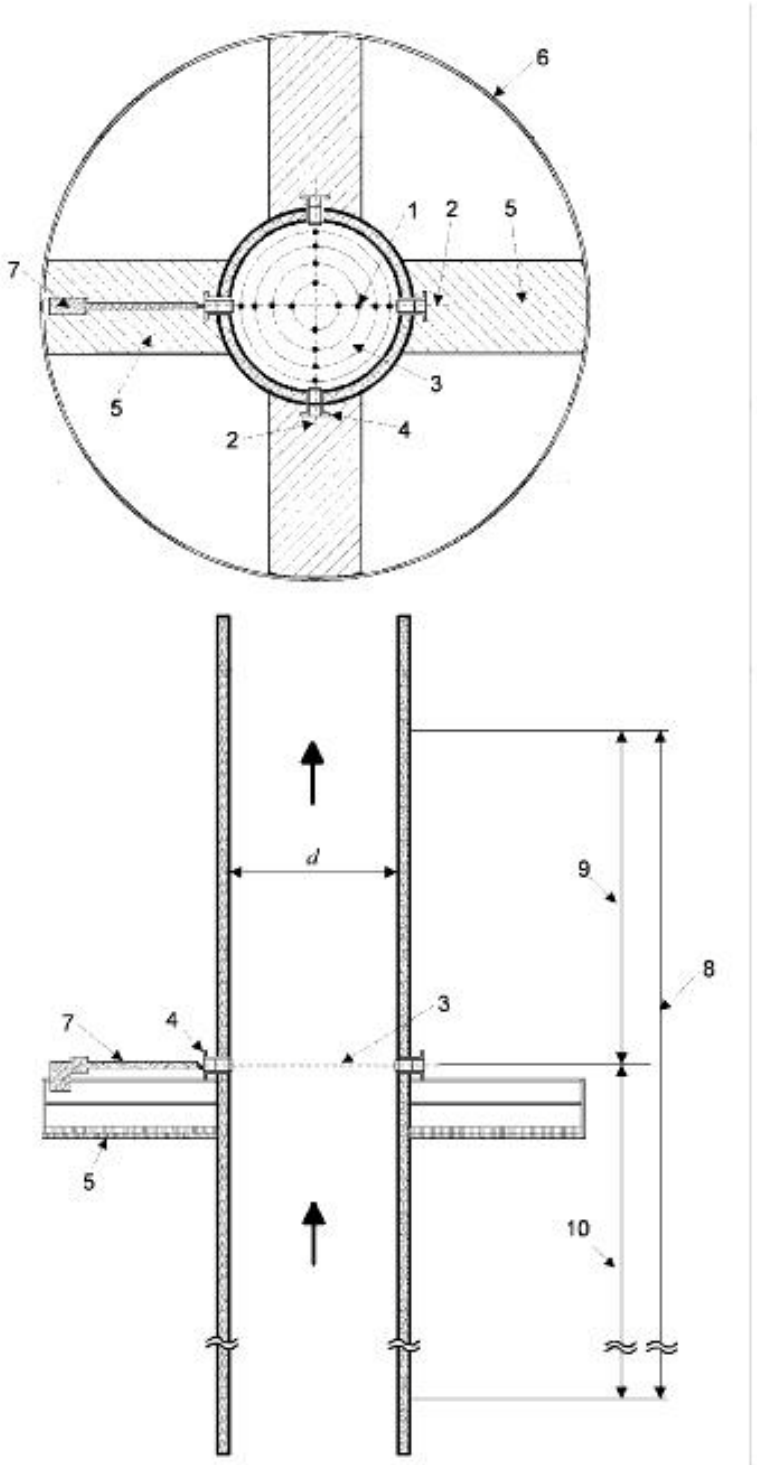


Figure B.1 Example of homogeneous (left) and inhomogeneous (right) profiles of mass concentration, velocity and mass flow density (reference DIN EN 15259.)



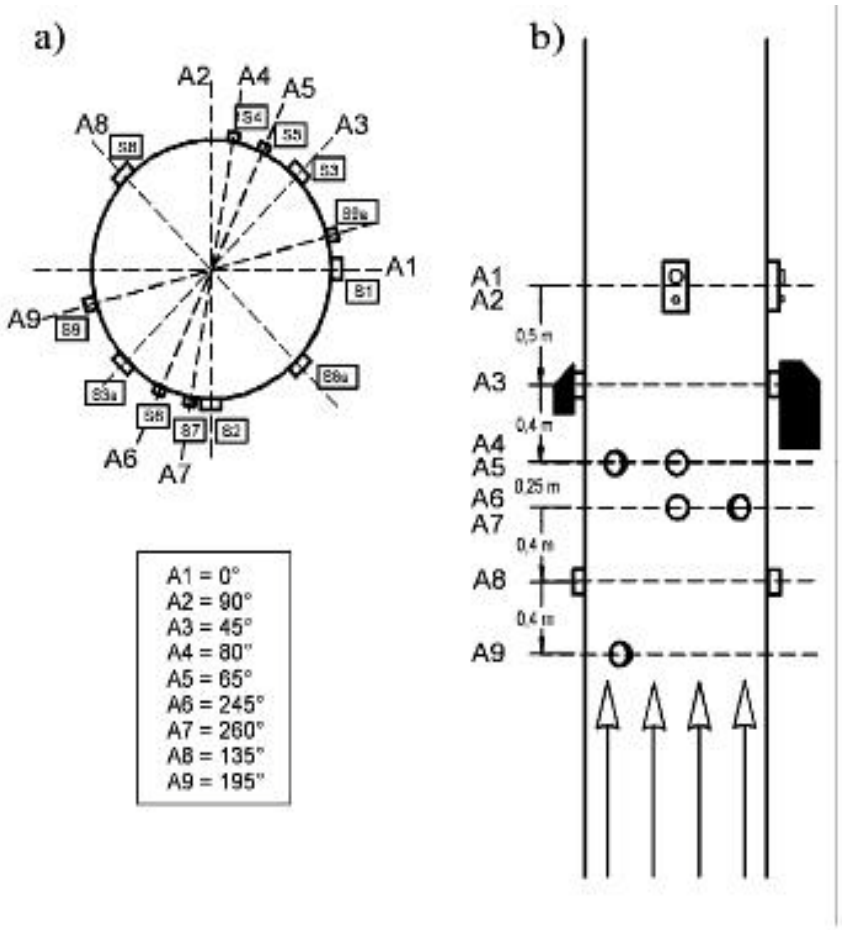
Key

1	measurement point
2	measurement line
3	measurement plane
4	measurement port
5	clearance area
6	measurement site
7	manual sampling train
8	measurement section
9	outlet section
10	inlet section

Symbols

d	internal duct diameter

Figure B.2 Illustration of measurement site and measurement section for full scale plant (reference DIN EN 15259).



Key

a) top view b) front view

Figure B.3 Sampling ports located in several measurement planes (reference DIN EN 15259)

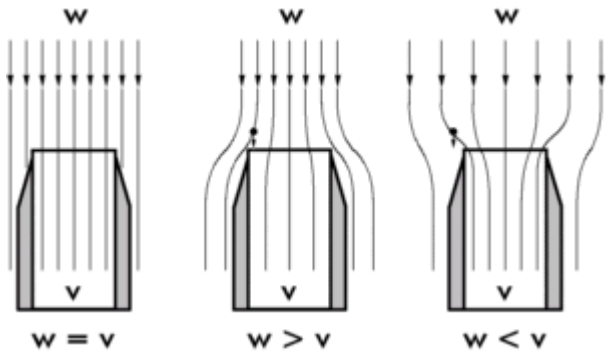


Figure B.4 Iso-kinetically sampling of gas with droplets / particles (drawing from http://www.photometer.com/en/abc/abc_052.htm)

Sampling material should be designed in corrosion resistant material. Possible corrosion may be caused by amines, organic acids from amine degradation, SO_2, CO_2 and NH_3. Material could be stainless steel, PTFE or rubber-coated steel.

B.4 Recovery and analyses

A number of analyses must be conducted prior to sampling and of the samples. Some of the relevant analyses and relevant validated methods are shown in Table B.2. Of specific interest is the analyses of potential hazardous compounds, which primarily are considered to organic compounds. However, it is important also to overview methods for sampling, and dust and metals are often included as part of stationary flue gas emission sampling regime.

For particle/dust sampling the dust in a gas sample collected with isokinetically flow-rate and measured volume is separated by a pre-weighed plane filter system, which is then dried and re-weighed. The filtering device can be placed inside or outside the duct (Figure B.5). The filters should be able to collect > 99.5 % of test aerosols with mean particle diameters of 0.3 µm. For most cases quartz fibre or PTFE filters have proven efficient. However, quartz fibre filters may have weak mechanical characteristics, while PTFE filters should not be exposed to temperatures above 230°C (EN 13284-1: 2002).

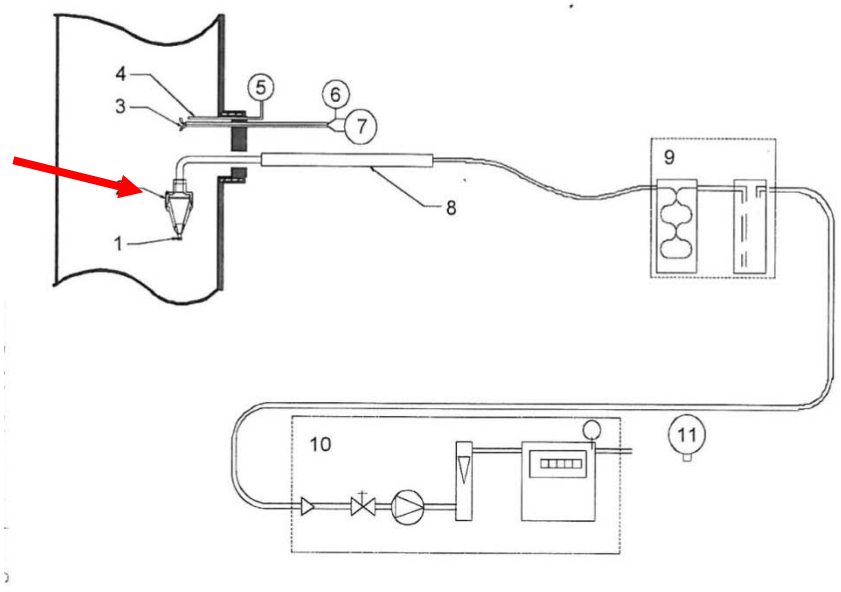
For heavy metal analyses flue gas should be isokinetically sampled, with dust collected in a filter (preferably PTFE), and the gas stream should then passed through a series of absorbers with absorption solutions. Both filters and absorption solutions are used for analyses (Figure B.6). Typical absorption system includes a series of three absorbers with solution of nitric acid/hydrogen peroxide, while filters are digested by a mixture of hydrofluoric acid and nitric acid (EN 14385:2004). Analyses are typically performed as AAS, ICP-OES or ICP-MS). Sampling and recovery should be performed so a detection limit of 1 $\mu g/m^3$ for individual elements is achieved. Field evaluations showed that absorption efficiencies for different elements were between 39 and 91 % (EN 14385:2004).

For sampling of organic components in flue gas a number of validated methods are available (Table B.2). The standards EN 1948, parts 1 to 3 describe sampling, extraction/recovery and analyses of polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The samples collected isokinetically

adsorbed or on particles are collected in a sampling train. There is a choice between three different test systems:
1) A filter/condenser method with an in-stack filter (e.g. quartz wool filter), with a downstream condenser (gas cooled down to < 20°C) and/or a solid adsorber.
2) A dilution system where the gas is collected with a heated probe, cooled rapidly down (< 40°C) with dry filtered air, and after air dilution PCDDs/PCDFs collected as particulates on filters, while gaseous PCDDs/PCDFs are collected on solid adsorbents.
3) Cooled probe method in which the sample passes a water-cooled probe (< 20°C) and the condensate is collected in a condensate flask, while filters and impingers and/or solid adsorbents and filters are used for collecting small particles, breaking aerosols and collecting gaseous PCDDs/PCDFs. The methods are shown schematically in Figure B.7). Typical filters used are quartz/quartz wool or glass fibre, while recommended solid adsorbers include XAD-2, polyurethane foam, or Porepak PS (EN-1948-1: 2006).

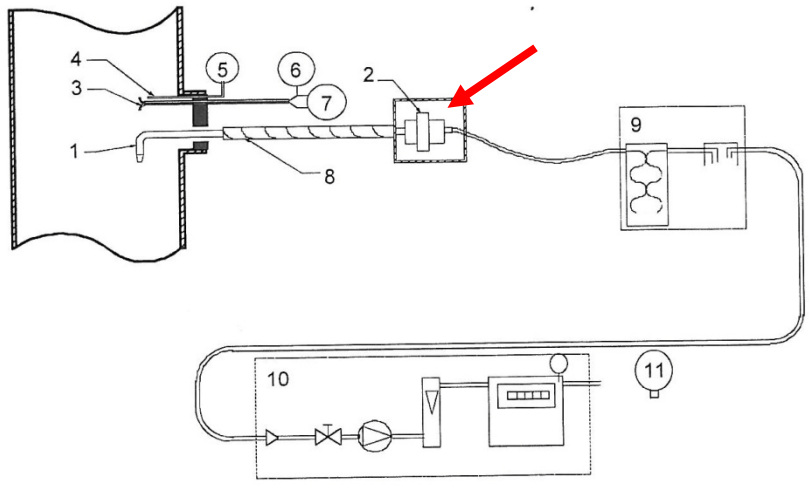
Table B.2 Flue gas sampling and analyses - relevant validated methods.

Samples / Analyses	Methods	References
Gas Flow Velocity	Standard Test Method for Average Velocity in a Duct (Pitot Tube Method) Stationary source emissions – Measurement of velocity and volume flowrate of gas streams in ducts	ASTM 3154-00 ISO 10780
Particulates	Stationary source emissions. Determination of low range mass concentration of dust. Automated measuring systems Stationary source emissions – Determination of low range mass concentration of dust – Part 1: Manual gravimetric method Particulate matter measurement. Dust measurements in flowing gases. Gravimetric determination of dust load Standard Test Methods for Sampling and Determination of Particulate Matter in Stack Gases Standard Test Method for Determination of Mass Concentration of Particulate Matter from Stationary Sources at Low Concentrations (Manual Gravimetric Method)	EN 13284 EN 13284-1:2005 VDI 2066 Part 1: 2006 ASTM D3685 / ASTM3685M-98 ASTM 6331-98
Metals	Emissions from stationary sources – Determination of the total emissions of As, Cd, Cr, Co, Cu, Mn, Ni, Pb, Sb; Tl and V Measurement of the total emissions of metals, semi-metals and their components - Manual measurement in flowing and emitted gases – Sampling system for particulate and filterable substances	EN 14385: 2005 VDI 3868-1: 1994
Organic compounds	Emissions from stationary sources – Determination of the mass concentrations of PCDD/PCDF and dioxinelike PCBs – Part 1: Sampling Emissions from stationary sources – Determination of the mass concentrations of PCDD/PCDF and dioxinelike PCBs – Part 2: Extraction and cleanup Emissions from stationary sources – Determination of the mass concentrations of PCDD/PCDF and dioxinelike PCBs – Part 3: Identification and quantification Stationary source emissions – Determination of the mass concentration of individual gaseous organic compounds – Activated carbon and solvent desorption method Determination of gaseous emissions – Measurement of aliphatic and aromatic aldehydes and ketones using the DNPH-procedure – Gas wash-bottle method Stationary source emissions – Determination of gas and particle-phase polycyclic aromatic hydrocarbons – Part 1: Sampling Stationary source emissions – Determination of gas and particle-phase polycyclic aromatic hydrocarbons – Part 2: Sample preparation, clean-up	EN 1948-1: 2006 EN 1948-2: 2006 EN 1948-3: 2006 EN 13649: 2002 VDI 3862-2: 2000 ISO 11338-1; 2003 ISO 11338-2; 2003



Key

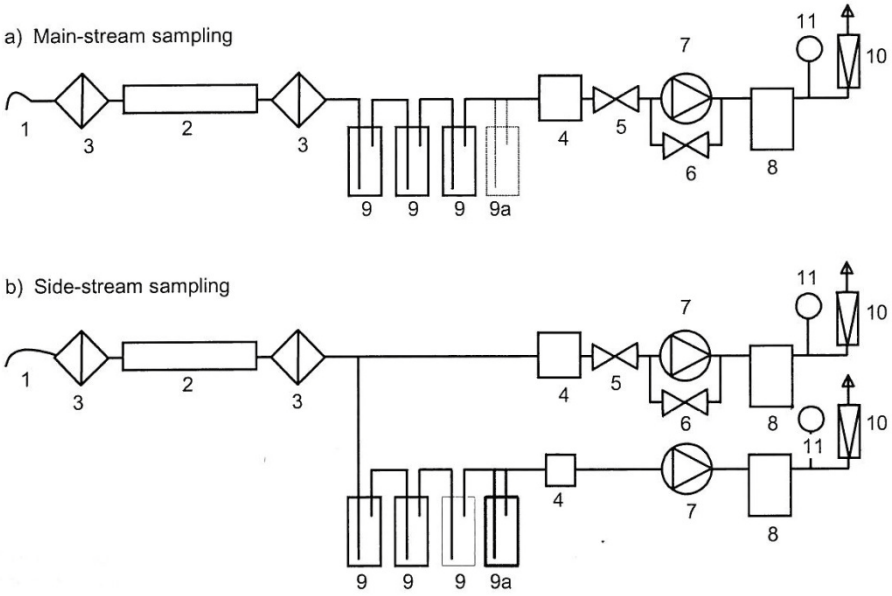
1 Entry nozzle
2 Filter holder
3 Pitot tube
4 Temperature sensor
5 Temperature indicator
6 Static pressure measurement
7 Dynamic pressure measurement
8 Support tube („in stack" device)
9 Cooling and drying system
10 Suction unit and gas metering device
11 Pressure gauge



Key

1 Entry nozzle
2 Filter holder
3 Pitot tube
4 Temperature sensor
5 Temperature indicator
6 Static pressure measurement
7 Dynamic pressure measurement
8 Suction tube („out-stack" device)
9 Cooling and drying system
10 Suction unit and gas metering device (see Figure 5)
11 Pressure gauge

Figure B.5 Examples of "in-stack" (upper) and "out-stack" (lower) dust filter sampling systems (Source: EN 13284-1:2004)



Key

1	Nozzle	7	Pump
2	Probe	8	Gas volume meter
3	Filter (either behind or in front of the probe)	9	absorber
		9a	Safety bottle (optional)
4	Dryer	10	Gas flow meter
5	Main-stream valve	11	Temperature and pressure measurement
6	By-pass valve		

Figure B.6 Isokinetic sampling system for heavy metal analyses (Source: EN 14385:2004).

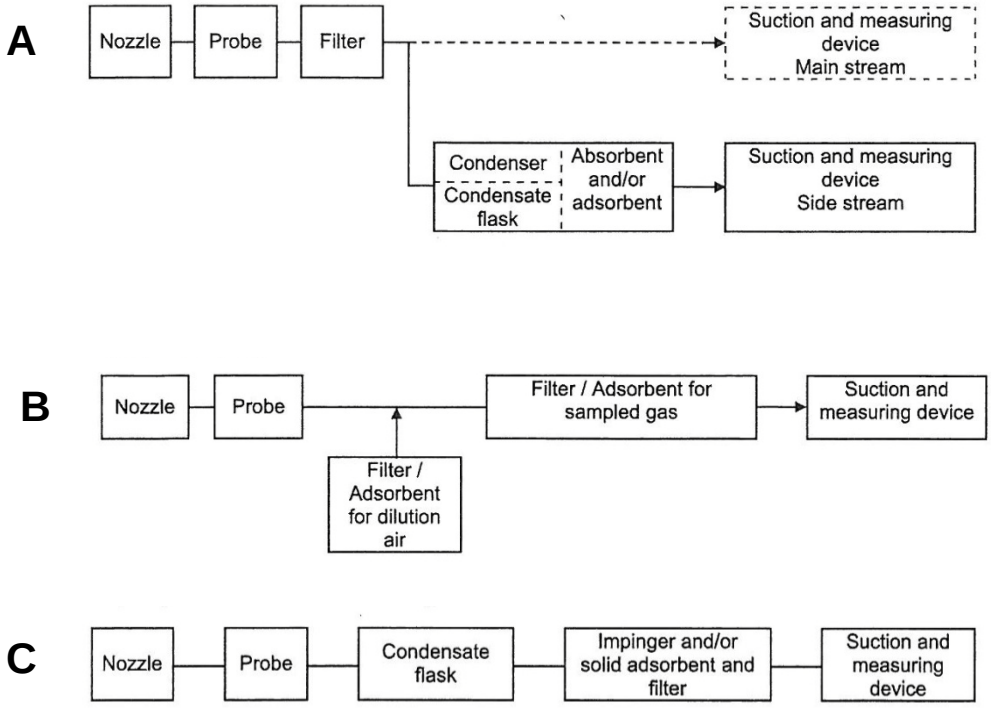


Figure B.7 Schematic presentations of filter/condenser method (A), dilution method (B) and cooled probe method (C) for polychlorinated compounds (Source: EN 1948-1: 2006).

Sampling and analyses of gas and particle-phase PAH are described in the ISO standards 11338-1 and 11338-2. Sampling may be performed isokinetically by one of three methods regarded as equivalent: 1) Dilution method, 2) the heated filter/condenser/adsorber method, and 3) the cooled probe/adsorber method. These are the same methods as described for the PCDDs/PCDFs sampling methods above (EN 1948-1: 2006), and as shown schematically in Figure B.7. The methods are suitable for different flue gas characteristics as shown in Table B.3. Typical filter units for particle trapping are glass fibre, quartz wool cartridge or cyclone, while adsorber materials are polyurethane foam and/or XAD-2

Table B.3 Applicability of dilution method, the heated filter/condenser/adsorber method, and the cooled probe/adsorber method for PAH sampling at different flue gas characteristics (Adopted from ISO standards 11338-1).

Flue gas characteristics	Dilution	Heated filter	Cooled probe
Temperature, °C	< 800	< 800	< 800
H_2O content, g/cm^3	< 600	< 500	< 300
Particulate matter conc., g/m^3	< 5	< 2	< 2
PAH concentration, $\mu g/Nm^3$	0.04 to 10 000	0.1 to 6000	0.002 to 30 000

The standard methods VDI 3862 consist of a series of 7 Guidelines for sampling and analyses of aldehydes, ether as total aldehydes /ketones or as individual components. Part 1 gives instructions for measurements of short-chain aldehydes by 3-methyl-2-benzothiazolinone hydrazone (MBTH) for total aldehyde determination. This method is particularly suitable for measurements of formaldehyde emissions. Parts 2 and 3 present methods for aliphatic and aromatic aldehydes and ketones to be measured as individual components. The substances are converted to corresponding hydrozones in impingers or cartridges with 2,4-nitrophenylhydrazine (DNPH), and the components can be directly analysed by HPLC-methods. Part 4 describes a formaldehyde determination method, in which formaldehyde is converted in an alkaline medium (AHMT). Part 5 describes a specific method for determination of lower aldehydes, where the components are converted to corresponding oxazolidines i tubes with 2-(hydroximethyl) piperidine (2-HMP) on fixed sorption material. After desorption (toluene), individual components are analysed by GC. Part 6 presents a acetylacetone method for formaldehyde determination in exhausts gas by wet-chemical photometric or fluorometric analyses. Part 7 describes a method to measure aliphatic and aromatic aldehydes and ketones individually, where the substances are converted to corresponding hydrozones in impingers with a hydrochloric DNPH solution.

In summary, all standard methods described above required some destructive methods for trapping of target analytes. A summary of these methods are shown in Table 5.4. Particulate compounds trapped on filters are subsequently destructed with acid for metal analyses. Filter materials and solid sorbents used for sampling organic components are extracted in solvents for concentration and analyses of organic components (e.g. diethylether/n-hexane or other solvents). Condensates are extracted with n-hexane. Samples are then concentrated before analyses (e.g. ISO11338-2:2003). Alternatively components in flue gas samples may be specifically converted to target analytes which can be directly analysed to high specificity (e.g. VDI 3862). Additional methods of relevance for analyses of target components from absorber flue gas include sampling for analyses of amines, ammonia, amides, alkylamines, nitrosamines and amines. All compound groups required the use of absorbants or solid adsorbents with subsequent solvent desorption (Table 5.4).

A valuable information source for extraction of specific compounds from gas samples:

http://www.osha.gov/dts/sltc/methods/toc_m.html

Table B.4 Methods for collection of inorganic and organic components in flue gas samples

Components	Particulate phase	Gaseous phase	Reference
Metals	Quartz fiber/PTFE filters	HNO_3/H_2O_2 absorber	EN 13284-1:2002
PCDD/PCDF	Quartz wool filters	Condensate (<20°C) and XAD-2/PU/Porepak PS adsorbents	EN1948-1:2006
PAH		Condensate (<20°C) and XAD-2/PU	ISO 1138-1
Aldehydes/ ketones	Glass fibre/ quartz wool filters	DNPH-conversion to hydrazones	VDI 3862-2
Ammonia	Glass fibre/ quartz wool filters	H_2SO_4 absorber	VDI 2461-2
Amides (acetamide)	Filter[A)]	Water absorber	DS/EN 689
Alkylamines (methylamine)	Filter[A)]	XAD-7 adsorber coated with NBD chloride in tetrahydrofuran	http://www.osha.gov/
Nitrosamines	Filter[A)]	Thermosorb-N/ Ascorbic acid-impregnated filters	Isconlab, internal procedures
Amines (MEA)	Filter[A)]	XAD-2 adsorber coated with NITC	MEL-20
	Filter[A)]		

[A)] *Filter quality not described*

B.5 Analytical and preparative sampling of volatile amines in a cold trap – A concept study

Background

The normal function of a cold trap mounted first in a sampling train is to remove water from the sampled flue gas before adsorption on a sampling tube. It has been observed that a volatile nitrosamine (NDMA) is trapped together with water in a cold trap.

Idea

The idea is that water soluble compounds will follow water in the trapping process on a cold trap. If correct, this principle can be used to develop an analytical and a preparative sampling procedure for other water-soluble and volatile compounds. Cold trap sampling will allow for sampling without a sampling medium like acidic absorption solutions or solid sampling tubes that eventually will interfere with the sample or experiments that are going to be performed with the sample. Thus, a clean and undiluted sample will result that easily can be stored at liquid nitrogen for subsequent analysis and/or experiments.

Aim of study

The aim of the study was to verify that the concept will work and to get rough preliminary data on the trapping ratio of compounds. These data will be very useful for the further work in H&ETQP Amine 1, Subtask 2 and H&ETQP Amine 3, Activity 01. The volatile compounds studied was ammonia and the alkylamines; methylamine, ethylamine and dimethylamine. All these compounds are relevant in flue gas sampling.

Experimental

The test mixture consisted of 1 mg/mL of ammonia and 10 ug/mL of each of the alkylamines methylamine, ethylamine and dimethylamine. Cold trapping was performed in an empty gas washing bottle at minus 20 and minus 75. A midget impinger with 20 mL 0.1 N H2SO4 absorption solution was mounted behind the cold trap in the sampling train. The concentrations of amines were analyzed in the water trapped in the cold trap and in the absorption solution by GC-MS after derivatization. The total amounts of amines were calculated in the trapped water and the absorption solution.

Results

The results from experiments performed at minus 20 and minus 70 ºC are shown in the Table B.5. Results are given as percentage of total amount of amine trapped by the cold trap and by the absorption solution.

Table B.5-1 Experiment performed at minus 20 °C:

Analyte	Amount in cold trap (percent)	Amount i adsorption solution (percent)
Ammonia	16	84
Methylamine	60	40
Ethylamine	54	46
Dimethylamine	64	36

Table B.5-2 Experiment performed at minus 75 °C:

Analyte	Amount in cold trap (percent)	Amount i adsorption solution (percent)
Ammonia	71	29
Methylamine	92	8
Ethylamine	85	15
Dimethylamine	92	8

Conclusions

The main conclusions are that: 1) the most volatile amines (ammonia and alkylamines) can be trapped by the cold trap, 2) alkylamines are trapped more efficiently than ammonia, and 3) the trapping ratio of compounds for the cold trap is increased with lowered temperature.

The experiment has given the information that was missing both for TQP ID 1 and 3.

SINTEF

Appendix C Report from NTNU/NILU: Emission Compound Toxicity – Human/mammalian toxicity

Appendix C:

Human / mammal toxicity – endpoints and hazard data

Contents

1 Human / mammal toxicity – endpoints and hazard data

1.1 Endpoints

1.1.1 Carcinogenic and Mutagenic effects (C and M)

NILU thoroughly investigated the mutagenic and carcinogenic effects of amine 3 chemicals. For this toxicity, results from individual studies were divided into two broad categories: mutagenic (genotoxic) and carcinogenic. A separate toxicity form (Table 1) was prepared for each chemical. A complete list can be found in Appendix H.

Name of the chemical	
Chemical Group	
CAS Number	
LD50 (mg/kg b.w.)	
Mutagenicity /Carcinogenicity	
Mutagenicity	**Toxnet:** 1) Model (result) 2) Model (result) **RTECS:** 1) Model/animal (result / #studies) 2) Model/animal (result / #studies) Comments/details **HSDB:** Comments/details
Carcinogenicity	**Toxnet:** 1) Model (result) 2) Model (result) **RTECS:** 1) Model/animal (result / #studies) 2) Model/animal (result / #studies) Comments/details **HSDB:** Comments/details
Additional Info	
Conclusions	
RTECS	
ICLUID	
GESAMP	
Current project summary sheet	
Current project conclusion	
Current project further work	

Table 1: Mutagenic/carcinogenic toxicity form

1.1.2 Reproductive effects (R)

NTNU carefully investigated the reproductive effects of amine 3 chemicals. For reproduction toxicity, results from individual studies were divided into three broad categories: male/female reproduction capacity, fetotoxicity and fetal development. A separate reproduction toxicity form (Table 2) was prepared for each chemical where number in bracket referred to study the report sequence in database. A complete list of reproduction toxicity forms for each chemical is provided in Appendix H.

Name of the chemical	
Chemical Group	
CAS Number	
Oral LD50 (mg/kg b.w.)	
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** Results from studies (sequence) **IUCLID:** Results from studies (sequence)
Fetotoxicity	Same as above
Fetal development	Same as above
Additional Info	
Conclusions	
RTECS	
IUCLID	
GESAMP	
Current project summary sheet	
Current project conclusion	
Current project further work	

Table 2: Reproduction toxicity form

1.1.3 Sensitization (S)

NTNU carefully investigated the sensitization effects of amine 3 chemicals. For sensitization, results from individual studies from RTECS were categorized into inhalation and percutaneous studies. For IUCLID, studies were divided into four broad categories: Invalid, sensitizing, non-sensitizing and ambiguous; with type of species being included as a subcategory. The different categorization for RTECS and IUCLID was done since RTECS provides studies which are positive only according to their criteria.

A separate sensitization form (Table 3) was prepared for each chemical where number in bracket referred to study the report sequence in database. Appendix A provides a complete list of sensitization forms for each chemical.

Name of the chemical	
Chemical Group	
CAS Number	
Oral LD50 (mg/kg b.w.)	
Sensitization	
Studies	**RTECS:** Inhalation: Percutaneous **IUCLID:** **Invalid** **Sensitizing** (species as subcategory, e.g. Guinea pig, Mice, Human) **Non sensitizing:** (species as subcategory, e.g. Guinea pig, Mice, Human) **Ambiguous** (species as subcategory, e.g. Guinea pig, Mice, Human)
Additional Info	
Conclusions	
RTECS	
IUCLID	
GESAMP	
Current project summary sheet	
Current project conclusion for S	
Current project further work for S	

Table 3: Sensitization form

1.2 Data for hazard assessment

1.2.1 Mutagenicity and Carcinogenicity data

The genotoxicity-mutagenicity/carcinogenicity data on each chemical were assessed (Table 4). Based on the assessment and the conclusions candidates for further testing were proposed. A complete list of mutagenicity and carcinogenicity data for each chemical is provided in Appendix H.

Chemical Group	Name of the chemical	CAS Numbers	Conclusions and recommendations
Amin	MEA	141-43-5	**Mutagenicity** **Toxnet** reports negative Ames tests (21 st), TOXNET, equivocal for Tryptophan reverse gene mutation assays (2 st). **RTECS** considers is as a mutagen. It was positive for several Human Lymphocyte SCE tests and cytogenic analysis, but the articles are unavailable (Biological Journal of Armenia). **IUCLID** reported tests are all negative. **Cacrinogenicity** No data reported on **Toxnet** or **RTECS.** ➔ **We recommend further testing on both genotoxicity and cancerogenicity to confirm M and C.***
NH3	NH3	7664-41-7	**Mutagenicity** **Toxnet** reported negative Ames tests (10 st) and tryptophan reverse gene mutations in E.coli (2 st). **RTECS** reported positive (?) responses in one Rec assay in E.Coli and in one rodent cytogenetic assay, but the articles are very old and unavailable (Biological Journal of Armenia). **Carcinogenicity** **Toxnet** reported it as a tumor promoter for nitrosoguanidine in rat; stomach tumor and stomach adenocacinoma . **RTECS** classifies it as tumorigen, with no references. ➔ However, since it is a common chemical, we recommend no further testing.
Aldehydes	Formaldehyde	50-00-0	**Mutagenicity** **Toxnet** reports no conclusion in Cytogenetic assays, HPRT mutations and UDS assays in mammalian cells,

			but positive response for SCE in vitro (3st), lower eukaryote tests (3st), prokaryote tests (1 st)and in insect tests (1 st). **RTECS** reports many positive (?) assays in human cells (10 st), mammalian cells (40 st), lower eukaryotes (3 st), insects (7 st) and prokaryotes (16st). **Carcinogenicity** **Toxnet** classified under IARC Group 1 - Carcinogenic to humans (**CPDB:** TD50, 1.35 mg/kg/day in Rat). ➔ **We recommend no further testing as C and M are confirmed.**
	Acetaldehyde	75-07-0	**Mutagenicity** **Toxnet** reported it as positive for in SCE in vivo, SCE in vitro (3 st) and Rec assay. **RTECS** reported many positive(?) tests in human cells (13 st), mammalian cells (23st), lower eukaryotes(4 st), insects (2 st) and prokaryotes (2st). **Cacrinogenicity** **Toxnet** classifies it as Cancerogenic. **IARC** classifies it as Group 2B - probably carcinogenic to humans (**CPDB:** TD50, mg/kg/day: 153^{m}, Rat, oral). ➔ **We recommend no further studies since C and M are confirmed.**
Amides	Acetamide	60-35-5	**Mutagenicity** **Toxnet** reported no mutagenicity in Ames tests (5 st), but positive for one in vivo micronucleus test that has later been questioned. **RTECS** reports positive (?) mutagenicity with different tests (5 st). **PubMed** reports two newer articles that conclude negative mutagenicity based on Ames tests, DNA repair tests and Micronucleus tests. **Carcinogenicity** **Toxnet** classifies it as Cancerogenic. **IARC** classifies it as Group 2B - probably carcinogenic to humans (**CPDB**: TD50, mg/kg/day: 180^{m} , Rat, oral) ➔ **We recommend no further studies.**
Alkylamines	Methylamine	74-89-5	**Mutagenicity** **Toxnet** reported negative Ames tests (16 st) and tryptophan reverse mutation tests (4 st) but positive TK mutation mammalian test (1 st). **RTECS** and **IUCLID** report positive rat dominant lethal test and mammalian somatic mutation test (?).

			Carcinogenicity **Toxnet and RTECS** report no data. ➔ **We recommend further studies on carcinogenicity to confirm C.**
	Dimethylamine	124-40-3	**Mutagenicity** **IUCLID and TOXNET** reported equivocal results in Ames tests (2 positive, 12 negative). **RTECS and IUCLID** reports positive results for cytogenic analysis (1 st) and SCE assay (1st). **Carcinogenicity** **Toxnet:** classified following **IARC Group 4 -** Not classifiable as a human carcinogen ➔ **No further testing is recommended.**
Nitrosamines	N-nitroso-dimethylamine	62-75-9	**Mutagenicity** **Toxnet** reported positive in Ames, CHO test , HPRT and TK, and Tryptophan gene mutation assays, in vitro Micronucleus, SCE , Lower eukaryotes-gene mutation, Rec assay and Sex-linked recessive lethal gene mutation. Equivocal for in vitro chromosomal aberrations and for in vivo micronuclei. No conclusion in dominant lethal test, spot test, and gene mutation tests. **RTECS** reported positive (?) tests in human cells (32 st), mammalian studies (130 st), lower eukaryotes (11 st), insects (8 st) and prokaryotes (15 st). **Carcinogenicity** **Toxnet** classified it as cancerogenic. **IARC** classified it as Group 2A - Probably carcinogenic to humans. (**CPDB:** TD50, mg/kg/day: 0,0959 mv, Rat, oral). ➔ **We recommend no further testing to confirm C or M.**
	N-nitrosodiethanolamine	1116-54-7	**Mutagenicity** **Toxnet** reported positive and negative results in Ames tests (34 st) and no conclusions for cytogenetic assays (micronucleus test and chromosome aberrations). RTECS reports positive results in 4 of 5 mammalian cell studies (5 st), and positive (?) results in lower eukaryotes (1 st), insects (1 st) and prokaryotes (3 st). **Carcinogenicity** **Toxnet** classified it as cancerogenic. **IARC** classifies it

			as Group 2B - Probably carcinogenic to humans. (**CPDB:** TD50, mg/kg/day: 3,17 [mv], Rat, oral) ➔ **We recommend no further testing to confirm C or M.**
	N-nitrosomorpholine	59-89-2	**Mutagenicity.** **Toxnet** show positive and negative response in Ames, positive in CHO, UDS, SCE, chromosomal aberration, micronucleus test in vitro and on recessive lethal gene mutation. **RTECS** reports positive (?) results in human cells (8st), mammalian cell studies (28 st), lower eukaryotes (5 st), insects (2 st) and prokaryotes (9 st). **Carcinogenicity** **Toxnet** classifies it as cancerogenic. IARC classifies it as as Group 2B - Probably carcinogenic to humans (**CPDB**: TD50, mg/kg/day: 0,109 [m] , Rat, oral) ➔ **M and C are confirmed, no further testing is recommended.**
Nitraminies	Dimethylanitramine	4164-28-7	**Mutagenicity** **Toxnet** reports both negative and positive Ames tests (2 st). **RTECS** reports positive Ames tests (2 st). **PubMed** literature reports positive results in one Ames test, and in various human and mammalian cells causing DNA damage. **Carcinogenicity** **Toxnet** reports two positive studies. **PubMed** reports 4 positive studies. **RTECS** reports 4 positive studies, and classifies it as carcinogen and tumorigen. Both male and female rats show a number of tumors in nasal cavity, respiratory tract, spinal cord and nerves, GIT, liver, kidney, RES, endocrine and mammary glands. **CPDB:** TD50, mg/kg/day: 0,547 [mv] , Rat, oral. ➔ **We recommend no further testing, C and M are confirmed.**
	Ethanolnitramine	74386-82-6	**Toxnet and RTECS;** no data on mutagenicity or carcinogenicity. ➔ **We therefore conclude that there is no data available and recommend it as a candidate for testing.**

	Methylnitramine	598-57-2	**<u>Mutagenicity</u>** **Toxnet** negative Ames tests (2 st). **PubMed** negative Ames tests (2st). **RTECS and PubMed** positive results in different assays detecting DNA damage in human and mammalian cells (2 st). **<u>Carcinogenicity</u>** **Toxnet and RTECS:** One positive study: nervous system cancers in rats, spinal cord and spinal nerve, female and male. Considered as carcinogen and tumorigen by **RTECS** criteria. **PubMed;** one positive study: neurogenic tumors oft he lumbar region of the spine in rats. **CPDB:** TD50, mg/kg/day: 17,4 ^{m} , Rat, oral. **Toxnet**: Tumorigenic: TDLo, 76 mg/kg/2Y (continuous) Rat, oral. ➔ **We recommend further testing for genotoxicity to confirm M.**

Table 4: Mutagenicity (genotoxicity)/Carcinogenicity data for amine 3 chemicals. m = There is more than one positive experiment in the species, and TD50 values from each positive experiment are used in the calculation of the reported, v = Variation is greater than ten-fold among statistically significant (two-tailed p<0.1) TD50 values from different positive experiments, TD50 = Median Toxic Dose; tumorigenic dose rate 50%, TDLo = Toxic Dose Low; lowest published toxic dose. * It should be stressed that though we suggest MEA for further testing (due to lack of knowledge,) this is temporary decision as further check with ongoing REACH dossiers which are still not known should be done.

1.2.2 Reproduction toxicity data

The reproduction data on each chemical was assessed (Table 5) and based on this, conclusions were made and candidates for further testing were proposed. A complete list of reproduction toxicity data for each chemical is provided in Appendix H.

Chemical Group	Name of the chemical	CAS Numbers	Conclusions and recommendations
Amin	MEA	141-43-5	RTECS has classified it as reprotoxic (R). Studies from IUCLID confirm maternal effects and developmental effects in some studies but not all. From the above studies we concluded that it has slight maternal toxicity but no fetotoxic or developmental toxicity. We therefore conclude that it is as no R and recommend no further testing.
NH3	NH3	7664-41-7	RTECS indicated that there is a lack of reproductive and developmental toxicology studies for this chemical. In addition, IUCLID states that no guideline test assessing teratology or reproductive effects in laboratory animals has been reported. We therefore conclude that there is lack of reproduction toxicity studies and limited data. However, since it is a common chemical, we recommend no further testing.
Aldehydes	Formaldehyde	50-00-0	RTECS classified it as R. Several studies in IUCLID indicated fetotoxicity but no statistically significant teratogenic effects. We therefore conclude that it has mainly fetotoxic effects but may exhibit maternal and developmental toxicity as well. We recommend no further testing if considered as a confirmed R.
	Acetaldehyde	75-07-0	RTECS classfied it as R. Stuides from IUCLID indicate no paternal toxicity studies. However, teratogenic and developmental effects are reported. We therefore conclude that it has mainly fetotoxic and developmental effects and confirm it as R. We therefore recommend no further testing.

Amides	Acetamide	60-35-5	RTECS data reported post-implantation mortality, specific developmental abnormalities in musculoskeletal system and fetotoxicity. IUCLID did not provide any chemical data sheet. We therefore concluded that there is lack of data on parental toxicity but studies indicate fetotoxic and developmental toxic effect. We therefore confirm it as R and recommend no further testing.
Alkylamines	Methylamine	74-89-5	No reprotox data from RTECS. IUCLID studies on reproduction, developmental and teratogenecity showed negative results on these parameters. We therefore confirm it as no R and recommend no further testing.
	Dimethylamine	124-40-3	No reprotox study is reported by RTECS. In IUCLID several studies indicating no developmental toxicity. We therefore confirm that it as no R and recommend no further testing.
Nitrosamines	N-nitrosodimethylamine	62-75-9	RTECS gave a range of studies indicating pre and post-implantation mortality, fetotoxicity, fertility, developmental abnormalities. From IUCLID no chemical data sheet is available. We therefore conclude that it mainly fetotoxic but may have developmental and paternal toxicity. We therefore confirm it as R and recommend no further testing.
	N-nitrosodiethanolamine	1116-54-7	RTECS has reported no data on reprotoxicity. From IUCLID no chemical data sheet is available. We therefore conclude that there is no data available and recommend it as a candidate for testing.
	N-nitrosomorpholine	59-89-2	RTECS has reported no data on reprotoxicity. From IUCLID no chemical data sheet is available. We therefore conclude that there is no data available and recommend it as a candidate for testing.
Nitramines	Dimethylanitramine	4164-28-7	RTECS has reported no data on reprotoxicity. The chemical was not found in IUCLID. We therefore conclude that there is no data available and recommend it as a candidate for testing.

	Ethanolnitramine	74386-82-6	RTECS has no data at all. The chemical was not found in IUCLID. We therefore conclude that there is no data available and recommend it as a candidate for testing.
	Methylnitramine	598-57-2	RTECS has no data at all. The chemical was not found in IUCLID. We therefore conclude that there is no data available and recommend it as a candidate for testing.

Table 5: Reproduction toxicity data for amine 3 chemicals

1.2.3 Sensitization data

The sensitization data on each chemical was assessed (Table 6) and based on this, conclusions were made regarding sensitization. A complete list of sensitization data for each chemical is provided in Appendix H.

Chemical Group	Name of the chemical	CAS Numbers	Conclusions
Amin	MEA	141-43-5	RTECS has classified it as a primary irritant and percutaneous data shows that it is a primary irritant in mammals. However, two studies from IUCLID have contradicting results. From the above studies we concluded that there is limited data on S.
NH3	NH3	7664-41-7	In RTECS there is no report on S. In IUCLID database, one study with open epicutaneous test indicated no effect. Several repeat dose inhalation studies show no effects at low concentrations. We therefore conclude that it is confirm no S.
Aldehydes	Formaldehyde	50-00-0	RTECS percutaneous toxicity data shows that it has sensitizing effect. Several studies in IUCLID indicated both positive and negative results. GESAMP classified it as a sensitizer. From the above studies we concluded that it is confirm S.
	Acetaldehyde	75-07-0	RTECS classfied it as primary irritant. Several studies from IUCLID indicated sensitization in humans with patch test. We therefore concluded it as confirm S.
Amides	Acetamide	60-35-5	In RTECS there is no report on S and the chemical was found in IUCLID inventory but no chemical data sheet is available We therefore conclude that there is no data on S.
Alkylamines	Methylamine	74-89-5	RTECS classified as primary irritant but there is no study report on S in both RTECS and IUCLID. We therefore conclude that there is limited data on S.

	Dimethylamine	124-40-3	RTECS classified as primary irritant and percutaneous toxicity data shows that is a sensitizer in rabbit and mammals. In IUCLID, 3 studies reported sensitization effects in guinea pig. GESAMP classified it as an S. We therefore conclude that it is a confirm S.
Nitrosamines	N-nitrosodimethylamine	62-75-9	In RTECS there is no report on S and the chemical was found in IUCLID inventory but no chemical data sheet is available. We therefore conclude that there is no data on S.
	N-nitrosodiethanolamine	1116-54-7	In RTECS there is no report on S and the chemical was found in IUCLID inventory but no chemical data sheet is available. We therefore conclude that there is no data on S.
	N-nitrosomorpholine	59-89-2	In RTECS there is no report on S and the chemical was not found in IUCLID inventory. We therefore conclude that there is no data on S.
Nitramines	Dimethylanitramine	4164-28-7	In RTECS there is no report on S and the chemical was not found in IUCLID inventory. We therefore conclude that there is no data on S.
	Ethanolnitramine	74386-82-6	Chemical not found in databases. We therefore conclude that there is no data available.
	Methylnitramine	598-57-2	In RTECS there is no report on S and the chemical was not found in IUCLID inventory. We therefore conclude that there is no data on S.

Table 6: Sensitization data for amine 3 chemicals

Appendix D Mammalian Toxicity Test Evaluation Forms

TEST METHOD EVALUATION-OECD 425

ENDPOINT: Acute oral toxicity

TEST METHOD NAME: Up and Down Procedure (UDP)

ENPOINT PARAMETER: Signs of toxicity and Mortality

REFERENCE: OECD 425

VALIDATION STATUS: YES OECD

IN VIVO / IN VITRO TEST METHOD: In Vivo

EXPOSURE ROUTE(S): Oral (Single dose by gavage). If single dose is not possible: smaller fractions within 24 hours.

ANIMAL / CELL CULTURE INFORMATION: Female Rats (8 and 12 weeks old)

NO. ANIMAL GENERATIONS TESTED: NA

TEST DURATION: 48 hrs to 14 days

INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Weighing balance for weight measurement, Microscope for gross pathology

TEST PRINCIPLES: Acute oral toxicity refers to adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours.

BRIEF TEST DESCRIPTION:

The test is divided into two parts:

a) Limit Test: It is a sequential test with 5 animals; performed when the test material is likely to be non toxic. Dose one animal at the test dose (2000/5000 mg/Kg).
Animal Dies: Conduct the main test to determine the LD50.

Animal Survives: Dose four additional animals sequentially so that a total of five animals are tested. (O=survival, X=death).

If 3 animals X, limit test – terminated; main test - performed.

The LD50 ≥ 2000/5000 mg/kg if ≥ 3 animal survival (OOOOO; OOOXO; OOOOX; OOOXX; OXOXO; OXOOO; OOXXO; OOXOO; OXXOO

The LD50 is ≤ 2000/5000 mg/kg if ≥ 3 animals die (OXOXX; OOXXX; OXXOX; OXXX)

b) Main Test: For each run, animals are dosed in a single ordered dose progression, one at a time, at 48 hrs. interval. The first animal is dosed a step below the level of the best estimate of LD50.
Upon survival/death: Dose for the next animal is increased/decreased by a progression factor of 3.2 times the original dose. The dose progression factor is the antilog of 1/(the estimated slope of the dose-response curve) (for e.g. a progression of 3.2 corresponds to a slope of 2). If no

information on the slope of the substance to be tested, a dose progression factor of 3.2 is used which gives a dose sequence of 1.75, 5.5, 17.5, 55, 175, 550, 2000 (or 1.75, 5.5, 17.5, 55, 175, 550, 1750, 5000 for specific regulatory needs). If no estimate of the substance's lethality is available, dosing should be initiated at 175 mg/kg.

The main test is stopped when one of the stopping criteria is met.

a) 3 consecutive animals survive at the upper bound;
b) 5 reversals occur in any 6 consecutive animals tested;
c) at least 4 animals have followed the first reversal and the specified likelihood-ratios exceed the critical value.

Results of the main test procedure serve as the starting point for a computational procedure for calculation of LD50 and a confidence interval.

Interval between treatment groups: Usually at 48 hr interval or determined by the onset, duration, and severity of toxic signs.

Observation: At least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days.

SPECIFIC PRECAUTIONS: Maximum dose volume for administration: 1mL/100g of body weight or 2 mL/100g body weight for aqueous solutions.

Fasting before dose administration: Rats-overnight; Mice- 3-4 hrs

Fasting after dose administration: Rats- 3-4 hrs; Mice- 1-2 hrs

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION: Mortality or moribund animals; nature, severity and duration of toxic effects (such as changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behaviour pattern. Observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma).

SUITABILITY EXPOSURE OF AIR SAMPLES: No

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

COMMENTS:

This form has been edited by:

Name	Organization	Date
Tore Syversen and Parvinder Kaur	NTNU	July-2010

TEST METHOD EVALUATION – OECD 471

ENDPOINT: Mutagenicity
TEST METHOD NAME: Bacterial Reverse Mutation Test
ENPOINT PARAMETER: The number of revertant colonies on both negative and positive control plates is the principal endpoint (prokaryote assay, reverse gene mutation by Ames test. Histidine forward and reverse gene mutation
REFERENCE: OECD TG 471
VALIDATION STATUS: OECD validated
IN VIVO / IN VITRO TEST METHOD: In vitro
EXPOSURE ROUTE(S): An appropriate minimal agar (e.g. containing Vogel-Bonner minimal medium E and glucose) and an overlay agar containing histidine and biotin or tryptophan, to allow for a few cell divisions, is used.
ANIMAL / CELL CULTURE INFORMATION: Salmonella typhimurium: TA98, TA100, TA1535, TA1537, or TA1538. Host-mediated assay
NO. ANIMAL GENERATIONS TESTED:
TEST DURATION: All plates in a given assay should be incubated at 37°C for 48-72 hours. After the incubation period, the number of revertant colonies per plate is counted.
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.):
TEST PRINCIPLES: Suspensions of bacterial cells are exposed to the test substance in the presence and in the absence of an exogenous metabolic activation system. The suspensions are mixed with an overlay agar and plated immediately onto minimal medium. The treatment mixture is incubated and then mixed with an overlay agar before plating onto minimal medium. For both techniques, after two or three days of incubation, revertant colonies are counted and compared to the number of spontaneous revertant colonies on solvent control plates.
BRIEF TEST DESCRIPTION: Suspensions of bacterial cells are exposed to the test substance in the presence and in the absence of an exogenous metabolic activation system. In the plate incorporation method, these suspensions are mixed with an overlay agar and plated immediately onto minimal medium. In the preincubation method, the treatment mixture is incubated and then mixed with an overlay agar before plating onto minimal medium. For both techniques, after two or three days of incubation, revertant colonies are counted and compared to the number of spontaneous revertant colonies on solvent control plates.
SPECIFIC PRECAUTIONS: It is recognised that certain classes of mutagens are not always detected using standard procedures such as the plate incorporation method or preincubation method. These should be regarded as "special cases" and it is strongly recommended that alternative procedures should be used for their detection. The following "special

cases" could be identified (together with examples of procedures that could be used for their detection): azo-dyes and diazo compounds , gases and volatile chemicals, and glycosides. A deviation from the standard procedure needs to be scientifically justified.

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:

SUITABILITY EXPOSURE OF AIR SAMPLES:

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

This form has been edited by:

Name	Organization	Date
Maria Dusinska and Evy Siversen	NILU	August -2010

TEST METHOD EVALUATION – OECD 473

ENDPOINT: Genetic toxicity/mutagenicity
TEST METHOD NAME: In vitro Mammalian Chromosome Aberration Test
ENPOINT PARAMETER: The percentage of cells with structural chromosome aberration(s) is the principal endpoint. Concurrent measures of cytotoxicity for all treated and negative control cultures in the main aberration experiment(s) are also endpoints in this project. Individual culture data is provided.
REFERENCE: OECD TG 473
VALIDATION STATUS: OECD validated
IN VIVO / IN VITRO TEST METHOD: In vitro
EXPOSURE ROUTE(S): Cell cultures are exposed to the test substance (liquid or solid) both with and without metabolic activation.
ANIMAL / CELL CULTURE INFORMATION:
NO. ANIMAL GENERATIONS TESTED:
TEST DURATION: The cells are exposed to the test substance for 3-6 hours, and sampled at a time equivalent to about 1.5 normal cell cycle length after the beginning of treatment. If negative results, both with and without activation, an additional experiment without activation is done with continuous treatment until sampling at a time equivalent to about 1.5 normal cell cycle lengths.
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.):
TEST PRINCIPLES: Cell cultures are exposed to the test substance both with and without metabolic activation. At predetermined intervals after exposure of cell cultures to the test substance, they are treated with a metaphase-arresting substance (e.g. Colcemid® or colchicine), harvested, stained and metaphase cells are analysed microscopically for the presence of chromosome aberrations.
BRIEF TEST DESCRIPTION: The in vitro chromosome aberration test may employ cultures of established cell lines, cell strains or primary cell cultures. Cell cultures are exposed to the test substance (liquid or solid) both with and without metabolic activation during about 1.5 normal cell cycle lengths. At least three analysable concentrations of the test substance are used. At each concentration duplicate cultures is normally used. At predetermined intervals after exposure of cell cultures to the test substance, the cells are treated with a metaphase-arresting substance, harvested, stained. Metaphase cells are analysed microscopically for the presence of chromosome aberrations.
SPECIFIC PRECAUTIONS: Care should be taken to avoid conditions which would lead to positive results which do not reflect intrinsic mutagenicity and may arise from changes in pH, osmolality or high levels of cytotoxicity.
CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:
SUITABILITY EXPOSURE OF AIR SAMPLES:

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

This form has been edited by:

Name	Organization	Date
Maria Dusinska and Evy Siversen	NILU	August -2010

TEST METHOD EVALUATION- OECD 474

ENDPOINT: Mutagenicity
TEST METHOD NAME: Mammalian Erythrocyte Micronucleus Test
ENPOINT PARAMETER: The frequency of micronucleated immature (polychromatic) erythrocytes is the principal endpoint. The frequency of micronucleated immature (polychromatic) erythrocytes is the principal endpoint. The number of mature (normochromatic) erythrocytes in the peripheral blood that contain micronuclei among a given number of mature erythrocytes can also be used as the endpoint of the assay when animals are treated continuously for 4 weeks or more.
REFERENCE: OECD TG 474
VALIDATION STATUS: OECD validated
IN VIVO / IN VITRO TEST METHOD: In vivo
EXPOSURE ROUTE(S): Usually administered by gavage using a stomach tube or a suitable intubation cannula, or by intraperitoneal injection. Other routes may be acceptable when justified.
ANIMAL / CELL CULTURE INFORMATION: Bone marrow of rodents or mice is recommended, but other appropriate mammals may also be used
NO. ANIMAL GENERATIONS TESTED:
TEST DURATION: With one single treatment of test substance: Samples of bone marrow are taken between 24 and 48 hours after treatment. Samples of peripheral blood are taken between 36 and 72 hours. If 2 or more daily treatments, samples should be collected once between 18 and 24 hours following the final treatment for the bone marrow and once between 36 and 48 hours following the final treatment for the peripheral blood.
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.):
TEST PRINCIPLES: Animals are exposed to the test substance by an appropriate route. If bone marrow is used, the animals are sacrificed at appropriate times after treatment, the bone marrow extracted, and preparations made and stained. When peripheral blood is used, the blood is collected at appropriate times after treatment and smear preparations are made and stained. For studies with peripheral blood, as little time as possible should elapse between the last exposure and cell harvest. Preparations are analyzed for the presence of micronuclei.
BRIEF TEST DESCRIPTION: The mammalian in vivo micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts, by analysis of erythrocytes as sampled in bone marrow and/or peripheral blood cells of animals, usually rodents (mice or rats). The purpose of the micronucleus test is to identify substances (liquid or solid) that cause cytogenetic damage which results in the formation of micronuclei containing lagging chromosome fragments or whole chromosomes. An increase in the frequency of micronucleated polychromatic erythrocytes in treated animals is an indication of induced chromosome damage. Animals are exposed to the test substance by an appropriate route (usually by gavage using a stomach tube or a suitable intubation cannula, or by intraperitoneal injection). Bone marrow and/or blood cells are collected, prepared and

stained. Preparations are analyzed for the presence of micronuclei. Each treated and control group must include at least 5 analysable animals per sex. Administration of the treatments consists of a single dose of test substance or two daily doses (or more). The limit dose is 2000 mg/kg/body weight/day for treatment up to 14 days, and 1000 mg/kg/body weight/day for treatment longer than 14 days.

SPECIFIC PRECAUTIONS: If there is evidence that the test substance, or a reactive metabolite, will not reach the target tissue, it is not appropriate to use this test. Weight variation of animals should be minimal and not exceed ± 20% of the mean weight of each sex.

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:

SUITABILITY EXPOSURE OF AIR SAMPLES:

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

This form has been edited by:

Name	Organization	Date
Maria Dusinska and Evy Siversen	NILU	August -2010

TEST METHOD EVALUATION – OECD 475

ENDPOINT: Genetic toxicity/mutagenicity
TEST METHOD NAME: Mammalian Bone Marrow Chromosome Aberration Test
ENPOINT PARAMETER: The mitotic index should be determined as a measure of cytotoxicity in at least 1000 cells per animal for all treated animals (including positive controls) and untreated negative control animals. The number of cells scored, the number of aberrations per cell and the percentage of cells with structural chromosome aberration(s) should be evaluated. Different types of structural chromosome aberrations should be listed with their numbers and frequencies for treated and control groups.
REFERENCE: OECD TG 475
VALIDATION STATUS: OECD validated
IN VIVO / IN VITRO TEST METHOD: In vivo
EXPOSURE ROUTE(S): Usually administered by gavage using a stomach tube or a suitable intubation cannula, or by intraperitoneal injection. Other routes may be accepted when justified.
ANIMAL / CELL CULTURE INFORMATION: Bone marrow cells of rodents are used.
NO. ANIMAL GENERATIONS TESTED:
TEST DURATION: Samples should be taken at two separate times on one day. The samples are taken between 12-18 hours after treatment. For optimum time of chromosome aberration detection, a later sample collection 24 hr after the first sample time is recommended. If dose regimens of more than one day are used, one sampling time at 1.5 normal cell cycle lengths after the final treatment should be used.
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.):
TEST PRINCIPLES:
BRIEF TEST DESCRIPTION: Animals are exposed to the test substance (liquid or solid) by an appropriate route. The animals are sacrificed at appropriate times after treatment. Prior to sacrifice, animals are treated with a metaphase-arresting agent. Chromosome preparations are then made from the bone marrow cells and stained, and metaphase cells are analysed for chromosome aberrations. Each treated and control group must include at least 5 analysable animals per sex. The limit dose is 2000 mg/kg/body weight/day for treatment up to 14 days, and 1000 mg/kg/body weight/day for treatment longer than 14 days.
SPECIFIC PRECAUTIONS: If there is evidence that the test substance, or a reactive metabolite, will not reach the target tissue, it is not appropriate to use this test. Weight variation of animals should be minimal and not exceed ± 20% of the mean weight of each sex.
CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:
SUITABILITY EXPOSURE OF AIR SAMPLES:

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

COMMENTS:

This form has been edited by:

Name	Organization	Date
Maria Dusinska and Evy Siversen	NILU	August -2010

TEST METHOD EVALUATION – OECD 476

ENDPOINT: Genetic toxicity/carcinogenicity
TEST METHOD NAME: In vitro Mammalian Cell Gene Mutation Test
ENPOINT PARAMETER: Gene mutations, HPRT assay. Data should include cytotoxicity and viability determination, colony counts and mutant frequencies for the treated and control cultures. Survival (relative cloning efficiencies) or relative total growth should be given. Individual culture data should be provided. Negative results need to be confirmed.
REFERENCE: OECD TG 476
VALIDATION STATUS: OECD validated
IN VIVO / IN VITRO TEST METHOD: In vitro
EXPOSURE ROUTE(S): Cells in suspension or monolayer culture are exposed to the test substance, both with and without metabolic activation, for a suitable period of time and subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection.
ANIMAL / CELL CULTURE INFORMATION: mammalian
NO. ANIMAL GENERATIONS TESTED:
TEST DURATION: Each locus has a defined minimum time requirement to allow near optimal phenotypic expression of newly induced mutants (HPRT and XPRT require at least 6-8 days, and TK at least 2 days).
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.):
TEST PRINCIPLES: The objective is to detect possible mammalian mutagens and carcinogens induced by chemical substances.
BRIEF TEST DESCRIPTION: Mammalian cells in suspension or monolayer culture are exposed to, at least four analysable concentrations of the test substance, both with and without metabolic activation, for a suitable period of time. They are subcultured to determine cytotoxicity and to allow phenotypic expression prior to mutant selection. It is recommended to utilise at least 106cells. Cytotoxicity is usually determined by measuring the relative cloning efficiency (survival) or relative total growth of the cultures after the treatment period. The treated cultures are maintained in growth medium for a sufficient period of time, characteristic of each selected locus and cell type, to allow near-optimal phenotypic expression of induced mutations. Mutant frequency is determined by seeding known numbers of cells in medium containing the selective agent to detect mutant cells, and in medium without selective agent to determine the cloning efficiency (viability). After a suitable incubation time, colonies are counted.
SPECIFIC PRECAUTIONS: Care should be taken to avoid conditions which would lead to results not reflecting intrinsic mutagenicity. Positive results which do not reflect intrinsic mutagenicity may arise from changes in pH, osmolality or high levels of cytotoxicity.

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:

SUITABILITY EXPOSURE OF AIR SAMPLES:

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

COMMENTS:
Compound tested: Dimethylnitrosamine, CAS: 62-75-9, Results: positive
Compound tested:
1) Benzaldehyde, CAS 100-52-7
2) Nitrosaminedimethylamine, CAS 62-75-9
3) Methylamine, CAS 74-89-5
Results: Positive

This form has been edited by:

Name	Organization	Date
Maria Dusinska and Evy Siversen	NILU	August -2010

TEST METHOD EVALUATION – OECD 487

ENDPOINT: Genotoxicity
TEST METHOD NAME: In Vitro Mammalian Cell Micronucleus Test
ENPOINT PARAMETER: Genotoxicity test for the detection of micronuclei (MN) in the cytoplasm of interphase cells
REFERENCE: OECD TG 487
VALIDATION STATUS: OECD validated
IN VIVO / IN VITRO TEST METHOD: In vitro
EXPOSURE ROUTE(S):
ANIMAL / CELL CULTURE INFORMATION: Various rodent cell lines (CHO, V79, CHL/IU, and L5178Y) and human lymphocytes The use of the human TK6 lymphoblastoid cell line (35), HepG2 cells (36) (37) and primary Syrian Hamster Embryo cells (38) has been described, although they have not been used in validation studies.
NO. ANIMAL GENERATIONS TESTED:
TEST DURATION:
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.):
TEST PRINCIPLES: This is a genotoxicity test for the detection of micronuclei 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 test substances in cells that have undergone cell division during or after exposure to the test substance.
BRIEF TEST DESCRIPTION: Cell cultures of human or mammalian origin are exposed to the test substance both with and without an exogenous source of metabolic activation unless cells with an adequate metabolizing capability are used. Concurrent solvent/vehicle and positive controls are included in all tests. During or after exposure to the test substance, the cells are grown for a period sufficient to allow chromosome or spindle damage to lead to the formation of micronuclei in interphase cells. For induction of aneuploidy, the test substance should ordinarily be present during mitosis. Harvested and stained interphase cells are analysed for the presence of micronuclei. Ideally, micronuclei should only be scored in those cells that have completed mitosis during exposure to the test substance or during the post-exposure period, if one is used. In cultures that have been treated with a cytokinesis blocker, this is achieved by scoring only binucleate cells. In the absence of a cytokinesis blocker, it is

important to demonstrate that the cells analysed are likely to have undergone cell division during or after exposure to the test substance.

SPECIFIC PRECAUTIONS: It is important to demonstrate that cell proliferation has occurred in both the control and treated cultures, and the extent of test substance-induced cytotoxicity or cytostasis should be assessed in the cultures (or in parallel cultures) that are scored for micronuclei.

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:

SUITABILITY EXPOSURE OF AIR SAMPLES:

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

COMMENTS:

This form has been edited by:

Name	Organization	Date
Maria Dusinska and Evy Siversen	NILU	September 2010

TEST METHOD EVALUATION- OECD 453

ENDPOINT: Chronic toxicity / Carcinogenicity
TEST METHOD NAME: Combined Chronic Toxicity / Carcinogenicity Studies
ENPOINT PARAMETER: General: Suvival data, body weight / changes, food consumption, toxicokinetic data, opthalmoscopy, haematology, clinical chemistry. Clinical findings: signs of toxicity, incidence of any abnormality, nature, severity and duration of clinical obersvation. Necropsy data: Terminal body weight, organ weights and their ratios, necropsy findings – incidence and severity of abnormalities. Histopathology: Non neoplastic histopathological findings, neoplastic histopathological findings, correlation between gross and microscopic findings, detailed description of all treatment-related histopathological findings including severity gradings, report of any peer review of slides.
REFERENCE: OECD TG 453
VALIDATION STATUS: OECD validated
IN VIVO / IN VITRO TEST METHOD: In vivo
EXPOSURE ROUTE(S): Focus on oral route. Dermal or inhalation need careful modifications
ANIMAL / CELL CULTURE INFORMATION: Primarily rodent species, by use of other species, appropriate modifications must be taken (OECD Guidance Document No 116)
NO. ANIMAL GENERATIONS TESTED:
TEST DURATION: Chronic phase: Normally 12 months, can be shorter (e.g.6 or 9) and longer (e.g. 18 or 24). Deviations from 12 months must be justified. Carcinogenicity phase: Normally 24 months
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.):
TEST PRINCIPLES: The objective of a combined chronic toxicity/carcinogenicity study is to identify carcinogenic and the majority of chronic effects, and to determine dose-response relationships following prolonged and repeated exposure.
BRIEF TEST DESCRIPTION: The rat is typically used for this study. For rodents, each dose group and concurrent control group intended for the carcinogenicity phase of the study should contain at least 50 animals of each sex, while for the chronic toxicity phase of the study should contain at least 10 animals of each sex. At least three dose levels should be used, in addition to the concurrent control group for both the chronic toxicity phase and the carcinogenicity phase of the study. For chronic phase, the test substance is administered daily in graduated doses to several groups of test animals, one dose level per group. For carcinogenicity, phase, the test substance is administered daily to several groups of test animals for a major portion of their life span. The observations permit the detection of neoplastic effects and a determination of carcinogenic potential as well as the general toxicity. The three main routes of administration are oral, dermal, and inhalation. The Test Guideline focuses

on the oral route of administration.

SPECIFIC PRECAUTIONS: Both sexes should be used. A sufficient number of animals should be used to have a throughout biological and statistical evaluation (e.g. for rodent – 50 animals of each sex)

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:

SUITABILITY EXPOSURE OF AIR SAMPLES: Requires modifications

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

COMMENTS:

This form has been edited by:

Name	Organization	Date
Tore Syversen and Parvinder Kaur	NTNU	July-2010

TEST METHOD EVALUATION-OECD 415

ENDPOINT: Reproduction Toxicity
TEST METHOD NAME: One-Generation Reproduction Toxicity Study
ENPOINT PARAMETER: Number and sex of pups, stillbirths, live births, Weight, Measurement of food consumption, Pathology: Gross necropsy and Histopathology (ovaries, uterus, cervix, vagina, testes, epididymides, seminal vesicles, prostate, coagulating gland, pituitary gland and target organs), Pertinent behavioural changes, signs of difficult or prolonged parturition and all signs of toxicity, including mortality.
REFERENCE: OECD 415
VALIDATION STATUS: Yes OECD
IN VIVO / IN VITRO TEST METHOD: In Vivo
EXPOSURE ROUTE(S): Diet or drinking water (recommended); Other routes (also acceptable)
ANIMAL / CELL CULTURE INFORMATION: Mice / Rat (5-9 weeks)
NO. ANIMAL GENERATIONS TESTED: F1
TEST DURATION: Males: 56 days; Rats 70 days. Females: 2 weeks prior to mating, continue 3-weeks mating period, pregnancy and up to the weaning of the F1 offspring.
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Microscope, Weighing machine
TEST PRINCIPLES: The test substance is administered in graduated doses to several groups of males for at least 1 complete spermatogenic cycle and females for at least two complete oestrous cycles. Endpoints of reproductive toxicity are measured in F1 and P generation animals.
BRIEF TEST DESCRIPTION: Animals are dosed according to either limit test (low toxicity in repeated-dose studies) or at 3 treatment groups (high/ intermediate/ low) and a control group. The animals are then mated according to 1:1 or 1:2 ratio. Each test and control group: 20 pregnant females at or near term. Animals which fail to mate: Evaluated to determine the cause of the apparent infertility. Litter without standardised litter: Normal rearing of progency until weaning. Litter with standardisation: on day 4, the size of each litter is adjusted to 4 males and 4 females Observations: once daily. Measurement of food consumption: Weekly- During pre-mating and mating periods; Daily-Pregnancy; Same day as the litters- After parturition and during lactation. Weight of the animals: P males and females (Day 1 of dosing and weekly) Weight of the pups: (Day 1, 4 and 7 and weekly, until termination of the study) Animals are then examined for gross necropsy and histopathology and all signs of toxicity, including mortality are reported.
SPECIFIC PRECAUTIONS:

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION: Evaluated in terms of observed effects, necropsy and microscopic findings. The dose of the test substance related to presence / absence, incidence / severity of abnormalities, including fertility, clinical abnormalities, body weight changes, effects on mortality and any other toxic effects. Provides estimation of a no-effect level and adverse effects on reproduction, parturition, lactation and postnatal growth.

SUITABILITY EXPOSURE OF AIR SAMPLES: Require modifications

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

COMMENTS: Extrapolation of the results of the study to man is valid to a limited degree, although it can provide useful information on no-effect levels and permissible human exposure.

It is not designed to determine specific cause and effects in all cases and will require modifications to study substances administered by the inhalation route.

This form has been edited by:

Name	Organization	Date
Tore Syversen and Parvinder Kaur	NTNU	July-2010

TEST METHOD EVALUATION-OECD 416

ENDPOINT: Reproduction Toxicity
TEST METHOD NAME: Two-Generation Reproduction Toxicity Study
ENPOINT PARAMETER: P and F1 generation: No. of fertile and pregnant animals, No. of animals showing signs of toxicity, description of signs of toxicity: time of onset, duration, and severity of any toxic effects. Integrity and performance of the male and female reproductive systems, including gonadal function, sperm count and motililty, the oestrus cycle, mating behaviour, conception, gestation, parturition, lactation, and weaning. F1 and F2 Pups: Number and sex of pups, stillbirths, live births, body and organ weight, growth and development, measurement of food and water consumption. Body and organ weight: P and F1 generation: uterus, ovaries, testes, epididymides (total and cauda), prostate, seminal vesicles with coagulating glands and their fluids (as one unit); brain, liver, kidneys, spleen, pituitary, thyroid and adrenal glands and known target organs; F1 and F2 pups: Brain, spleen and thymus Histopathological examination and necropsy findings with special attention to the organs of the reproductive system.(ovaries, uterus, cervix, vagina, testes, epididymides, seminal vesicles, prostate, coagulating gland, pituitary gland and target organs).
REFERENCE: OECD 416
VALIDATION STATUS: Yes OECD
IN VIVO / IN VITRO TEST METHOD: In Vivo
EXPOSURE ROUTE(S): oral (by diet, drinking water or gavage) unless another route of administration (e.g. dermal or inhalation) are more appropriate
ANIMAL / CELL CULTURE INFORMATION: Rat, Parental males and females (5-9 weeks old), F1 males and females (weaning)
NO. ANIMAL GENERATIONS TESTED: F1 and F2
TEST DURATION: Parental animals: During growth and for at least 1 complete spermatogenic cycle in males (56 days-mice and 70 days-rat) and females during growth and for several complete oestrous cycles. Dosing is continued during mating, resulting pregnancies, weaning of their Fl offspring and to the Fl offspring during their growth into adulthood, mating and production of an F2 generation, until the F2 generation is weaned.
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Microscope, Weighing machine
TEST PRINCIPLES: The test substance is administered in graduated doses to several groups of males during growth and for at least 1 complete spermatogenic cycle (56 days-mice and 70 days-rat) and females during growth and for several complete oestrous cycles. Administration to the parental (P) animals is done during mating, resulting pregnancies, weaning of their Fl offspring and to the Fl offspring during their growth into adulthood, mating and production of an F2 generation, until the F2 generation is weaned. Clinical observations and pathological examinations: Performed on all animals for signs of toxicity with special emphasis on the integrity and performance of the male and female reproductive systems and on the growth and development of the F1 and F2 offspring.
BRIEF TEST DESCRIPTION: Animals are dosed for at least 10 weeks before the mating period, according to either limit test (1000 mg/kg b.w./ day) or at 3 dose levels (descending sequence) and a concurrent control with the highest dose level chosen with the aim to induce toxicity but not death or severe suffering. Dose: 2-4 fold intervals/ 7-days-a-week basis. The animals are then mated according to 1:1 ratio. Dosing is continued during the 2 week mating period, continue throughout pregnancy and up to the weaning of the F1 offspring and continue until termination. Test and control group: Sufficient number of animals to yield preferably not less than 20 pregnant females at or near parturition.

Control group: Untreated group or a vehicle-control group with highest volume of vehicle being used.

Observation: Pairs without progeny are evaluated to determine the apparent cause of the infertility, Assess the integrity and performance of the male and female reproductive systems and in addition study the growth and development of the F1 and F2 generation.

Results: Estimation of a no-effect level and an understanding of adverse effects on reproduction, parturition, lactation, postnatal development including growth and sexual development. Provide information on the effects of repeated exposure to a substance during all phases of the reproductive cycle. Can be used in assessing the need for further testing of a chemical and to provide information on permissible human exposure.

SPECIFIC PRECAUTIONS: Mating of siblings should be avoided, F1 offspring should not be mated

until they have attained full sexual maturity, stability of the test substance in the vehicle should be determined, Constant volume not exceeding 1 ml/100g b.w. should be used at all dose levels, constant dietary concentration in terms of the b.w. should be used, Gavage studies: Dose should be given at similar times each day, and adjusted to maintain a constant dose level in terms of animal b.w. with respect to placental distribution and last trimester of pregnancy.

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION: Evaluated in terms of observed effects, necropsy and microscopic findings. The dose of the test substance related to presence / absence, incidence / severity of abnormalities, including fertility, clinical abnormalities, body weight changes, effects on mortality and any other toxic effects. Provides estimation of a no-effect level and adverse effects on reproduction, parturition, lactation and postnatal growth.

SUITABILITY EXPOSURE OF AIR SAMPLES: -

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

COMMENTS: Extrapolation of the results of the study to man is valid to a limited degree, although it can provide useful information on no-effect levels and permissible human exposure.

Males of the P generation need not be included in the evaluation: If data on spermatogenesis are available (e.g. 90 day study).

This form has been edited by:

Name	Organization	Date
Tore Syversen and Parvinder Kaur	NTNU	July-2010

TEST METHOD EVALUATION-OECD 422

ENDPOINT: Reproduction/Developmental Toxicity
TEST METHOD NAME: Combined Repeated Dose Toxicity Study with the Reproduction / Developmental Toxicity Screening Test
ENPOINT PARAMETER: Toxic effects in P generation: No. of animals at the start, during and end of the test, the time of death, No. of fertile animals and pregnant females, time of onset, duration, and severity of toxic effects such as No. of implantations, post-implantation loss, counting of corpora lutea, duration of gestation, sensory and motor assessments, haematological test, clinical biochemistry, signs of difficult or prolonged parturition and all signs of toxicity including mortality. Gross necropsy and histopathology, microscopic findings of the male genital tract. Body weight changes and organ weight data, Food and water consumption, pertinent behavioural changes Toxic effects in F1 generation: No. and sex, stillbirths, live births, runts, post natal growth, body weight changes and presence of grossly visible abnormalities.
REFERENCE: OECD 422
VALIDATION STATUS: Yes OECD
IN VIVO / IN VITRO TEST METHOD: In Vivo
EXPOSURE ROUTE(S): Oral (gavage, diet or drinking water); Other routes require modifications
ANIMAL / CELL CULTURE INFORMATION: Rat; Other species require modifications
NO. ANIMAL GENERATIONS TESTED: F1
TEST DURATION: 54 days
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Microscope, Weighing machine
TEST PRINCIPLES: The study will provide evaluations of reproduction/developmental toxicity associated with administration of repeated doses. In particular, since emphasis is placed on both general toxicity and reproduction/developmental toxicity endpoints, the results of the study will allow for the discrimination between reproduction/developmental effects occurring in the absence of general toxicity and those which are only expressed at levels that are also toxic to parent animals. It could provide an indication of the need to conduct further investigations and could provide guidance in the design of subsequent studies.
BRIEF TEST DESCRIPTION: The test substance is administered in graduated doses to at least 3 test groups and a control group of males (at least 4 weeks) and females (approximately 54 days). Dosing is done based on information from any existing toxicity and toxicokinetic data in 10 animals/ group/ sex. The highest dose level- inducing toxic effects but not death or severe suffering. Thereafter, a descending sequence of dose levels dosage related response and no-observed-adverse effects (NOAEL) at the lowest dose level. Two to four fold intervals between dosages is used. If in the limit test, no observable toxic effects are observed at 1000mg/Kg/b.w/day , then a full study using several dose

levels is not necessary.
Mating is done at full sexual maturity (Sprague Dawley rats 10 weeks, Wistar rats -12 weeks) at 1:1 ratio. No. of pregnant females: 8/ group.
Termination of study Males: 28 days; Females: Day 3 post-partum, or 24-26 days for (non copulated females)
Observation: General clinical observation (Daily); Measurement of food/water consumption during pre-mating, pregnancy and lactation (Weekly); Weight of the P males and females (Day 1 of dosing and weekly, at termination), Weight of the pups: (Day 0 or 1, 4), Weight of pregnant females: (Day 0 or 7, 14 and 20 and within 24 hours of parturition (day 0 or 1 post-partum) and day 4 post-partum. duration of gestation, Number and sex of live pups, still births, live births, runts, Haematology, clinical biochemistry, Functional observations (at the end of the study), Pathology: Gross necroscopy and histopathology (at the end of the study). Effect of the substance on fertility, pregnancy, maternal and suckling behaviour, and growth and development of the F1 offspring.

SPECIFIC PRECAUTIONS:
Maximum dose volume for administration: 1mL/100g of body weight or 2 mL/100g body weight for aqueous solutions.
Diet: constant dietary concentration (ppm) or a constant dose level in terms of the animals' body weight
Gavage: given at similar times each day, and adjusted at least weekly to maintain a constant dose level in terms of animal body weight

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION: The dose of the test substance related to presence / absence, incidence / severity of abnormalities, including gross lesions, identified target organs, infertility, clinical abnormalities, affected reproductive and litter performance, body weight changes, effects on mortality, histopathology of the testis and epididymus, fertility data and any other toxic effects.

SUITABILITY EXPOSURE OF AIR SAMPLES: Require modifications

GENERAL JUDGEMENT OF TEST:

SCORE OF TEST AIR SAMPLES: ☐ WATER SAMPLES: ☐

COMMENTS: Offers only limited means of detecting post-natal manifestations of prenatal exposure, or effects that may be induced during post-natal exposure.

This form has been edited by:

Name	Organization	Date
Tore Syversen and Parvinder Kaur	NTNU	July-2010

Appendix E Ecotoxicity Test Evaluation Forms

TEST METHOD EVALUATION
Ecotoxicity

ENDPOINT: Acute toxicity, inhibition of growth
TEST METHOD NAME: Freshwater Algae and Cyanobacteria, Growth Inhibition Test
ENPOINT PARAMETER: ECx (e.g. EC50) and/or LOEC and NOEC.
REFERENCE: OECD guidelines for the testing of chemicals 201, adopted 23 March 2006
VALIDATION STATUS: This guideline is based on OECD guideline 201 (rev. 1984), ISO 8692 (1993), ISO/DIS 14442 (1998), ISO 5667-16 (1998), extensive practical experience, scientific progress in the field of algal toxicity studies, and extensive regulatory use
IN VIVO / IN VITRO TEST METHOD: In vivo
EXPOSURE ROUTE(S): Surface
EXPOSURE VOLUME(S): Variable, depending on test design and measurement instruments. The test vessels must be of dimensions that allow a sufficient volume of culture for analytical determinations and a sufficient mass transfer of CO2 from the atmosphere.
ANIMAL / CELL CULTURE INFORMATION (INCLUDING NO. ANIMALS/CELL CULTURE CONC.): Several species of non-attached microalgae (e.g. *Pseudokirchneriella subcapitata)* and cyanobacteria may be used. Examples of suitable strains are given in the guideline
NO. GENERATION OF ORGANISM TESTED: Effects over several generations can be assessed.
TEST DURATION: Normally 72 hours. Shorter or longer test durations may be used if validity criteria are met.
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Culturing vessels/flasks, culturing apparatus, temperature and light measurement instruments, apparatus to determine algal biomass (electronic particle counter, a microscope with counting chamber, flow cytometer, fluorimeter, spectrophotometer or colorimeter), etc.
TEST PRINCIPLES AND BRIEF METHOD DESCRIPTION The purpose of this test is to determine the effects of a substance on the growth of freshwater microalgae and/or cyanobacteria. For full expression of the system response to toxic effects (optimal sensitivity), the cultures are allowed unrestricted exponential growth under nutrient sufficient conditions and continuous light for a sufficient period of time to measure reduction of the specific growth rate.

Based on results from a range-finding test, a final definitive test with at least five different concentrations of the test solution, arranged in a geometric series with a factor not exceeding 3,2 is prepared. The test design should include three replicates at each test concentration. An inoculum culture of exponentially growing test organisms is added to each test vessel, and exposed to the test substance over a period of normally 72 hours. Algal biomass (often measured by surrogate parameters like cell volume, fluorescence, optical density, etc.) in each test vessel is determined at least daily during the test period.

The system response is the reduction of growth in a series of algal cultures (test units) exposed to various concentrations of a test substance. Growth and growth inhibition are quantified from measurements of the algal biomass as a function of time, and the response is then evaluated as a function of the exposure concentration in comparison with the average growth of unexposed control cultures. Growth is expressed as the logarithmic increase in biomass (average specific growth rate) during the exposure period. From the average specific growth rates recorded in a series of test solutions, the concentration bringing about a specified x % inhibition of growth rate (e.g. 50%) that is determined and expressed as the ECx (e.g. EC50). Also, the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC) may be statistically determined.

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS

CRITERIA FOR VALIDITY AND HAZARD EVALUATION CLASSIFICATION:
For the test to be valid, the following performance criteria should be met:

- The biomass in the control cultures should have increased exponentially by a factor of at least 16 within the 72-hour test period.
- The mean coefficient of variation for section-by-section specific growth rates (days 0-1, 1-2 and 2-3, for 72-hour tests) in the control cultures must not exceed 35%
- The coefficient of variation of average specific growth rates during the whole test period in replicate control cultures must not exceed certain strain specific limits.
- The pH of the control medium should not increase by more than 1.5 units during the test.

When possible, the 95% confidence limits for each estimate should be determined. It is desirable to test a reference substance like 3,5-dichlorophenol used in the international ring test, or potassium dichromate for green algae, at least twice a year.

SUITABILITY EXPOSURE OF AIR SAMPLES: No. This guideline is most easily applied to water-soluble substances. For testing of substances that are volatile, strongly adsorbing, coloured, having a low solubility in water or substances that may affect the availability of nutrients or minerals in the test medium, certain modifications of the described procedure may be required (e.g., closed system, conditioning of the test vessels).

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Ecotoxicity

ENDPOINT: Acute test, immobilisation
TEST METHOD NAME: *Daphnia* sp., Acute Immobilisation Test
ENPOINT PARAMETER: EC50 after 24h (optional) and 48h.
REFERENCE: OECD Guidelines for testing of chemicals 202, adopted 13 April 2004
VALIDATION STATUS: This guideline revision is based upon OECD 211 (1998) with references to ISO 6341(1996), EPA OPPTS 850.1010 (1996), EPS 1/RM/11 (1996)
IN VIVO / IN VITRO TEST METHOD: In vivo
EXPOSURE ROUTE(S): Direct uptake across surface and oral
EXPOSURE VOLUME(S): At least 2 ml of test solution should be provided for each animal (i.e. a volume of 10 ml for five daphnids per test vessel)
ANIMAL / CELL CULTURE INFORMATION (INCLUDING NO. ANIMALS/CELL CULTURE CONC.): Preferred test species is *Dapnhia magna* Straus, but other Daphnia species can be used. In order to reduce variability, the animals should be less than 24 hours old, not be first brood progeny and derive from a healthy stock showing no signs of stress. All organisms used for a particular test should have originated from cultures established from the same stock of daphnids, maintained in culture conditions (light, temperature, and medium) similar to those to be used in the test.
NO. GENERATION OF ORGANISM TESTED: One
TEST DURATION: 48 hours
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Oxygen-meter, pH-meter, temperature control, equipment for determination of total organic carbon concentration (TOC), chemical oxygen demand (COD)and hardness, etc.
TEST PRINCIPLES AND BRIEF METHOD DESCRIPTION: Young daphnids are exposed to the test substance at a range of concentrations for a period of 48 hours. At least five test concentrations should be used, arranged in a geometric series with a separation factor preferably not exceeding 2.2. Immobilisation is recorded at 24 hours and 48 hours and compared with control values. The results are analysed in order to calculate the EC50 at 48h. Determination of the EC50 at 24h is optional.

As far as possible, the use of solvents, emulsifiers or dispersants should be avoided. A range-finding test may be conducted to determine the range of concentrations for the definitive test unless information on toxicity of the test substance is available. Natural water (surface or ground water), reconstituted water or dechlorinated tap water are acceptable as holding and dilution water if daphnids will survive in it for the duration of the culturing, acclimation and testing without showing signs of stress (high mortality, presence of males andephippia, delay in the production of the first brood, discoloured animals, unusual behaviour such as trapping at surface of water etc).

Test vessels are filled with appropriate volumes of dilution water and solutions of test substance before daphnids are introduced. At least 20 animals, preferably divided into four groups of five animals each, should be used at each test concentration and for the controls. The test may be carried out using semi-static renewal or flow-through system. The temperature should be within the range of 18 - 22°C, and a 16-hour light and 8-hour dark cycle is recommended. The pH should remain in the range 6-9. The daphnids should not be fed during the test

Each test vessel should be checked for immobilised daphnids after 24 and 48 hours exposure. Those animals that are not able to swim within 15 seconds, after gentle agitation of the test vessel are considered to be immobilised, even if they can still move their antennae. In addition, any abnormal behaviour or appearance should be reported. The dissolved oxygen and pH are measured at the beginning and end of the test in the control(s) and in the highest test substance concentration.

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS

CRITERIA FOR VALIDITY ANDHAZARD EVALUATION CLASSIFICATION:
For a test to be valid, the following performance criteria apply:

- Not more than 10 % of the daphnids should show immobilisation or other signs of disease or stress in the controls.
- The dissolved oxygen concentration at the end of the test should be ≥3 mg/l in control and test vessels.
- EC50 is calculated with 95% confidence limits (p = 0.95).

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Nam	Organization	Date

TEST METHOD EVALUATION
Ecotoxicity

ENDPOINT: Acute toxicity, mortality

TEST METHOD NAME: Fish, Acute Toxicity Test

ENPOINT PARAMETER: LC50

REFERENCE: OECD Guideline for testing of chemicals 203, adopted 17.07.92

VALIDATION STATUS: OECD

IN VIVO / IN VITRO TEST METHOD: In vivo

EXPOSURE ROUTE(S): Directly through skin, gills, oral.

EXPOSURE VOLUME(S): Variable, depending on species and exposure tanks

ANIMAL / CELL CULTURE INFORMATION (INCLUDING NO. ANIMALS/CELL CULTURE CONC.):
One or more species may be used, the choice being at the discretion of the testing laboratory. Examples of fish recommended for testing are given in the guideline. The fish should be in good health and free from any apparent malformation, and must be obtained and held in the laboratory at least 12 days before they are used for testing. They must be held in water of the quality, temperature and photperiode to be used in the test for at least seven days immediately before, and least 80% O_2 of ASV. The fish should be fed three times per week or daily until 24 hours before the test is started. Following a 48-hours settling-in period, mortalities are recorded and the following criteria applied:

- greater than 10% of population in seven days: rejection of entire batch
- between 5 and 10% of population: acclimatisation continued for seven additional days
- less than 5% of population: acceptance of batch

NO. GENERATION OF ORGANISM TESTED: One

TEST DURATION: 96 hours

INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Oxygen meter, equipment for determination of water hardness, adequate temperature control, tanks made of chemically inert material and of a suitable capacity, etc.

TEST PRINCIPLES AND DESCRIPTION:
The fish are exposed to the test substance preferably for a period of 96 hours. Mortalities are recorded at 24, 48, 72 and 96 hours and the concentration which kill 50% of the fish (LC50) are determined where possible.

There should be at least five concentrations of the test substance in a geometric series with a factor preferably not exceeding 2.2. A range-finding test properly conducted before the definitive test enables the choice of the appropriate concentration range. Stock solutions of substances of low water solubility may be prepared by ultrasonic dispersion etc. If necessary, vehicles such as organic solvents, emulsifiers or dispersants of low toxicity to fish may be used. The test should be carried out without adjustment of pH.

At least 7 fish must be used at each test concentration and in the controls. There should be maximum loading of 1.0 g fish/litre for static and semi-static test. For flow-through systems higher loading can be accepted. The fish are inspected at least after 24, 48, 72 and 96 hour, when mortality and visible abnormalities are recorded. Fish are considered dead if there is no visible movement (e.g. gill movements) and if touching of the caudal peduncle produces no reaction. Visible abnormalities that should be recorded as loss of equilibrium, swimming behaviour, respiratory function, pigmentation, etc. Observations at three and six hours after the start of the test are desirable. Dead fish are removed when observed and mortalities are recorded. Measurement of pH, dissolved oxygen and temperature should be carried out at least daily.

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS

CRITERIA FOR VALIDITY AND HAZARD EVALUATION CLASSIFICATION:
For a test to be valid the following conditions should be fulfilled:

- Mortality in the controls should not exceed 10 % (one fish if less than ten are used) at the end of the test
- Constant conditions should be maintained as far as possible throughout the test. If necessary, semi-static or flow-through procedures should be used.
- The dissolved oxygen concentration should be ≥60%of the air saturation value throughout the test.
- There should be evidence that the concentration of the substance being tested has been satisfactorily maintained (≥80 % of the nominal concentration) over the test period. If the deviation is greater than 20%, results should be based on measured concentrations

The cumulative percentage mortality for each exposure period is plotted against concentration. Normal statistical procedures are employed to calculate LC50 with 95% confidence limits (p=0,95). When data obtained are inadequate for the use of standard statistical methods, the highest concentration causing no mortality and the lowest concentration producing 100% mortality should be used as an approximation for the LC50.).

SUITABILITY EXPOSURE OF AIR SAMPLES: No

PECIFIC PRECAUTIONS AND COMMENTS: Animal testing.

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Ecotoxicity

ENDPOINT: Acute toxicity, mortality

TEST METHOD NAME: Earthworm, Acute Toxicity Test

ENPOINT PARAMETER: LC50

REFERENCE: OECD Guideline for Testing of Chemicals 207, adopted 4 April 1984

VALIDATION STATUS:OECD

IN VIVO / IN VITRO TEST METHOD: In vivo

EXPOSURE ROUTE(S): Direct contact, skin and oral

EXPOSURE VOLUME(S): No information

ANIMAL / CELL CULTURE INFORMATION (INCLUDING NO. ANIMALS/CELL CULTURE CONC.):
The recommended test species of earthworm is *Eisenia foetida*. Although this is not a typical soil species, it occurs in soil rich in organic matter, and its susceptibility to chemicals resembles that of true soil-inhabiting species. *Eisenia foetida* has a short life cycle, hatching from cocoons in 3 to 4 weeks, and reaching maturity in seven to eight weeks at 20°C. It is very prolific, available commercially and can be bred readily in a wide range of organic waste materials. *Eisenia foetida* exists in two races which some taxonomists have separated into species. *E. foetida foetida*, and *E. foetida andre.* Other species may be used if the necessary methodology is available. Worms should be adult (at least two months old with clitellum) with an individual weight of 300 to 600 mg.

NO. GENERATION OF ORGANISM TESTED: One

TEST DURATION: Filter paper test: 72 h. Artificial soil test: 14 days

INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Filter paper, artificial soil substrate, glass test containers , illuminated cabinet/chamber controllable to ± 2°C with a light intensity of 400 to 800 lux, other standard laboratory equipment.

TEST PRINCIPLES AND DESPRIPTION:
This guideline includes two tests: a simple paper contact toxicity test (an optional initial screen test), and an artificial soil test that gives toxicity data more representative of natural.

Filter paper test:
The test substance is dissolved in water (if soluble up to a concentration of 1000 mg/l) or in a suitable organic solvent, to give a range of known concentrations. The solution is evaporated to dryness in a flat-bottomed glass vial lined with filter paper. Then, 1 ml of deionised water is added to each vial to moisten the filter paper. For each treatment, ten replicates, each consisting of one worm per vial, are

the minimum requirement. Tests are done in the dark at 20° and for a period of 48 hours with a further optional mortality assessment after 72 hours. Worms are classified as dead when they do not respond to a gentle mechanical stimulus to the front end. Any behavioural or pathological symptoms should be reported.

The artificial soil test:
The artificial soil test involves keeping earthworms in samples of a precisely defined artificial soil to which a range of concentrations of the test substance has been applied. Normally, five concentrations in a geometric series, at a ratio of at most 2.0. Mortality is assessed 7 and 14 days after application. One concentration resulting in no mortality and one resulting in total mortality should be used. A preliminary range-finding test is optional.

An emulsion or dispersion of the test substance in deionised water (or a suitable organic solvent if insoluble in water) is mixed with artificial soil or sprayed evenly over it. For each test, 750 g weight of the test medium is placed into each glass container and ten conditioned earthworms are placed on the test medium surface. Four replicates for each treatment are recommended. The containers are covered with perforated plastic film to prevent the test medium from drying and kept under the test conditions, 20° in continuous light, for 14 days. The mortality is assessed by emptying test medium onto a glass tray or plate, sorting worms from the medium and testing their reaction to a mechanical stimulus at the front end. After the 7-day assessment worms and medium are replaced in the test container. Any behavioural or pathological symptoms should be reported. At the end of the test the moisture content of the test medium should be assessed. Mortality and concentration data should be reported, and the median lethal concentration (LC50) and its confidence limits estimated.

HSE CONSIDERATIONS: Reference and test substance should be handled according to MSDS.

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:
For either test to be valid, the mortality in the controls should not exceed 10% at the end of either test. Results should include LC50, highest concentration causing no mortality, lowest concentration causing 100% mortality, average live weight, description of obvious physical or pathological symptoms or distinct changes in behaviour, moisture content of artificial soil at start and end and pH. LC50 of a reference substance should be determined occasionally as a means of assuring that the laboratory test conditions are adequate and have not changed significantly.

SUITABILITY EXPOSURE OF AIR SAMPLES: No. This Test Guideline can be used for substances that are either insoluble or soluble in water, although the method of application differs.

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Ecotoxicity

ENDPOINT: Visual assessment of seedling emergence, dry shoot weight (alternatively fresh shoot weight) and in certain cases shoot height. Assessment of visible detrimental effects on different parts of the plant
TEST METHOD NAME: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test
ENPOINT PARAMETER: ECx, ERx, NOEC, LOEC
REFERENCE: OECD Guidelines for the testing of chemicals 208, adopted 19 July 2006
VALIDATION STATUS: OECD. References to ISO 11269-1(1993), ISO 11269-2 (1995), ASTME 1963-98 (2002), US EPA FIFRA 40CFR (1982), US EPA OPPTS series 850 (1996), et al.
IN VIVO / IN VITRO TEST METHOD: In vivo
EXPOSURE ROUTE(S): seed surface, roots
EXPOSURE VOLUME(S): Depending on the test design
ANIMAL / CELL CULTURE INFORMATION (INCLUDING NO. ANIMALS/CELL CULTURE CONC.): The species selected should be reasonably broad, e.g., considering their taxonomic diversity in the plant kingdom, distribution, abundance, species specific life-cycle characteristics and region of natural occurrence, to develop a range of responses. A list of characteristics of the possible test species that should be considered in the selection, and some of the historically most used test species and potential non-crop species are summarized in this test guideline.
NO. GENERATION OF ORGANISM TESTED: One
TEST DURATION: 21 days, or more
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Environment chambers, phytotrons, or greenhouses. Apparatus for measuring/monitoring temperature, light, humidity, carbon dioxide concentration etc.
TEST PRINCIPLES AND BRIEF DESCRIPTION OF THE METHOD: The test assesses effects on seedling emergence and early growth of higher plants following exposure to the test substance in the soil (or other suitable soil matrix), it does not cover chronic effects or effects on reproduction (i.e. seed set, flower formation, fruit maturation). The test can be conducted in order to determine the dose-response curve, or at a single concentration/rate as a limit test according to the aim of the study.

The test conditions should approximate those conditions necessary for normal growth for the species and varieties tested. The emerging plants should be maintained under good horticultural practices in controlled growth facilities (recording of temperature, humidity, carbon dioxide concentration, light (intensity, wave length, etc) and light period, means of watering, etc.) to assure good plant growth as judged by the control plants of the selected species. Depending on the expected route of exposure and on physical properties as water solubility etc, the test substance is either incorporated into the soil or applied to the soil surface. Seeds of the same species are planted in pots. During the observation period, usually 14 to 21 days after 50% emergence of the seedlings in the control group, the plants are observed frequently (at least weekly and if possible daily) for emergence and visual phytotoxicity and mortality. At the end of the test, measurement of percent emergence and biomass of surviving plants should be recorded, as well as visible detrimental effects on different parts of the plant. The latter include abnormalities in appearance, e.g. stunted growth, chloris, discoloration, mortality, and effects on plant development. The final biomass can be measured using final average dry shoot weight of surviving plants, or alternatively fresh shoot weight. The height of the shoot may be another endpoint. A uniform scoring system for visual injury should be used to evaluate the observable toxic responses.

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS.

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:
In order for the test to be valid, the following criteria must be met in the controls:

- the seedling emergence is ≥70%
- the seedlings do not exhibit visible phytotoxic effects (e.g. chlorosis, necrosis, wilting, leaf and stem deformations) and the plants exhibit only normal variation in growth and morphology for that particular species
- the mean survival of emerged control seedlings is ≥90% for the duration of the study
- environmental conditions for a particular species are identical, growing media contain the same amount of soil matrix/support media/substrate from the same source.

Recorded endpoints are subjected to statistical analysis to determine ECx or ERx and their confidence limits. Where regression analysis, standard errors are required for the regression equation and individual parameter estimate. NOEC and LOEC can be calculated. A reference substance may be tested at regular intervals, to verify that performance of the test and the response of the particular test plants and the test conditions have not changed significantly over time.

SUITABILITY EXPOSURE OF AIR SAMPLES:
No, this test does not address plants exposed to vapours of chemicals.

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Ecotoxicity

ENDPOINT: Reproduction, number of living offspring per parent animal alive at termination.
TEST METHOD NAME: *Daphnia magna* Reproduction Test
ENPOINT PARAMETER: ECx, LOEC and/or NOEC.
REFERENCE: OECD Guidelines for testing of chemicals 211, adopted 3 October 2008
VALIDATION STATUS: This guideline is based on OECD Guideline 202, partII (1982), research activities due to the identification of the reasons for variable test results, ring-tests performed in 1992 and 1994, EPA/600/4-90/027F (1993), ASTM E729-88a(1988) et.al
IN VIVO / IN VITRO TEST METHOD: In vivo
EXPOSURE ROUTE(S): Directly, across surface
EXPOSURE VOLUME(S): At least 2 ml of test solution should be provided for each animal.
ANIMAL / CELL CULTURE INFORMATION (INCLUDING NO. ANIMALS/CELL CULTURE CONC.): The species to be used in the test is *Daphnia magna* Straus. Preferably, the clone identified by genotyping and belongs to Clone A (originated from IRCHA in France). At the start of the test, the animals should be less than 24 hours old and must not be first brood progeny. They should be derived from a healthy stock (i.e. showing no signs of stress such as high mortality, presence of males and ephippia, delay in the production of the first brood, discoloured animals, etc) that is maintained in culture conditions (light, temperature, medium, feeding and animals per unit volume) similar to those to be used in the test.
NO. GENERATION OF ORGANISM TESTED: Two
TEST DURATION: 21 days
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Test vessels, oxygen-meter, pH-meter, adequate apparatus for temperature control, equipment for the determination of TOC and COD, equipment for the determination of hardness of the water, apparatus for the control of the lighting regime and measurement of light intensity.
TEST PRINCIPLES AND DESCRIPTION: The primary objective of the test is to assess the effect of chemicals on the reproductive output of *Daphnia magna*. Young female *Daphnia* (the parent animals), are exposed to the test substance added to water at a range of concentrations. The test duration is 21 days. At the end of the test, the total number of living offspring produced per parent animal alive at

the end of the test is assessed. The reproductive output of the animals exposed to the test substance is compared to that of the control(s) in order to determine LOEC and hence the NOEC. In addition, the data are analysed using a regression model in order to estimate the concentration that would cause a x % reduction in reproductive output, i.e. ECx (e.g. EC50, EC20 or EC10). Other substance-related effects on parameters such as growth (e.g. length), and possibly intrinsic rate of increase, may also be examined.

Test solutions of the chosen concentrations are usually prepared by dilution of a stock solution Normally there should be at least five test concentrations arranged in a geometric series with a separation factor preferably not exceeding 3.2.The use of organic solvents or dispersants may be required in some cases, but every effort should be made to avoid the use of such materials. Parent animals are maintained individually, one per test vessel, with 50 - 100 ml of medium in each vessel. For semi-static tests, at least 10 animals individually held at each test concentration and in the control series. For flow-through tests, 40 animals divided into four groups of 10 animals at each test concentration. The offspring produced by each parent animal should preferably be removed and counted daily from the appearance of the first brood to prevent them consuming food intended for the adult. It is only the number of living offspring that needs to be counted, but the presence of aborted eggs or dead offspring should be recorded. Mortality among the parent animals should be recorded preferably daily, at least at the same times as offspring are counted. Growth measurements are highly desirable since they provide information on possible sublethal effects which may be more useful than reproduction measures alone. Other parameters that can be measured or calculated include time to production of first brood (and subsequent broods), number and size of broods per animal, number of aborted broods, presence of male neonates or ephippia and possibly the intrinsic rate of population .

Oxygen concentration, temperature, hardness and pH values should be measured at least once a week, in fresh and old media, in the control(s) and in the highest test substance concentration. During the test, the concentrations of test substance are determined at regular intervals.

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS.

CRITERIA FOR HAZARD EVALUATION CLASSIFICATION:
For a test to be valid, the following performance criteria should be met in the control(s):

- the mortality of the parent animals does not exceed 20% at the end of the test
- the mean number of live offspring produced per parent animal surviving at the end of the test is > 60.

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

Appendix F Biodegradation Test Evaluation Forms

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Primary biodegradation
TEST METHOD NAME: DOC Die-Away Test
ENPOINT PARAMETER: Dissolved Organic Carbon (DOC)
REFERENCE: OECD Guidelines for testing of chemicals 301A adopted 17 July 1992
VALIDATION STATUS: This guideline is related to OECD 301E, ISO Standard 7827, ASTM E1279-89, and US-EPA test 835-3170
SUITABILITY: Test substances with water solubility up to 100 mg/L
MEDIUM: Freshwater amended with mineral medium
INOCULUM: Activated sludge preconditioned to test conditions, but not pre-adapted to test substance Alternative: secondary effluent of sewage or surface water(inoculum in surface water may be concentrated)
TEST SUBSTANCE CONCENTRATION: 10-40 mg DOC/L
REFERENCE SUBSTANCE(S): Aniline, sodium acetate, sodium benzoate
CONTROLS: Test substance inhibition control (mixture of test and reference substances) Abiotic control (test substance in sterilised un-inoculated medium (sterile-filtered and with biocide) Adsorption control (test substance, inoculum and biocide)
TEST PRINCIPLE: Test substance in mineral medium is incubated at 22°C for 28 days. Degradation is measured by frequent DOC-analyses. Degree of biodegradation in determined by calculation of DOC-concentrations as percentage of initial concentration present
TEST DURATION: 28 days
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): DOC-analyser, shaking machine, filtration apparatus, Dissolved oxygen-meter; centrifuge, pH-meter
TEST DESCRIPTION: Inoculum is preconditioned 5-7 days at test temperature. Mineral medium (800 ml) with 10-40 mg DOC/L is added to 2-L conical flasks.

A number of 8 flasks are used; Flasks 1&2 with test substance and inoculum, flaska 3&4 with only inoculum, flasks 5 with reference substance, flask 6 abiotic control, flask 7 adsorption control and flask 8 inhibition control. DOC is measured in duplicate from each bottle appr. 5 times during the biodegradation period. Samples are filtered and analysed at the sampling day (alternatively stored at 2-4°C for max 48 hours or below -18°C for a longer period.

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS

VALIDITY OF TEST:
For a test to be valid, the following performance criteria apply:
- Pass level for ready biodegradability: 70 % DOC removal
- Pass levels must be reached with the "10-days" window (period from 10 % degradation to before 28 days)
- Pass level for reference substance (70 % DOC removal) should be reached within 14 days after start
- Differences between extremes of replicates should be < 20 %
- Inhibition test should show < 35 % inhibition of reference substance DOC removal

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Ultimate biodegradation
TEST METHOD NAME: CO_2 Evolution Test
ENPOINT PARAMETER: CO_2-analyses
REFERENCE: OECD Guidelines for testing of chemicals 301B adopted 17 July 1992
VALIDATION STATUS: This guideline is related to ISO/DIN 9439
SUITABILITY: Test substances with water solubility up to 100 mg/L, poorly soluble or adsorbing
MEDIUM: Freshwater amended with mineral medium
INOCULUM: Activated sludge preconditioned to test conditions, but not pre-adapted to test substance Alternative: secondary effluent of sewage or surface water(inoculum in surface water may be concentrated)
TEST SUBSTANCE CONCENTRATION: 10-20 mg DOC/L or TOC/L
REFERENCE SUBSTANCE(S): Aniline, sodium acetate, sodium benzoate
CONTROLS: Test substance inhibition control (mixture of test and reference substances) Abiotic control (test substance in sterilised un-inoculated medium (sterile-filtered and with biocide)
TEST PRINCIPLE: Test substance in mineral medium is incubated at 22°C for 28 days with continuous aeration of CO_2-free air at controlled rate. Degradation is determined from frequent measurements of CO_2 trapped in BaOH or NaOH. Trapped CO_2 is measured by titration of residual OH or as inorganic carbon (IC). Degree of biodegradation in determined by calculation of CO_2 relative to ThCO_2. Biodegradation may be supplemented by DOC-analyses at the start and the end of the degradation period.
TEST DURATION: 28 days
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Device for measuring CO_2, DOC-analyser (optional), magnetic stirrers, gas-adsorption bottles, device for controlling air flow, apparatus for CO_2 scrubbing, device for determination of CO_2 (titrimetrically or IC analyser.

TEST DESCRIPTION:
Inoculum is preconditioned 5-7 days at test temperature.
Mineral medium (2400 ml) with 10-20 mg DOC/L is added to 5-L flasks. Inoculum is added to give appr. 30 mg/L
A number of 7 flasks are used; Flasks 1&2 with test substance and inoculum, flasks 3&4 with only inoculum, flasks 5 with reference substance, flask 6 abiotic control, and flask 7 inhibition control.
CO_2-free air is bubbled through the flasks (30-100 ml/min). Analyses of CO_2 should be done every second day the first 10 days, then at least every fifth day.
On the measuring day titrate BaOH adsorbers with 0.05 M HCl and phenolphthalein as indicator. Alternatively, with NaOH as absorber, inject absorber into the IC-part of a carbon analyser. At the end of the test (28 days) analyse DOC.

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS

VALIDITY OF TEST:
For a test to be valid, the following performance criteria apply:
- Pass level for ready biodegradability: 60 % $ThCO_2$
- Pass levels must be reached with the "10-days" window (period from 10 % degradation to before 28 days)
- Pass level for reference substance (60 % $ThCO_2$) should be reached within 14 days after start
- Differences between extremes of replicates should be < 20 %
- Inhibition test should show < 25 % inhibition of reference substance CO_2 increase

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Ultimate and primary biodegradation
TEST METHOD NAME: Modified MITI Test (I)
ENPOINT PARAMETER: Biological oxygen demand (BOD) and DOC
REFERENCE: OECD Guidelines for testing of chemicals 301C adopted 17 July 1992
VALIDATION STATUS: Validated
SUITABILITY: Test substances with water solubility up to 100 mg/L, poorly soluble, adsorbing or volatile
MEDIUM: Freshwater amended with mineral medium
INOCULUM: Inocula from at least 10 separate source mixed thoroughly together – mainly polluted areas (sewage treatment works, industrial wastewater, rivers, lakes, seas.
TEST SUBSTANCE CONCENTRATION: 100 mg /L
REFERENCE SUBSTANCE(S): Aniline, sodium acetate, sodium benzoate
CONTROLS: “Non-biotic” control (test substance in water without inoculum
TEST PRINCIPLE: Test substance in mineral medium is incubated at 25°C for 28 days with continuous stirring and with specifically grown, but unadapted inoculum. Oxygen consumption is measured automatically over the test period while CO_2 is absorbed by soda lime. Biodegradation is expressed as oxygen uptake related to ThOD. Primary biodegradation may be determined by DOC-analyses.
TEST DURATION: 28 days
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Automated electrolytic BOD-meter or respirometer equipped with bottles, constant temperature room or water bath, membrane filtration asembly (optional), carbon analyser (optional)
TEST DESCRIPTION: Inocula are mixed, floating matter removed, and pH adjusted to 7. Inoculum is aerated for appr. 24 hours, 1/3 of the volume is then replaced by equal volume of 0.1% glucose/peptone/KPO_4. Procedure is repeated every day to maintain inoculum. A number of 6 flasks are used; Flasks 1 is test substance in water (100 mg/L), flasks 2, 3&4 test

substance in mineral medium (100 mg/L), flask 5 reference compound in mineral medium (100 mg/L9 and flask 6 mineral medium alone.
Inoculum (30 mg suspended solids) are added to flasks 2, 3, 4, 5 and 6. After assembling the units O_2 is read in all flasksby appropriate automated method. At the end of the test period pH and DOC are measured (nitrate and nitrite may be measured if nitrification is anticipated).

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS

VALIDITY OF TEST:
For a test to be valid, the following performance criteria apply:
- Pass level for ready biodegradability: 60 % ThOD
- Pass levels must be reached with the "10-days" window (period from 10 % degradation to before 28 days)
- Pass level for reference substance (60 % ThOD) should be reached within 14 days after start
- Differences between extremes of replicates should be < 20 %
- Inhibition test should show < 25 % inhibition of reference substance CO_2 increase

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Ultimate biodegradation
TEST METHOD NAME: Closed Bottle Test
ENPOINT PARAMETER: Biological oxygen demand (BOD)
REFERENCE: OECD Guidelines for testing of chemicals 301D adopted 17 July 1992
VALIDATION STATUS: Validated Related to ISO 10707
SUITABILITY: Test substances with water solubility up to 100 mg/L, poorly soluble, adsorbing or volatile
MEDIUM: Freshwater amended with mineral medium
INOCULUM: Inocula - secondary effluents from domestic sewage (alternatively surface water from river or lake); preconditioning 5-7 days to test conditions (not to test substance)
TEST SUBSTANCE CONCENTRATION: 2-5 mg /L (up to max 10 mg/L)
REFERENCE SUBSTANCE(S): Aniline, sodium acetate, sodium benzoate
CONTROLS: Inoculum blank (only inoculum, no test substance) Test substance inhibition control (mixture of Test and reference substances)
TEST PRINCIPLE: Test substance in mineral medium is incubated at 20°C for 28 days in closed air-tight bottles (no air bubbles present) and with a relatively small number of microorganisms from a mixed population. Oxygen consumption is measured at least weekly and is corrected for uptake by the inoculum blank. Biodegradability is expressed as the percentage of ThOD (alternatively as the percentage of COD).
TEST DURATION: 28 days
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Dissolved oxygen meter, constant temperature room or water bath, closed bottles (BOD bottles)
TEST DESCRIPTION: Mineral solutions with test or reference substances, and with inocula, are distributed on BOD-bottles as follows; at least 10 bottles with test substance and inoculum, 10 bottles with only

inoculum (blanks), 10 bottles with reference substance and inoculum, 6 bottles with inhibition control. Air bubbles are completely removed during distribiuion. Dissolved oxygen (DO) in 0-time samples are measured imediately. Duplicate samples are withdrawn every week or preferably every 3-4 days for DO measurements (the latter frequency allows for the 10-day window of ready biodegradability determination). BOD is determined by subtracting for oxygen depletion in inoculum blanks. Percentage biodegradation is determined as the percentage BOD related to ThOD or COD for the test substance.

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS

VALIDITY OF TEST:
For a test to be valid, the following performance criteria apply:
- Pass level for ready biodegradability: 60 % ThOD
- Pass levels must be reached with the "10-days" window (period from 10 % degradation to before 28 days)
- Pass level for reference substance (60 % ThOD) should be reached within 14 days after start
- Differences between extremes of replicates should be < 20 %
- Inhibition test should show < 25 % inhibition of reference substance CO_2 increase

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Primary biodegradation
TEST METHOD NAME: Modified OECD Screening Test
ENPOINT PARAMETER: Dissolved Organic Carbon (DOC)
REFERENCE: OECD Guidelines for testing of chemicals 301E adopted 17 July 1992
VALIDATION STATUS: This guideline is related to OECD 301A, ISO Standard 7827, ASTM E1279-89, and US-EPA test 835-3170
SUITABILITY: Test substances with water solubility up to 100 mg/L
MEDIUM: Freshwater amended with mineral medium
INOCULUM: Secondary effluent of sewage
TEST SUBSTANCE CONCENTRATION: 10-40 mg DOC/L
REFERENCE SUBSTANCE(S): Aniline, sodium acetate, sodium benzoate
CONTROLS: Test substance inhibition control (mixture of Test and reference substances) Abiotic control (test substance in sterilised un-inoculated medium (sterile-filtered and with biocide) Adsorption control (test substance, inoculum and biocide)
TEST PRINCIPLE: Comparable to OECD 301A (DOC Die-Away Test) but require a lower concentration of microorganisms. Test substance in mineral medium is incubated at 22°C for 28 days. Degradation is measured by frequent DOC-analyses. Degree of biodegradation in determined by calculation of DOC-concentrations as percentage of initial concentration present
TEST DURATION: 28 days
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): DOC-analyser, shaking machine, filtration apparatus, Dissolved oxygen-meter; centrifuge, pH-meter
TEST DESCRIPTION: Inoculum may be preconditioned 5-7 days at test temperature and is used in concentrations of 0.5 ml effluent/L mineral medium. Mineral medium (800 ml) with 10-40 mg DOC/L is added to 2-L conical flasks. A number of 8 flasks are used; Flasks 1&2 with test substance and inoculum, flasks 3&4 with only

inoculum, flasks 5 with reference substance, flask 6 abiotic control, flask 7 adsorption control and flask 8 inhibition control. DOC is measured in duplicate from each bottle appr. 5 times during the biodegradation period. Samples are filtered and analysed at the sampling day (alternatively stored at 2-4°C for max 48 hours or below -18°C for a longer period.

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS

VALIDITY OF TEST:
For a test to be valid, the following performance criteria apply:
- Pass level for ready biodegradability: 70 % DOC removal
- Pass levels must be reached with the “10-days” window (period from 10 % degradation to before 28 days)
- Pass level for reference substance (70 % DOC removal) should be reached within 14 days after start
- Differences between extremes of replicates should be < 20 %
- Inhibition test should show < 35 % inhibition of reference substance DOC removal

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Ultimate biodegradation (optional primary biodegradation)
TEST METHOD NAME: Manometric Respirometry Test
ENPOINT PARAMETER: Biological oxygen demand (BOD)
REFERENCE: OECD Guidelines for testing of chemicals 301F adopted 17 July 1992
VALIDATION STATUS: Validated
SUITABILITY: Test substances with water solubility up to 100 mg/L, poorly soluble, adsorbing or volatile
MEDIUM: Freshwater amended with mineral medium
INOCULUM: Activated sludge preconditioned to test conditions, but not pre-adapted to test substance Alternative: secondary effluent of sewage or surface water(inoculum in surface water may be concentrated)
TEST SUBSTANCE CONCENTRATION: 100 mg (giving at least 50-100 mg ThOD/L)
REFERENCE SUBSTANCE(S): Aniline, sodium acetate, sodium benzoate
CONTROLS: Inoculum blank (only inoculum, no test substance) Reference (reference compound and inoculum) Abiotic control (Test substance and biocide) Inhibition control (test substance and reference substance mixed)
TEST PRINCIPLE: Test substance in mineral medium is incubated with constant stirring for up to 28 days in closed flasks. The consumption of oxygen is determined either by measuring the quantity of oxygen (produced electrolytically) required to maintain constant gas volume in a respirometer flask, or from the change in volume or pressure (or a combination of the two) in an apparatus. Evolved CO_2 is absorbed by KOH or another suitable absorbent. The amount of oxygen taken up by the microorganisms is expressed as percentage of ThOD (or COD). Primary biodegradation may also optionally be determined if DOC-analyses are performed at the start and end of the test.
TEST DURATION: Up to 28 days

INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.):
Respirometer, temperatrure control, membrane filtration assembly (optional) and DOC analyser (optional)

TEST DESCRIPTION:
Mineral solutions with test or reference substances (100 mg(L), and with inocula, are introduced in at least duplicate in respirometer flasks. CO_2-absober (e.g. KOH) is added to the CO_2 absorber compartments of the flasks. The flasks are distributed as follows: Flasks 1&2-test substance and inocukum, flasks 3&4 – only inoculum, flask 5 – reference compound and inoculum, flask 6 – abiotic control, flask 7 – inhibition control. Activated sludge or other inocula are added in concentrations of solids less than 30 mg/L. The equipmenmt is assembled and incubated air-tight at selected temperature. With an automated respirometer a continuous record of oxygen uptake is recorded until the 10—day window is achieved. For non-automated systems daily recordings are made for up to 28 days. Samples may be withdrawn at the start and end of the test for DOC-analyses. Calculate BOD and determine this as percentage of ThOD (or COD).

HSE CONSIDERATIONS: Reference and test substances should be handles according to MSDS

VALIDITY OF TEST:
Oxygen uptake in inoculum blank should be < 60 mg/L in 28 days (higher uptake require
If pH is outside the range 6 to 8.5 and oxygen uptake in by the test substance is < 60 mg/L a new test may be conducted with lower concentration of test substance

Other validity criteria:
- Pass level for ready biodegradability: 60 % ThOD
- Pass levels must be reached with the “10-days” window (period from 10 % degradation to before 28 days)
- Pass level for reference substance (60 % ThOD) should be reached within 14 days after start
- Differences between extremes of replicates should be < 20 %
- Inhibition test should show < 25 % inhibition of reference substance CO_2 increase

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Primary biodegradation
TEST METHOD NAME: Inherent Biodegradability: Modified SCAS Test
ENPOINT PARAMETER: Dissolved Organic Carbon (DOC)
REFERENCE: OECD Guidelines for testing of chemicals 302A adopted 12 May 1981
VALIDATION STATUS: Validated. This guideline is also related to ISO Standard 9887, ASTM E1625-94, US-EPA test 835-3210 and US-EPA test 835-5045
SUITABILITY: Test substances with water solubility at least 20 mg/L
MEDIUM: Freshwater amended with mineral medium
INOCULUM: Activated sludge from a sewage treatment plant.
TEST SUBSTANCE CONCENTRATION: 20 mg mg/L
REFERENCE SUBSTANCE(S): May be used; no specific chemicals recommended
CONTROLS: Sewage sample without test substance as reference for DOC measurements
TEST PRINCIPLE: Activated sludge from a sewage treatment plant is placed in an aeration unit (SCAS) for 23 hours. Aeration is stopped, sludge allowed to settle and supernatant liquid removed. Sludge remaining in the chamber is mixed with an aliquot of test substance and the cycle repeated. Biodegradation is established by determination of DOC in the supernatant and compared to DOC in a control sewage sample without test substance.
TEST DURATION: up to 12 weeks
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): DOC-analyser, system for semi-continuous activated sludge (SCAS)
TEST DESCRIPTION: Activated sludge (150 ml) from a sewage treatment plant is placed in a semi-continuous activated sludge(SCAS) aeration unit for 23 hours. Aeration is stopped, sludge allowed to settle and 100 ml

supernatant liquid removed and new 100 ml sludge introduced. Aeration is restarted and the unit fed daily until a clear supernatant liquor is obtained on settling (may take 2 weeks) by which DOC in supernatant liquor should be < 12 mg/L. Sludge remaining in the chamber is mixed with an aliquot of test substance and the cycle repeated. Individual settled sludges are mixed and 50 ml of the composite sludge added to each unit.

100 ml settled sewage are added to the control units and 95 ml plus 5 ml of the test compound stock solution (400 mg/L) to the test unit. Aeration is started for 23 hours, the sludge allowed to settle for 45 minutes, and the supernatant drawn off and analysed for DOC. This fill and draw procedure is repeated throughout the test.

Percentage biodegradability is determined as DOC depletion corrected for DOC in control unit.

HSE CONSIDERATIONS: Test substances (and reference substance in used) should be handles according to MSDS

VALIDITY OF TEST:
For a test to be valid, the following performance criteria apply:
- Pass level for inherent biodegradability: 20 % DOC removal
- Pass level for ultimate biodegradability is 70 % DOC removal

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Primary biodegradation
TEST METHOD NAME: Inherent Biodegradability: Zahn-Wellens/EMPA Test
ENPOINT PARAMETER: Dissolved Organic Carbon (DOC)
REFERENCE: OECD Guidelines for testing of chemicals 302B adopted 17 July 1992
VALIDATION STATUS: Validated. This guideline is also related to ISO Standard 9888, and US-EPA test 835-3200
SUITABILITY: Test substances with water solubility at least 50 mg DOC/L
MEDIUM: Freshwater amended with mineral medium
INOCULUM: Activated sludge from a sewage treatment plant.
TEST SUBSTANCE CONCENTRATION: 50-400 mg mg/L
REFERENCE SUBSTANCE(S): Ethylene glycol, diethylene glycol, lauryl sulfonate, aniline.
CONTROLS: Inoculum blank: Sewage sample without test substance as reference for DOC measurements Procedure control: Reference compound with inoculum
TEST PRINCIPLE: A mixture containing test substance in mineral medium and a relatively large amount of activated sludge is agitated and aerated at 20-25°C for up to 28 days. Biodegradation is monitored by DOC (or COD) analyses of samples taken out daily. DOC/COD corrected for DOC/COD in inoculum blanks are used for determination of biodegradability.
TEST DURATION: up to 28 days
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): DOC-analyser, stirring systems, aeration system (compressed air)
TEST DESCRIPTION: Activated sludge (BOD_5 < 25 mg/L) is washed twice with mineral medium and sludge separated by centrifugation. The sludge should be used within 6 hours. To cylindrical glass vessels (1 - 5 L) add 500 ml mineral medium, test substance and inoculum to

reach 50 to 400 mg DOC/L and 0.2-1.0 g dry matter/L. A final volume between 1 and 5 L is added up with mineral medium (normally 2 L is necessary) Set up 5 test vessels as follows: 2 vessels with test substance and inoculum (test suspension), 2 vessels with inoculum alone /inoculum blank) and 1 vessel with reference substance and inoculum (procedure control).

Run the test for up to 28 days at 20-25°C. Aerate with humidified air and ensure that suspension does not settle. Sampling are performed daily for determination of DOC or COD.

Percentage biodegradability is determined as DOC (or COD) depletion corrected for DOC (or COD) in blank.

HSE CONSIDERATIONS: Test substances (and reference substance in used) should be handles according to MSDS

VALIDITY OF TEST:
For a test to be valid, the following performance criteria apply:
- Pass level for inherent biodegradability: 20 % DOC removal
- Pass level for ultimate biodegradability is 70 % DOC removal

Biodegradation of reference compound(s) should be 70 % within 14 days

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Ultimate biodegradation
TEST METHOD NAME: Inherent Biodegradability: Modified MITI Test (II)
ENPOINT PARAMETER: Biological oxygen demand (BOD)
REFERENCE: OECD Guidelines for testing of chemicals 302C adopted 12 May 1981
VALIDATION STATUS: Validated.
SUITABILITY: : Test substances with water solubility up to 100 mg/L, poorly soluble, adsorbing or volatile
MEDIUM: Freshwater amended with mineral medium
INOCULUM: Activated sludge from a sewage treatment plant.
TEST SUBSTANCE CONCENTRATION: 30 mg mg/L
REFERENCE SUBSTANCE(S):): Aniline, sodium acetate, sodium benzoate
CONTROLS: “Non-biotic” control (test substance in mineral water without inoculum) Reference (reference substance in mineral medium with inoculum) Blank control (Mineral medium with inoculum – no test substance)
TEST PRINCIPLE: Test substance in mineral medium is incubated at 25°C for 28 days in an automated closed BOD system with continuous stirring and with specifically grown, but unadapted inoculum. Oxygen consumption is measured automatically over the test period while CO_2 is absorbed by soda lime. Biodegradation is expressed as oxygen uptake related to ThOD. Primary biodegradation may be determined by DOC-analyses.
TEST DURATION: 14 - 28 days
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Automated BOD system DOC-analyser (optional)
TEST DESCRIPTION: An automated BOD-system with 6 bottles are used. The system includes: Bottle 1 – Deionized water and test substance, bottles 2, 3, and 4 – mineral medium with test substance and inoculum, bottle

5 – mineral medium with reference substance and inoculum, bottle 6 – mineral medium with inoculum.

Activated sludge and surface water are sampled from 10 different locations (e.g. city sewage plant, industry sewage plant, 3 rivers, one lake and 2 inland seas). Inocula are mixed, floating matter removed, and pH adjusted to 7. Inoculum is aerated for appr. 24 hours, 1/3 of the volume is then replaced by equal volume of 0.1% glucose/peptone/KPO_4 (synthetic sewage). Culturing is conducted at 25°C. Procedure is repeated every day to maintain inoculum.
A number of 6 flasks are used; Flasks 1 is test substance in water (30 mg/L), flasks 2, 3&4 test substance in mineral medium (30 mg/L), flask 5 reference compound in mineral medium (30 mg/L9 and flask 6 mineral medium alone.
Inoculum (100 ppm suspended solids) are added to flasks 2, 3, 4, 5 and 6. After assembling the units O_2 is read in all flasksby appropriate automated method. At the end of the test period pH and DOC are measured (nitrate and nitrite may be measured if nitrification is anticipated).

HSE CONSIDERATIONS: Test substances (and reference substance in used) should be handles according to MSDS

VALIDITY OF TEST:
Biodegradation of reference compound(s) should be 40 % after 7 days and 65 % within 14 days

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

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Name	Organization	Date

TEST METHOD EVALUATION
Biodegradability

DEGRADABILITY: Primary biodegradability (transformation) in Soil
TEST METHOD NAME: Aerobic and Anaerobic Transformation in Soil
ENPOINT PARAMETER: Measurement of test substance or (or $^{14}CO_2$ evolution if ^{14}C-labelled test compound is used)
REFERENCE: OECD Guidelines for testing of chemicals 307 adopted 24 April 2002
VALIDATION STATUS: Validated.
SUITABILITY: Pure test compound with known solubility characteristics, or ^{14}C-labelled test substance with the ability to be dissolved in water or in acetone
MEDIUM: Soil, three different types. The examples below are from OECD 304A. a) Alfisoil (pH 5.5-6.5, organic C 1-1.5 %, clay 10-20 %, cation exchange capacity 10-15 mval), b) Spodosoil (pH 4.0-5.0, organic C 1.5-3.5 %, clay < 10 %, cation exchange capacity < 10 mval), c) Entisoil (pH 6.6-8.0, organic C 1-4 %, clay 11-25 %, cation exchange capacity > 10 mval)
INOCULUM: Soil (see above)
TEST SUBSTANCE CONCENTRATION: E.g. appr. 1 mg test substance per kg soil in a 10 cm layer (?). For radioactive test substances: 100 µL of test compound with radioactivity of 37-185 KBq/100 µl (ca. 1-5 µCi/100 µl).
REFERENCE SUBSTANCE(S): Recommended (no specific chemicals described)
CONTROLS: Sterilised soil and samples for abiotic degradation control. Soil without test substance added.
TEST PRINCIPLE: Soils samples are treated with test substance and incubated in the dark in Biometer-type flasks or in flow-through systems under controlled laboratory conditions (constant temperature and soil moisture). After appropriate time intervals soil samples are extracted and analysed for the parent substance and for transformation products (volatile compounds may also be analysed). If ^{14}C-labelled test substance are used mineralisation may be determined as release of $^{14}CO_2$ from the test substance is measured by means of alkali absorption and liquid scintillation counting.
TEST DURATION: Up to 120 days at 20 ± 2°C (10 ± 2°C may be used for colder climates), or after 90 % depletion
INSTRUMENT REQUIREMENTS (EXPOSURE, MEASUREMENTS ETC.): Analytical instrument for

quantification of parent test substances (e.g. GLC, HPLC, TLC-equipment), liquid scintillation counter (if ^{14}C-labelled test substances are used), instruments for identification purposes (e.g. MS, GC-MS, HPLC-MS, NMR), oxidiser for combustion of radioactive material (if necessary), centrifuge, extraction apparatus (e.g. Soxhlet), instrumentation for concentration (e.g. Rotavapor), water bath, mechanical mixing device

TEST DESCRIPTION:
For transformation studies under aerobic conditions soil moisture must be adjusted (pF between 2.0 and 2.5), while soil is flooded for anaerobic conditions.

About 50-200 g soil (dry weight) is placed in each incubation flask, and the soil treated with test substance. Test substance may be dissolved in water, in an organic solvent (the solvent must be evaporated before application and not interfere with biodegradation or analytical results), or be added to the soil as a solid. The soil is thoroughly mixed with the test substance (small aliquotes of the soil should be tested for homogeneous application of test substance). The test substance treatment rate should correspond to the highest relevant application rate, and to an appropriate depth in the soil (e.g. 10 cm depth).

Also untreated soil samples (no test substance added) are treated in the same manner. If test substance is dissolved in solvent this control should include solvent treated in the same way as the test substance.

The flasks with treated soil are either attached to a flow-through system or incubated as individual entities (Biometer flasks).

Duplicate samples are collected at appropriate times and analysed for test substance and/or transformation products. Sampling frequency may be every 7 day during the first month and every 14 day after one month. A typical test sampling may be: day 0, 2, 3, 7, 14, 21, 28, 2 months, 3 months, etc.). Biodegradation rates are calculated by first-order rate kinetics, and including calculations of transformation half-life (DT_{50}). Major transformation products may be identified, if possible.

If different temperatures are used, transformation rates should be described as a function of temperature, using the Arrhenius relationship.

HSE CONSIDERATIONS: Test substances (and reference substance in used) should be handled according to MSDS. Special precautions should be used for the handling of radioactive material

VALIDITY OF TEST:
None described.

SUITABILITY EXPOSURE OF AIR SAMPLES: No

SPECIFIC PRECAUTIONS AND COMMENTS:

This form has been edited by:

Name	Organization	Date

Appendix G **Report on health and environmental Risk Assessment**

Hazard and risk assessment approaches

Contents

1. Introduction

Risk assessments vary widely in scope and application. Some look at single risks in a range of exposure scenarios whereas others are site-specific and look at the range of risks posed by an installation. In broad terms risk assessments are carried out to examine the effects of an agent on humans (Health Risk Assessment) and ecosystems (Ecological Risk Assessment). Risk assessment is carried out to enable a risk management decision to be made. Risk management is the decision-making process through which choices can be made between a range of options which achieve the "required outcome", and should result in risks being reduced to an "acceptable" level (EEA, 1998).

2. Risk assessment for human health

The aim of a risk assessment is to combine characteristics of chemical hazards with expected exposure scenarios. The product is to establish a guideline for safe use of the chemical. The guideline can be a regulation as to its safe use in products or exposure limits in food, air and water. Risk assessment is a combination of the likelihood of an occurrence and the severity of the consequences. Thus, it is based on the hazard profile of a chemical as well as a set potential exposure scenarios. A risk assessment is therefore connected to what is considered as critical effects of the chemical as well as the intended use. For a given hazardous chemical the guideline will differ considerably depending on e.g. the population for which it is intended. As an example; we would not recommend the use of a carcinogen in children's toy but we accept it as an intermediary product in industry where the workers are properly educated and protected. In conclusion, the risk assessment is not a uniform exercise but rather directed to specific situations or populations.

In the current project chemicals are to be release to the atmosphere. The plant is part of a large petrochemical based industrial area situated in a rural area. The population of potential exposure will reflect any population as to gender, age and health status. We should therefore consider exposure as air contamination to the general public, and any guideline should have the general population rather than the occupationally exposed as its "target" population.

Some of the flue emission substances may precipitate and find their way through water or the food chain to people. Thus, exposure may go beyond direct inhalation. However, in the present – first attempt – risk assessment we have addressed this as an inhalation problem only.

Whenever we have had to do an "expert opinion" on matters that cannot be readily converted into some classification we have chosen to be very conservative and make potential errors on the safe side. Thus, the reader should assume that any later revisions resulting from more data should increase rather than decrease the ambient air standard that we suggest in this chapter.

The chemicals that have been identified as potential releases from the plant represent a variety of health effects:

- Irritant to skin and airways
- Sensitizers to skin and airways
- Systemic toxicity after oral, percutaneous and inhalation exposure
- Carcinogenic
- Mutagenic
- Reproductive effects

Some of these effects can only be observed after exposure to relatively high concentrations during acute exposure.

Exposure to the general population around an industrial facility like Mongstad is characterized as low-level continuous exposure and it is not expected that acute effects should be observed. We therefore need to focus on compounds causing effects that may occur after long-term and low level exposure. Such effects include carcinogenesis, mutagenesis and changes in fertility and reproduction and inhalation sensitization.

The aim of risk assessment in the current project is:

- determine the critical effect
- determine a no effect dose for the most critical effect
- suggest an ambient air standard
- compare the ambient air standard with the expected flue gas concentration

The critical effect

This project specifies a total of 13 substances. The toxic effects noted after a database and literature search includes all endpoints presented in the introduction above. However, there are two chemical groups that deserve special attention:

- nitrosamines which include both acute and serious long term effects
- nitramines where data is very sparse, but long term serious effects is to be expected.

We suggest that genetic effects (mutagenic and carcinogenic) and effects on reproduction are considered as the critical effects for the risk assessment.

1.1 Risk assessment for mutagenic/carcinogenic compounds following ECHA and REACH

Guidelines

There are several Guidelines for Risk assessment (RA) of chemicals and substances. Generally, the recommended risk assessment techniques take into account the inherent toxicity of a substance as well as the type and degree of exposure. Risk models incorporates assumptions that will nearly always over predict health risks and thus to assure the outcome is protective of health. REACH (Annex I, 1.0.1) defines

the Derived No-Effect Level (DNEL), i.e. the level of exposure above which humans should not be exposed. In the risk characterisation, the exposure of each human population likely or known to be exposed is compared with the appropriate DNEL. The risk to humans is considered to be adequately controlled if the exposure levels estimated do not exceed the appropriate DNEL (REACH Annex I, 6.4).

Whereas the former legislation on new and existing substances required a comprehensive RA and a risk characterisation (RC) for all relevant toxicological effects, REACH requires a RC for the leading health effect (i.e., the toxicological effect that results in the most critical DNEL) for a given exposure pattern (such as duration, frequency, route of exposure and exposed human population) which is associated with an exposure scenario. In case of flue gas compounds both mutagenicity/carcinogenicity as well as reproductive toxicity are consider as worst effects endpoints.

Derivation of the dose-descriptor

The data for calculating dose-descriptors come preferentially from lifetime oral or inhalation studies according to Annex V of Directive 67/548/EEC or other accepted guidelines (e.g. OECD guidelines). For a substance considered genotoxic any tumour type observed in an animal bioassay is taken as relevant to humans and as starting point for a dose-descriptor determination, unless evidence to deviate from this approach is considered sufficiently convincing. This also concerns non-genotoxic carcinogens with tumour promoting mode of action.

The specific dose descriptors:

LD50:	Median lethal dose. The dose causing 50 % lethality
LOAEL:	Lowest Observed Adverse Effect Level
NOEL:	No Adverse Effect Level (NOEL) - the highest dose with no toxic effects
BMD:	The Benchmark dose; BMD concept involves fitting a mathematical model to dose-response data. The BMD is defined as the dose causing a predetermined change in response.
BMD10:	The Benchmark-dose associated with a 10% response (for tumours upon lifetime exposure after correction for spontaneous incidence, for other effects in a specified study)
BMDL10:	Defined as the lower 95% confidence dose of a Benchmark-dose representing a 10% tumor response upon lifetime exposure, i.e. the lower 95% confidence dose of a BMD10.
ED10:	Effective dose 10 %; a dose representing an increased incidence of 10 % due to a specific exposure (e.g., to a chemical).
TD50:	The median toxic dose of a drug or toxin is the dose at which toxicity occurs in 50% of cases
DNEL:	Derived no effect-level (DNEL)
DMEL:	Derived Minimal-Effect Level; For non-threshold effects, the underlying assumption is that a no-effect-level cannot be established and a DMEL therefore expresses an exposure level corresponding to a low, possibly theoretical, risk, which should be seen as a tolerable risk.
T25:	The dose-descriptor value T25 is defined as the chronic dose rate that will give 25% of the animals' tumours at a specific tissue site and is calculated from the tumour incidence at the selected tumorigenic using linear intrapolation or extrapolation (Dybing et al. 1997).

In the European Guidelines the T25 dose-descriptor is in use for concentration limits of carcinogens, for non-threshold carcinogens and for risk characterization of chemicals in general (Annex I of Directive 67/548/EEC, EC, 1998; EC Regulation 1488/94; EC, 1994; SCCNFP/00690/03). The BMD10 has regulatory use as its lowest confidence value BMDL10 for the assessment of risks of food ingredients (EFSA, 2005) and comparable dose descriptor, ED10 (LED10), in cancer risk assessment practice by Environmental Protection Agency (US EPA, 2005).

For derivation of the DNEL (or equivalent dose descriptor T25, ED10, BMD10) the leading health effect for a given exposure pattern (exposure route, population and duration) needs to be selected. For some compounds DNEL cannot be derived either because not available data, data for one or several endpoints are missing or because of non-threshold effects. This is especially the case for the endpoints mutagenicity and carcinogenicity when involving a non-threshold mode of action (REACH Annex I, 1.4.1). Due to the high uncertainties in establishing safe exposure levels, for the non-threshold compounds, a substantially different approach is needed in relation to assessing risk. RA is usually derived, if possible, from long-term animal studies but more recently in vitro studies and more adequate human epidemiological studies data are recommended to be used to determine the NOEL and DNEL. A large safety factor is then added — to arrive at a safe level for humans.

The safety (assessment) factor

The safety (assessment) factor (AF) is built in partly to account for the differences between animals and humans, and also to allow for the variability between different populations, and individual variations among people, such as age, genetic background, health and how well nourished they are. The safety factor also account for many uncertainty factors, such as the variability in the experimental information and or inter and intra-species variation (including individual susceptibility); the nature and severity of the effect; the sensitivity of the human (sub-) population to which the quantitative and/or qualitative information on exposure applies, etc. DNELs must consider populations (workers, consumers, general population), exposure routes (inhalation, dermal/eye, oral) and duration of exposure.

1.2 The worst case approach

In some cases we do not have appropriate data for each compound in order to develop DNEL or DMEL values as described by REACH. Alternatives are then:

- Read-across from similar substances
- Use of established standards, e.g. OEL

Both these procedures have been discussed and accepted by REACH as valid for preliminary risk assessments (Guideline document chapter R4, section R.4.3.2.2 for grouping of substances and chapter R8, appendix R8-13 for the use of OEL)

When there is an urgent need to develop a preliminary risk assessment a hazard profile for a group of substances can be developed on the basis of worst case criteria. For a given group of chemicals which have the same functional group of toxicity we may combine the data from individual members of that group into a hazard profile. We may then use whatever exposure standard is available (occupational or general public) for individual members of that group to develop an interim exposure standard which applies to all members of that chemical group. Applying such a procedure may cause some chemicals to

receive a stricter regulation than needed – it is thus an approach where you will err on the safe side. In the present case such an approach can be applied to nitrosamines and nitramines. It should be reiterated that this is a provisional and temporary risk assessment until such time that appropriate data is available.

3. Risk assessment for the environment

3.1 Summary

Environmental risk assessment involves the assessment of the risks posed by the presence of substances released to the environment, on all living organisms in the variety of ecosystems which make up the environment. Environmental risk assessment methodology has been developed from methods established for human health. Health risk assessment is concerned with individuals and morbidity and mortality, where environmental risk assessment is concerned with populations and communities and the effects of substances on a number of endpoints, usually mortality. Environmental risk assessment must take into account a high number of organisms; all with varying sensitivities to chemicals and various groups have different exposure scenarios. Because of the difficulty in obtaining toxicity data on all organisms in an ecosystem, the usual practice is to use data on selected organisms as representatives for the whole system (EEA, 1998).

There are a number of challenges involved when performing an environmental risk assessment, i.e.:

- Determining the effects at population and community level;
- Selection of end-points;
- Selection of species representative for the system;
- The selection of field, laboratory, mesocosm and microcosm tests;
- The incorporation of resilience and recovery factors of the ecosystem

3.2 Definitions of environmental hazard and environmental risk

The use of the terms ‘hazard’ and ‘risk’ are frequently confused and therefore misused with the field of environmental assessment. This section aims to define the difference between the two terms, and how they will be used within this document. The term hazard is commonly defined as "the potential to cause harm". A hazard can be defined as "a property or situation that in particular circumstances could lead to harm" (EEA, 1998). The term risk is used in everyday language to mean "chance of damage / disaster". When used in the process of risk assessment it has specific definitions, the most commonly accepted being "The combination of the probability, or frequency, of occurrence of a defined hazard and the magnitude of the consequences of the occurrence" (EEA, 1998). Therefore, the following summary of hazard and risk can be used:

- Hazard is the potential to cause harm.
- Risk is the likelihood of harm.

If all other factors are equal, especially the exposures and the organisms subject to them, then the risk is proportional to the hazard. However, all other factors are rarely equal. Today risk-based assessment approaches are preferred prior to hazard-based approaches. This is partly due to the recognition that for many environmental issues a level of zero risk is unobtainable or not necessary for human and environmental protection and that a certain level of risk can be accepted.

3.3 Constituent elements of environmental risk assessment

The process of environmental risk assessment described by the OECD (OECD, 2010) and EU (EU TGD, 2003) includes four steps:

- hazard identification
- hazard characterisation
- exposure assessment
- risk characterisation

The first two steps are regarded as the process of 'hazard assessment'.

Hazard assessment
The OECD (OECD, 2010), describes the process of environmental hazard assessment as follows:

"Identifying and characterising the inherent properties of chemical substances is basically the first step of environmental risk assessment. Environmental hazard assessment (hazard identification and hazard characterisation) involves gathering or generating and evaluating data of chemical substances and concluding on their inherent eco-toxicological effects and environmental fate."

Exposure assessment
The OECD (OECD, 2010), describes the process of environmental exposure assessment as follows:

"Exposure assessment involves estimating or predicting the extent of exposure of chemicals to the target species and/or the environment through its production, use and disposal."

Risk characterisation
The OECD (OECD, 2010), describes the process of environmental exposure assessment as follows:

"Risk characterisation is the qualitative and, wherever possible, quantitative determination of the probability of occurrence of the adverse effects of chemicals to the environment under predicted exposure conditions. This process is based on outcomes of the previous steps, i.e. environmental hazard and environmental exposure assessment."

This final step is a combination of environmental hazard and environmental exposure as indicated in *Figure 1*. This implies that there is no direct relation between hazard and risk; a chemical with a high potential hazard may have a small environmental risk if the (probability of) environmental exposure is very small. Accordingly a chemical with a low potential hazard may have a have a high environmental risk if the exposure is high. The present chapter focuses on identifying the potential hazard of the CO_2 capture plant releases to air.

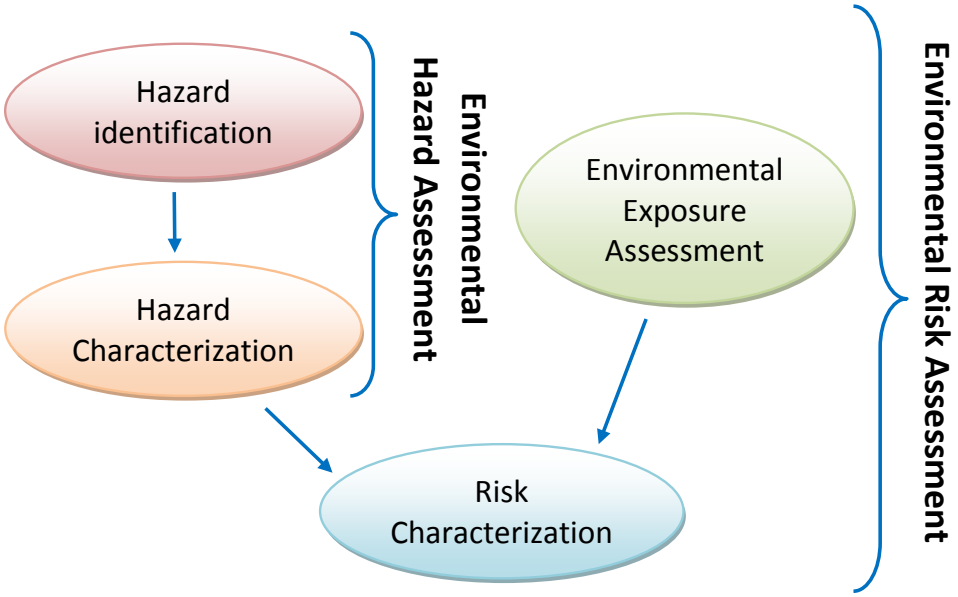

Figure 1. The main steps in risk assessment (adopted from OECD)

3.4 Review of available environmental risk assessment methods

This section will provide an overview of the main environmental risk assessment methods available. These have all been prepared and adopted by widely known government bodies such as the European Union and the USA.

3.4.1 US Environment Protection Agency (EPA) guidelines

The US Environmental Protection Agency has published a number of guidelines for risk assessments, where the most relevant guidelines are:

- Guidelines for exposure assessment (EPA/600/Z-92/001 May 1992)
- Guidelines for Reproductive Toxicity Risk Assessment (EPA/630/R-96/009 October 1996)
- Guidelines for Carcinogen Risk Assessment (EPA/630/P-03/001F March 2005)
- Guidelines for Developmental Toxicity Risk Assessment (EPA/600/FR-91/001 December 1991)
- Guidelines for Ecological Risk Assessment (EPA/630/R-95/002F April 1998)
- Supplementary Guidance for Conducting Health Risk Assessment of Chemical Mixtures (EPA/630/R-00/002 August 2000)

As described by EPA, Environmental Risk Assessments are typically iterative processes and include a number of steps:

- Problem Formulation
- Analysis
    - Characterization of exposure
    - Characterization of effects
- Risk characterization

- Communication of results
- Risk management

This is schematically shown in *Figure 2*.

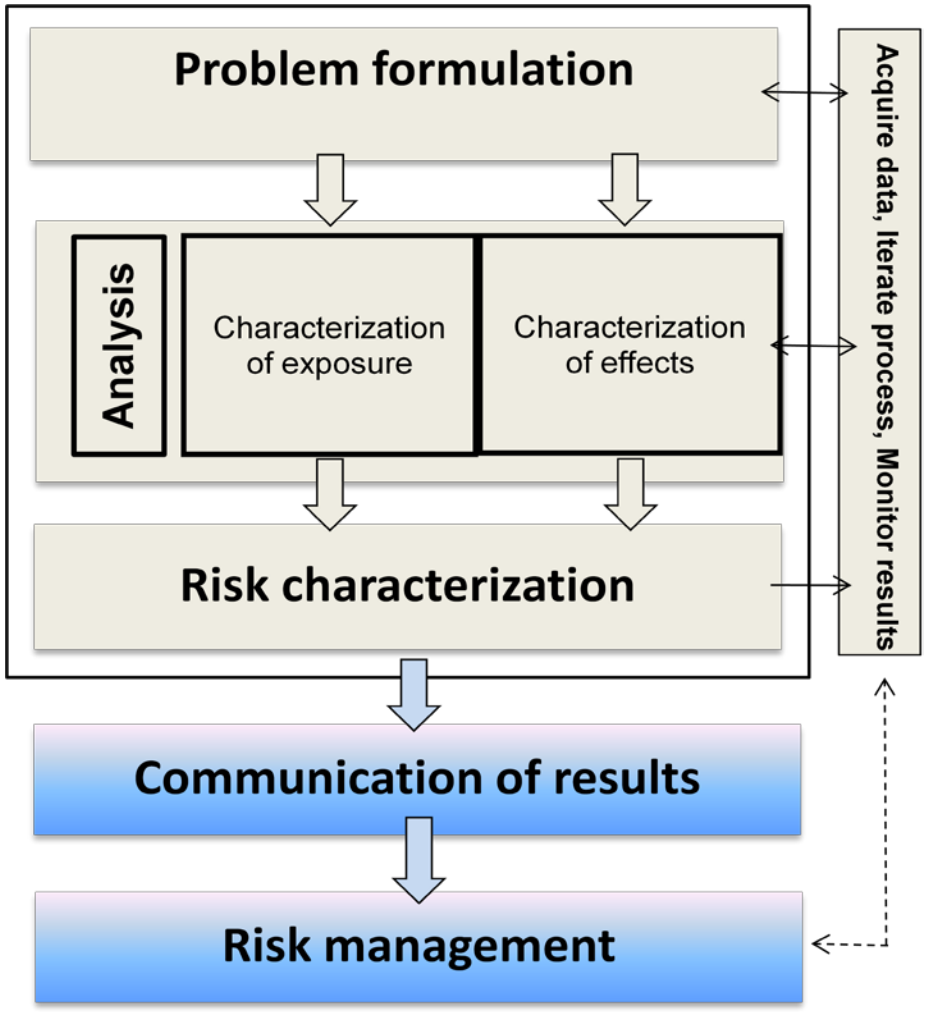

Figure 2. Typical steps in ecological risk assessment. Adopted from EPA/630/R-95/002F.

These guidelines can be regarded as a flexible framework, enabling users to establish risk assessment methods suited for specific problems. There are no described methods that can be used directly for amine-based carbon capture processes without carrying out the necessary steps involved. The methodology described by EPA is a very good basis for establishment of Risk Assessments, and is in accordance with the more operative PEC/PNEC approach described in the EU TGD (EU TGD, 2003).

3.4.2 European Union Technical Guidance Document (EU TGD)

3.4.2.1 Summary

Risk assessment is based upon a 'risk characterisation' which is derived from an 'effects assessment' and an 'exposure assessment':

- Effects Assessment involves the identification of the hazard based on its physico-chemical properties, ecotoxicity and intended use, and the estimation of a Predicted No Effect Concentration (PNEC), derived from ecotoxicity data and the application of assessment factors.

- Exposure Assessment involves the calculation of a Predicted Environmental Concentration (PEC). This is derived using monitoring data, realistic worst cases scenarios and predictive modelling techniques. It is a complex task and should consider release, degradation, and transport and fate mechanisms.

- Risk Characterisation involves the calculation of a quotient - the PEC/PNEC ratio. If the ratio is less than 1 the substance is considered to present no risk to the environment in a given scenario.

3.4.2.2 Description of risk assessment

The environmental risk assessment approach outlined in EU TGD (EU TGD, 2003) addresses the concern for the potential impact of individual substances on the environment by examining both exposures resulting from discharges and/or releases of chemicals and the effects of such emissions on the structure and function of the ecosystem. Three specific approaches are described, representing quantitative, qualitative and PBT which describes the potential for a chemical to be persistent, bioaccumulative and toxic. Of most relevance to the current TQP Amine 3 project is the quantitative approach. This provides a PEC/PNEC estimation for the environmental risk assessment of a substance by comparing compartmental concentrations (predicted environmental concentration; PEC) with the concentration below which unacceptable effects on organisms will most likely not occur (predicted no effect concentration; PNEC).

Dependent on the PEC/PNEC ratio the decision whether a substance presents a risk to organisms in the environment is taken. If it is not possible to conduct a quantitative risk assessment, either because the PEC or the PNEC or both cannot be derived, a qualitative evaluation is carried out of the risk that an adverse effect may occur. PEC values are derived for local as well as regional situations, each of them based on a number of specific emission characteristics with respect to time and scale. As a consequence, the comparison of PNEC values for the different environmental compartments with different PEC values for different exposure scenarios can lead to a number of PEC/PNEC ratios.

In some cases, the quantitative risk assessment approach may not provide sufficient confidence that the environmental compartment or targets considered are sufficiently protected. The PBT assessment is then used with the aim of identifying these cases.

3.4.2.3 Defining environmental compartments for risk assessment

The EU TGD states that the environmental risk assessment has been developed, and should therefore be carried out, for the following compartments:

Inland risk assessment:

- aquatic environment (including sediment)
- terrestrial environment
- atmosphere

Marine risk assessment:

- aquatic environment (including sediment)

In addition to the three primary environmental compartments (aquatic, terrestrial and atmosphere) effects relevant to the food chain (secondary poisoning) are considered. Also effects on the microbiological activity of sewage treatment systems are considered.

The methodologies implemented have as aim the identification of acceptable or unacceptable risks. This identification provides the basis for the regulatory decisions, which follow from the risk assessment.

3.4.2.4 Exposure assessment: Preparation of predicted environmental concentrations (PECs)

Environmental exposure assessment is completed by the determination of PEC values. PECs can be derived from available measured data (if available) and/or model calculations. Relevant measured data from substances with analogous use and exposure patterns or analogous properties, if available, should also be considered when applying model calculations. Preference should be given to adequately measured, representative exposure data where these are available. Consideration should be given to whether the substance being assessed can be degraded, biotically or abiotically, to give stable and/or toxic degradation products. Where such degradation can occur, the assessment should give due consideration to the properties (including toxic effects) of the products that might arise.

For new substances, it is unlikely that information will be available on such degradation products and thus only a qualitative assessment would normally be possible. No measured environmental concentrations will normally be available for new substances. Therefore, concentrations of a substance in the environment must be estimated. Measured concentrations can have a considerable uncertainty associated with them, due to temporal and spatial variations. Therefore, both measurement and modelling approaches complement each other in the complex interpretation and integration of the data.

For the release estimation of substances, a distinction is usually made between substances that are emitted through point sources at specific locations and substances that enter the environment through diffuse releases. Point source releases have a major impact on the environmental concentration on a local scale (PEClocal) and also contribute to the environmental concentrations on a larger scale (PECregional).

When determining a PEC for new substances at base-set level, or at the 10 tonnes per annum production level, estimates will usually focus on the generic local environment to which releases may occur. In the case of persistent and/or highly toxic chemicals, however, a regional assessment may still be relevant at low tonnages. Therefore, derivation of a PECregional is required, unless it can be justified that a regional assessment is not relevant for the substance at these low tonnages.

PEClocal is calculated on the basis of a daily release rate, regardless of whether the discharge is intermittent or continuous. It represents the concentration expected at a certain distance from the source on a day when discharge occurs. In principle, degradation and distribution processes are taken into consideration for the PEClocal. However, because of the relatively small spatial scale, only one or two key processes typically govern the ultimate concentration in a compartment.

The PECregional takes into account the further distribution and fate of the chemical upon release. It also provides a background concentration to be incorporated in the calculation of the PEClocal. The PECregional is assumed to be a steady-state concentration of the substance.

For the derivation of PECs at the local and regional scale, a standardised generic environment, with default values is used as specified in the EU TGD. The characteristics of the real environment will, obviously, vary in time and space. When more specific information is available on the location of the emission sources, this information can be applied in refinement of the PEC by deviating from the default values.

There are a large number of environmental fate processes which can significantly effect the concentration of a chemical released to the environment. These processes are discussed and evaluated as part of the environmental exposure assessment part of the risk assessment in the EU TGD. Briefly, these include:

- Partition coefficients
    - Adsorption to aerosol particles
    - Volatilisation (partitioning between air and water)
    - Adsorption/desorption (partitioning between solids and water in soil, sediment and suspended matter)

- Abiotic and biotic degradation processes
    - Hydrolysis
    - Photolysis in water
    - Photochemical reactions in the atmosphere
    - Biodegradation in a sewage treatment plant
    - Biodegradation in surface water, sediment and soil

Based on this information, local PECs and regional PECs are then derived for all environmental compartments, including groundwaters using a variety of parameters:

- PEClocal for the atmosphere
    - Local concentration in air during emission episode
    - Annual average local concentration in air
    - Total deposition flux (annual averge)

- PEClocal for the aquatic compartment
    - Local concentration in surface water during emission episode
    - Annual average local concentration in surface water

- PEClocal for sediment
    - Local concentration in sediment during emission episode

- PEClocal for the soil compartment
    - Local concentration in agricultural soil (averaged over a certain time period)
    - Local concentration in grassland (averaged over a certain time period)
    - Percentage of steady-state situation (to indicate persistency)

- Concentration in groundwater
    - Local concentration in groundwater

- PECregional
    - Regional computations are done by means of multimedia fate models based ont he fugacity concept

The EU TGD (EU TGD, 2003) describes the relationship between the local emission routes and the subsequent distribution processes, which may be relevant for the different environmental compartments. For each compartment, specific fate and distribution models are applied (*Figure 3*). As the emissions from the Mongstad CO_2 capture plant are expected to be to the atmosphere only, some of the environmental compartments do not need to be included into the risk assessment. Based on the information that there will be no liquid emissions to the local area (including to sewage treatment plants), risk assessment of the surface water and sediment compartments will not be necessary. This is because the primary route of distribution to these environmental compartments is via dilution in the effluent from sewage treatment plants.

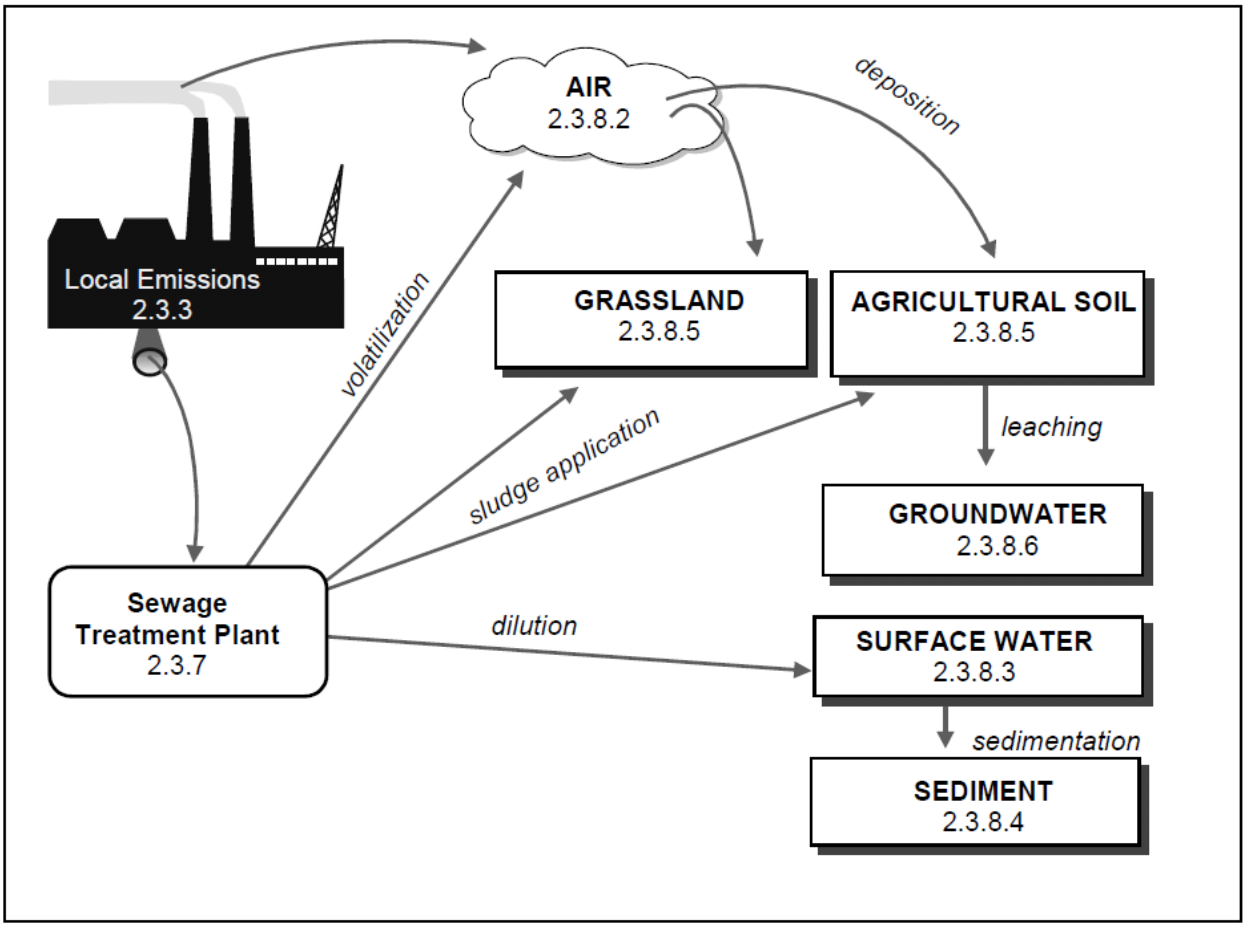

Figure 3. Local relevant emission and distribution routes (adopted from EU TGD, 2003)

3.4.2.5 Effects assessment: Preparation of predicted no effect concentrations (PNECs)

Effects assessment comprises the following steps of the risk assessment procedure:

- Hazard identification: The aim of the hazard identification is to identify the effects of concern.
- Dose (concentration) - response (effect) assessment: At this step the predicted no effect concentration (PNEC), shall, where possible, be determined.

The environmental effects assessment is completed by the determination of PNEC values. A PNEC value has to be derived for each of the environmental compartments specified in section 1.3.4.2.3. PNECs can be derived from available measured data (if available) and/or model calculations. It is typical to start the effects assessment process with an evaluation of the available ecotoxicological data. A PNEC is regarded as a concentration below which an unacceptable effect will most likely not occur. In principle, the PNEC is calculated by dividing the lowest short-term L(E)C50 or long-term NOEC (no effect concentration) value by an appropriate assessment factor. See section 1.3.4.2.6 for a description of assessment factor and their use in risk assessment.

A detailed assessment of the environmental risk is typically only feasible for the water compartment, especially for new substances. This is due tot he fact that most ecotoxicity studies are performed using aquatic organisms. Often, no ecotoxicity data will be available for sediment-dwelling organisms, as

appropriate test systems and standardised guidelines are still under development. In this case, the equilibrium partitioning method is proposed as a screening method for derivation of a $PNEC_{sed}$ to compensate for this lack of toxicity data. Similarly, few toxicity data are typically available for the soil compartment. If test data are lacking, the equilibrium partitioning method can be used to derive a $PNEC_{soil}$. Biotic and abiotic effects, such as acidification, are addressed for the atmosphere. In view of the lack of suitable data and the fact that no adequate methods are available yet to assess both types of effects, a provisional strategy is described in the EU TGD. Finally, chemicals showing bioaccumulation and biomagnification may pose an additional threat due to exposure of organisms higher in the food chain, e.g. top predators. This phenomenon is called 'secondary poisoning' and has to be addressed if a chemical fulfils several criteria, e.g. indication of a bioaccumulation potential.

In particular for new and existing substances where the data sets are restricted to acute toxicity testing with only three trophic levels, there may be effects of substances that are not so well characterised in the assessment, such as:

- Adverse effects for which no adequate testing strategy is available yet (e.g. neurotoxicity, behavioural effects and endocrine disrupting effects);
- Specific effects in some taxa that cannot be modelled by extrapolation of the data of other taxa (for example the specific effect of organotin compounds on molluscs).

The EU TGD recognises that experience with several of the described effects assessment methods is lacking. Thus, assessments by use of these types of methods can be uncertain. However, the methods presented in the EU TGD make it possible to identify if the compartment under consideration is possibly “of concern” and whether further data, e.g. testing on relevant organisms for that compartment, should be obtained.

The calculation of PNECs for the different environmental compartment is approached in slightly different ways. A brief overview of the method for calculating the PNEC for the aquatic is given below. The reader of this report is refered to the EU TGD document for more detailed calculations of this PNEC and those for other environmental compartments.

For the aquatic environment, a PNEC is derived that, if not exceeded, ensures an overall protection of the environment. Certain assumptions are made concerning the aquatic environment which allow, however uncertain, an extrapolation to be made from single-species short-term toxicity data to ecosystem effects. It is assumed that ecosystem sensitivity depends on the most sensitive species and, protecting ecosystem structure protects community function. It is generally accepted that protection of the most sensitive species should protect structure, and hence function.

When the pool of data from which to predict ecosystem effects is very limited (e.g. only short-term toxicity data are available), assessment factors must be used. In this case, the effect assessment performed with assessment factors can be supported by a statistical extrapolation method is the database on Species Sensitivity Distributions (SSDs) is sufficient for its application. If a large data set from long-term tests for different taxonomic groups is available, statistical extrapolation methods may be used to derive a PNEC. The method should be applied to all reliable available NOECs from chronic/long-term studies. The NOEC is defined as “the highest concentration tested at which the measured parameter shows no significant inhibition”. Ecotoxicity data for a broad range of taxonomic groups (described in the EU TGD) is required. Confidence can be associated with a PNEC derived by statistical extrapolation if the database contains at least 10 NOECs (preferably more than 15) for different species covering at least 8 taxonomic groups. The EU TGD states that the concentration corresponding with the point in the SSD

profile below which 5% of the species occur should be derived as an intermediate value in the determination of a PNEC.

PNEC is calculated using the following equation:

$$PNEC = \frac{5\%SSD(50\%c.i.)}{AF}$$

Where:
SSD is the Species Sensitivity Distribution
50%*c.i.* is a 50% confidence interval
AF is an appropriate assessment factor between 5 and 1.

3.4.2.6 Assessment factors

For most substances, the pool of data from which to predict ecosystem effects is very limited as, typically, only short-term toxicity data are available. In these circumstances, it is recognised that, while not having a strong scientific validity, empirically derived assessment factors must be used. Assessment factors have also been proposed by the US EPA and OECD (1992). In applying such factors, the intention is to predict a concentration below which an unacceptable effect will most likely not occur. It is not intended to be a level below which the chemical is considered to be safe. However, again, it is likely that an unacceptable effect will not occur. The assessment factors reflect the degree of uncertainty in extrapolation from laboratory toxicity test data for a limited number of species to the 'real' environment. Assessment factors applied for long-term tests are smaller as the uncertainty of the extrapolation from laboratory data to the natural environment is reduced. For this reason long-term data are preferred to short-term data.

In establishing the size of these assessment factors, a number of uncertainties must be addressed to extrapolate from single-species laboratory data to a multi-species ecosystem. These are best summarised under the following headings:

- intra- and inter-laboratory variation of toxicity data.
- intra- and inter-species variations (biological variance).
- short-term to long-term toxicity extrapolation.
- laboratory data to field impact extrapolation.

The size of the assessment factor depends on the confidence with which a PNEC value can be derived from the available data. This confidence increases if data are available on the toxicity to organisms at a number of trophic levels, taxonomic groups and with lifestyles representing various feeding strategies. Thus lower assessment factors can be used with larger and more relevant datasets than the base-set data. Essentially, the more limited the existing data set is the higher the assessment factor. For example, when calculating the $PEC_{aquatic}$ for the freshwater aquatic compartment where only short-term toxicity data are available, an assessment factor of 1000 should be applied on the lowest L(E)C50 of the relevant available toxicity data. This is irrespective of whether or not the test species is a standard test organism. It follows that a lower assessment factor should be applied on the lowest NOEC derived in long-term tests with a relevant organism.

A different set of assessment factors are used in the calculation of PNECs for different environmental compartments. However, they are not used in calculating the effects assessment in the air compartment and marine aquatic compartment, or for assessment of secondary poisoning (both marine aquatic and normal aquatic compartments). The following environmental compartments use assessment factors which are presented in *Table 1*-*Table 4*:

- Aquatic compartment, $PEC_{aquatic}$ (*Table 1*)
- Sediment, $PEC_{sediment}$ (*Table 2*)
- Terrestrial compartment, PEC_{soil} (*Table 3*)
- Marine sediment compartment, $PEC_{marine\ sediment}$ (*Table 4*)

Table 1: ***Assessment factors used to derive a $PNEC_{aquatic}$ (EU TGD, 2003).***

Available data	**Assessment factor**
At least one short-term L(E)C50 from each of three trophic levels of the base-set (fish, Daphnia and algae)	1000 [a)]
One long-term NOEC (either fish or Daphnia)	100 [b)]
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50 [c)]
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10 [d)]
Species sensitivity distribution (SSD) method	5-1 (to be fully justified case by case) [e)]
Field data or model ecosystems	Reviewed on a case by case basis [f)]

Table 2: ***Assessment factors used to derive a $PNEC_{sediment}$ (EU TGD, 2003).***

Available test result	**Assessment factor**
One long term test (NOEC or EC10)	100
Two long term tests (NOEC or EC10) with species representing different living and feeding conditions	50
Three long term tests (NOEC or EC10) with species representing different living and feeding conditions	10

Table 3: ***Assessment factors used to derive a $PNEC_{soil}$ (EU TGD, 2003).***

Information available	Assessment factor
L(E)C50 short-term toxicity test(s) (e.g. plants, earthworms, or microorganisms)	1000
NOEC for one long-term toxicity test (e.g. plants)	100
NOEC for additional long-term toxicity tests of two trophic levels	50
NOEC for additional long-term toxicity tests for three species of three trophic levels	10
Species sensitivity distribution (SSD method)	5 – 1, to be fully justified on a case-by-case basis (cf. main text)
Field data/data of model ecosystems	case-by-case

Table 4: ***Assessment factors used to derive a $PNEC_{marine\ sediment}$ (EU TGD, 2003).***

Available test result s	Assessment factor
One long-term freshwater sediment test	1000
Two long-term freshwater sediment tests with species representing different living and feeding conditions	500
One long-term freshwater and one saltwater sediment test representing different living and feeding conditions	100
Three long-term sediment tests with species representing different living and feeding conditions	50
Three long-term tests with species representing different living and feeding conditions including a minimum of two tests with marine species	10

The assessment factors presented in *Table 1-Table 4* above should be considered as general factors that under certain circumstances may be changed. In general, justification for changing the assessment factor could include one or more of the following:

- Evidence from structurally similar compounds (evidence from a closely related compound may demonstrate that a higher or lower factor may be appropriate).
- Knowledge of the mode of action including endocrine disrupting effects (some substances, by virtue of their structure, may be known to act in a non-specific manner).

- The availability of test data from a wide selection of species covering additional taxonomic groups other than those represented by the base-set species.
- The availability of test data from a variety of species covering the taxonomic groups of the base-set species across at least three trophic levels. In such a case the assessment factors may only be lowered if these multiple data points are available for the most sensitive taxonomic group.

3.4.2.7 Environmental Impact Factor (EIF)

The Environmental Impact Factor (EIF) risk assessment method is a Norwegian initiative. It has been developed for use as an indicator of the environmental risk caused by regular emissions to sea (marine environment). The EIF approach was originally developed in conjunction with operators on the Norwegian Continental Shelf (NCS) and the Norwegian Authorities with the aim of reducing the environmental impacts from produced water releases (including drill cuttings and mud releases) down to a level of "zero harmful effects". In addition to risk assessment, the EIF approach therefore provides a tool for reducing the environmental impacts of emissions. Essentially, the EIF can be used to measure the environmental benefit achieved when alternate measures are considered for reducing environmental impacts. The EIF method is able to achieve this because it gives a quantitative measure of the environmental risks involved when effluents are discharged to sea, and is thus able to form a basis for reduction of impacts in a systematic and a quantitative manner.

The EIF method is based on a PEC/PNEC approach similar to that described in the EU Technical Guidance Document (EU TGD, 2003). Briefly, the concentration for each compound discharged into the recipient is compared to a concentration threshold for that compound. When the predicted (modelled) environmental concentration (PEC) is larger than the predicted no-effect concentration (PNEC), an "unacceptable" environmental risk for damage is encountered. When the PEC is lower than the PNEC threshold, the environmental risk is considered to be "acceptable". An outline of the EIF method applied to produced water discharges is given in Johnsen et. al. (2000). The method is based on calculation of the EIF using the numerical model DREAM (Dose related Risk and Effect Assessment Model) developed by SINTEF, with financial support from StatoilHydro, ENI, Total, ExxonMobil, Petrobras, ConocoPhillips, and Shell.

3.4.2.8 Environmental risk and the EIF

As the EIF method is based upon the use of PEC and PNEC values, the reader is referred to the detailed description and definitions of these terms provided by the EU TGD (EU TGD, 2003) and described in section 1.3.4.2 above. However, once the PEC and PNEC values have been determined these can then be used in the calculation of the EIF. The EIF for a single component or component group is related to the recipient water volume where the ratio PEC/PNEC exceeds unity. The ratio PEC/PNEC is related to the probability of exceeding the PNEC level according to a method developed by Karman et. al. (1994) and also published by Karman and Reerink (1997). When the PEC/PNEC ratio equals 1, a risk exists for impacts to species representing the most sensitive 5%. *Figure 4* shows the relationship between the PEC/PNEC ratio and the probability of environmental impact.

The EIF method has the advantage over other risk assessment methods in that it can calculate risk contributions from a sum of chemicals and/or natural compounds in the recipient. This means that the EIF approach is capable of assessing the risk associated with complex mixtures. However, this is most

effective where the mode of toxicity is the same for all compounds present in the mixture, and that the toxicity is additive.

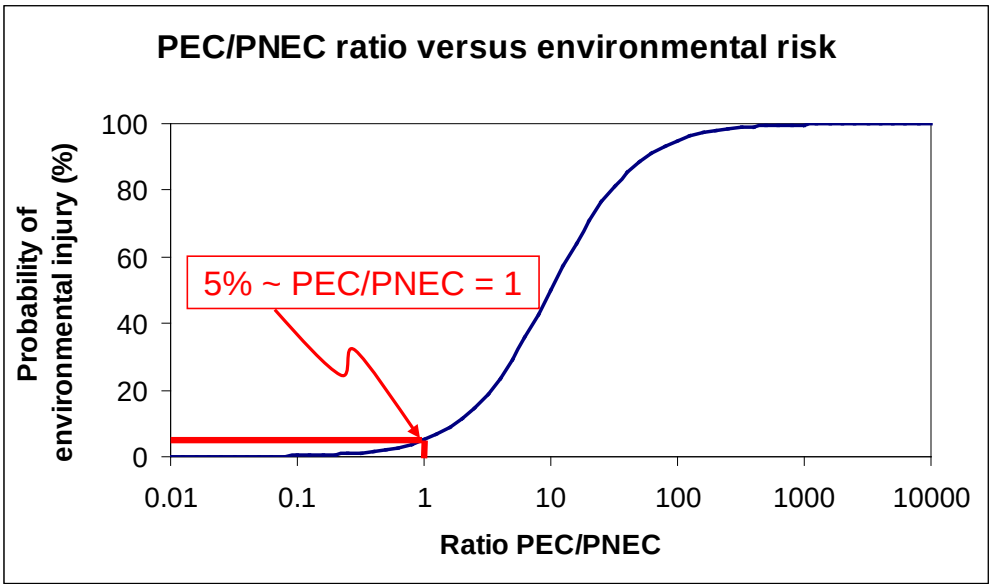

Figure 4. Relationship between the PEC/PNEC level and the risk level (in %) for impact to biota, based on Karman et. al. (1994). A PEC/PNEC ratio of 1 corresponds to a level at which there exists a possibility of impact to the 5% most sensitive species.

An attractive feature of the EIF approach is that the method is able to discriminate among the various contributors to environmental risk. Thus it is possible to separate a chemical product into its constituents and calculate the EIF contribution from each of them. The results of the calculations can then be used to improve the product in terms of replacing the constituents in the product with the largest contribution to the EIF. This capability provides useful information when comparing alternative proposed methodologies for reducing environmental risks associated with a discharge.

In addition to the marine environment, EIFs have also been developed for terrestrial and atmospheric environmental compartments. However, these additional EIFs have not yet been fully developed to the same level as the marine environment EIF. Owing to this limitation, it is suggested that the EIF risk assessment method is not the most suitable for use with CO_2 capture plant emissions. This is because these emissions are expected to be predominantly to atmospheric and terrestrial environments rather than the marine environment.

3.5 Recommendation of a risk assessment method for TQP Amine 3

The environmental risk assessment approach recommended in this report is that outlined in the EU Technical Guidance Document (EU TGD, 2003). It is suggested that this method currently offers the most suitable approach in the case of TQP Amine 3, although it should be stated there are some limitations with the method, which are discussed below in section 1.3.6. It is therefore recommended that new or revised risk assessment methods which become available in the future should be evaluated within the

context of TQP Amine 3. The method is based on the relationship between predicted environmental concentration and the predicted no effect concentration, the PEC/PNEC approach.

This risk assessment approach is recommended for use within TQP Amine 3 for the following reasons:

- *Quantifiable.* The method is well described, quantitative, transparent, and allows quantitative documentation of improvements.
- *Public acceptance.* The method is well established within the EU and already used as a basis for evaluating and comparing toxicity of regular releases into the marine environment. In Norway the PEC/PNEC based Environmental Impact Factor (EIF) is used as a standard for evaluating and comparing the toxicity of releases of complex mixtures from petroleum activities (produced water and drilling mud). The basic concept of the EIF-method is to calculate the volume (water) or area (seabed) where PEC/PNEC is lager than 1 which in both cases is used as a quantitative value for a potential environmental impact.
- *Flexibility.* The method is flexible in that components can be changed and evaluated without affecting the evaluation of existing components. Furthermore, the method is suitable for the assessment of both single chemicals and complex mixtures.
- *Incorporation of new knowledge.* When it becomes available, new and improved knowledge on toxicity and environmental concentrations may easily be incorporated into an existing assessment. This means the uncertainty of the risk assessment can continuously be refined and reduced.
- *Identification of knowledge gaps and prioritizing of research needs*. The method itself may be used to identify knowledge gaps as well as to prioritise research tasks and technology development. The method may, with a few assumptions, be used to pinpoint components that have the largest potential contribution to toxicity.
- *Suitability*. As the guidance has been developed mainly from the experience gained on individual organic substances, it is directly relevant in the current scenario which is concerned with the emission of organic chemicals into the environment.
- *Location specific*. It is recognised that exposure estimation is subject to variation due to topographical and climatological variability. When more specific information on the emission of a substance is available, it may well be possible to refine the generic or site-specific assessment.

3.6 Limitations of the EU TGD approach

This report recommends that the EU TGD PEC/PNEC approach is the best suited for providing the most reliable risk assessment of the emissions associated with amine solvent-based CO_2 capture plants. However, this approach still has some limitations which may affect the ultimate performance of the risk assessment. The main issue surrounds the fact that the EU TGD is designed only for the testing of single chemicals and not mixtures. The emissions for the CO_2 capture facility are expected to be a complex mixture of chemicals representing many different chemical groups with a broad range of physical and chemical properties. Unfortunately, such a complex mixture, or even simple mixtures (e.g. synthetic mixtures containing chemicals of interest) cannot be studied using this risk assessment approach. As a result the EU TGD risk assessment approach should be completed individually for all chemicals in the emission which are identified as hazardous.

It should be noted that this same issue regarding the risk assessment of mixtures is a major limitation in all of the available risk assessment methods reviewed for this project. The exception is the environmental impact factor (EIF) method described in section 1.3.4.3. As a result, it is recommended that the EIF approach is considered in the future as this has the capacity to address risk assessment of mixtures. At present the risk assessment method is not fully developed sufficiently to be used in the assessment of emissions for a CO_2 capture plant. In particular, the EIFs for terrestrial and atmospheric environmental compartments are not fully developed and therefore limited. The EIF method is, although capable of assessing mixtures, is based on the information for single chemicals (e.g. that used in, and required for the EU TGD). Therefore, it is suggested that the EU TGD approach is used in the immediate future as the data generated can be incorporated into the EIF approach at a later stage.

An additional limitation to the EU TGD risk assessment method is the use of assessment factors. Again, this limitation is one that is shared with all other risk assessment methods reviewed in this document (e.g. US EPA and EIF). Although essential for completing the risk assessment, assessment factors incorporate a large degree to caution in order to ensure that a false result is not generated for a chemical. As a result, the environmental concentrations and toxicity effect limit ranges which are considered environmentally acceptable/safe are often broader than necessary. In conclusion, it is recommended that the EU TGD is currently the best of the available models despite these limitations.

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Appendix H Detailed information on Health data for Emission Chemicals

Appendix: Summary, C, M, R and S Sheets for amine 3 chemicals.

CAS - No.	Name
141-43-5	**Ethanol, 2-amino- (MEA)**

EU-Risk phrases	Xn; R20/21/22 - C; R34
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	Yes
RTECS file	KJ5775000; Last updated 200911
IUCLID file	Yes
REACH file	
Other sources	• **TOXNET:** Carcinogenicity and mutagenicity studies

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**1**	1720	Rat	RTECS
2	**1**	700	Mice	RTECS
3	**1**	500	Mice	RTECS
4	**1**	1000	Rabbit	RTECS
5	**1**	620	Guinea pig	RTECS
6	**1**	500-10200	Several	IUCLID many studies, maily BASF

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-2000 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1			Rabbit	Relaible data RTECS-LD50= 1mL/kg; Administration onto skin (Union Carbide Data Sheet)
2	**1**	1025	Rabbit	IUCLID BASF

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1			Mice	RTECS –Lethal concentration= >2420 mg/m3/2H (Labor Hygiene and Occupational Diseases)
2			Cat	RTECS –Lethal concentration= >2420 mg/m3/2H (Labor Hygiene and Occupational Diseases)
3	-	0.145	Guinea pig	IUCLID 0.58 mg/l 1 hour. BASF data. However, several LC0 values are higher. Probably not an inhalation hazard apart from acute effects in airways when exposed to relatively high conc.'s.

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive
3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1	**2**	**Moderate** RTECS-Union Carbide Data Sheet/ Open irritation test/ Rabbit- 505mg

2	**3B**	**Corrosive** IUCLID

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1	**3**	**Severe** RTECS-American Journal of Ophthalmology/ Standard Draize Test/ Rabbit- 250µg
2	**3**	Corrosive IUCLID

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C		Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R		Immunotoxic - I	
Mutagenic - M	**M?**	Photosensitizer - P		Sensitizing - S	**S?**

Source/comment:

C: Carcinogenicity: RTECS and TOXNET- NA

M: Mutagenicity RTECS classified IUCLID reports a number of studies – all negative. The RTECS rating is questionable, since only based on two untraceable articles from Russian translation. Require confirmation before M-rating.

study no	proposed rating	source / kind of study / animal species
1	Positive ?	RTECS, Human Lymphocyte, Cytogenetic analysis,100 umol/L, Biological Journal of Armenia, 1986.
2	Positive ?	RTECS, Human Lymphocyte, Sister chromatid exchange, 1 mmol/L, Cytology and Genetics, 1987.
3	All negative	TOXNET, Ames test (21 st), Japan Chemical Industry Ecology, 1996.
4	Negative and positive	TOXNET, Tryptophan reverse gene mutation assay (2 st), E.coli, Japan Chemical Industry Ecology, 1996.
5	All negative	IUCLID reports a number of studies.

R: Reprotoxicity RTECS classified. IUCLID confirm maternal effects and developmental effects in some studies but not all. Further examinations of record show not an R**.** Slight maternal toxicity but no fetotoxic or developmental toxicity.

S: Senzitization RTECS: Classified as primary irritant, Percutaneous data indicating primary irritant in mammals. IUCLID: Two contradicting studies in guinea pig.

Remarks

ACGIH TLV TWA-3 ppm; STEL 6 ppm. Applies also in Norway-Jan 1999, OEL-Denmark: 2.5 mg/m3 2002

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date
1	1	3	3A	3		2009-04

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
Ethanol, 2-amino- (MEA)	141-43-5		RTECS+ IUCLID+ GESAMP+ CPDB- IRIS- TOXNET+	1	1	3	M? S?	OEL: 2.5 mg/m3 Skin and eye: Severely irritating **C: No data available** **M: Need more data** **Confirm no R** **S: Need more data** **Need further testing for M and C**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Ethanol, 2-amino (MEA)**
Chemical Group	**Amines**
CAS Number	**141-43-5**
LD50 (mg/kg b.w.) (from TOXNET)	**10.2 g/kg (Rat oral)** **67 mg/kg (Rat ip)** **225 mg/kg (Rat iv)** **1750 mg/kg (Rat im)**
Mutagenicity /Carcinogenicity	
Mutagenicity	**Toxnet:** 1. Ames test (21 st), TOXNET, Japan Chemical Industry Ecology, 1996 (**all negative**). 2. Tryptophan reverse gene mutation assay (2 st), TOXNET, Japan Chemical Industry Ecology, 1996 (**negative and positive**). **RTECS:** 1. Human Lymphocyte, Sister chromatid exchange, 1 mmol/L, Cytology and Genetics, 1987. (**positive?)** 2. Human Lymphocyte, Cytogenetic analysis,100 umol/L, Biological Journal of Armenia, 1986. (**positive?).** **IUCLID:** Several studies; (**all negative**). **HSDB:** Irritating to skin, eyes, respiratory system. MEA inhalation by humans has been reported to cause immediate allergic responses of dyspnea and asthma and clinical symptoms of acute liver damage and chronic hepatitis.
Carcinogenicity	**Toxnet:** No data **RTECS:** No data
Additional Info	
Conclusions	
RTECS	Classified as a mutagen. The RTECS rating is questionable, since only based on two untrackable articles from □ussian translation. Require confirmation before M-rating.
TOXNET	No conclusions on mutagenicity. No data on carcinogenicity.
ICLUID	IUCLID reports a number of studies – all negative.
GESAMP	
Current project summary sheet	
Current project conclusion for M and C	Possible mutagen (M?). Carcinogenicity no data
Current project further work for M and C	We recommend further testing on both genotoxicity and carcinogenicity to confirm M and C.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Monoethanolamine (MEA)**
Chemical Group	Amine
CAS Number	141-43-5
Oral LD50 (mg/kg b.w.)	1720 (rat); 500 (mice)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** Maternal-decrease in mean body weight at highest dose (3) Slight maternal toxicity at highest dose (4) **IUCLID:** Lethargic effect (3) Maternal food intake and body weight reduction (5, 6, 7, 14, 16) Maternal death at the highest dose (7, 8) Blood discharge from vagina (7) Other clinical signs (8) Effects on skin (13, 14, 15, 16) No effect (1, 2, 4, 9, 10, 11, 12)
Fetototoxicity	**RTECS:** Stunded fetus and fetal death (1) No effect (3,4) **IUCLID:** Increase in no. of dead foetus (3, 7) Decrease in number of viable litter (8) No effect (4, 5, 6, 9, 10, 11, 12, 13, 14, 16)
Fetal development	**RTECS:** Musculoskeletal (1) Urogenital (2) No effect (3,4) **IUCLID:** Malformations (3) No effect (4, 5, 6, 9, 10, 11, 12, 13, 14, 16)
Additional Info	Present in hair dye (22%)
Conclusions	
RTECS	Classified as reprotox
IUCLID	Confirm maternal effects and developmental effects in some studies but not all.
GESAMP	Not classified as reprotoxic
Current project summary sheet	**M?**
Current project conclusion for R	**Confirm no R. Slight maternal toxicity but no fetotoxic or developmental toxicity. Not a reprotoxic compound**
Current project further work for R	**Only maternal effects confirmed. Not a reprotoxin. No further testing needed**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Monoethanolamine (MEA)**
Chemical Group	Amine
CAS Number	141-43-5
Oral LD50 (mg/kg b.w.)	1720 (rat); 500 (mice)
Sensitization	
Studies	**RTECS:** Percutaneous data: primary irritant in mammals **IUCLID:** **Sensitizing:** Guinea pig: with open epicutaneous test (1) **Non sensitizing:** Guinea pig: (1)
Additional Info	
Conclusions	
RTECS	Classified as primary irritant
IUCLID	Two contradicting studies in IUCLID in guinea pig
GESAMP	Not classified as S
Current project summary sheet	**M?**
Current project conclusion for S	**S? Need more data**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
7664-41-7	**Ammonia**

EU-Risk phrases	R10/23/34/50; T; N
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	Yes
RTECS file	BO0875000, Last updated 200911
IUCLID file	Yes
REACH file	
Other sources	• **IRIS:** Oral, inhalation and carcinogenicity data • **TOXNET:** Carcinogenicity and mutagenicity studies

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**1**	350	rat	IUCLID Repsol Quimica, S.A. 1981
2			human	RTECS- TDLo= 15µl/Kg, changes in structure or function of esophagus, American Journal of Emergency Medicine

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-200 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1				

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1	**3**	1.42	Rat	RTECS-2000 ppm/4H Toxicology of Drugs and Chemicals, 1969
			Rat	Subchronic inhalation study; LOAEL= 0.0177 mg/l (Broderson et al., 1976) Increased severity of rhinitis and pneumonia with respiratory lesions
2	**3**	0.75	Mice	RTECS-4230 ppm/1H Behavioral – tremor, convulsions and ataxia. (Federation of American Societies for Experimental Biology, 1982)
3	**3**	1.6	Mice	RTECS-4600 mg/m3/2H (Hazardous substances. Inornanic substances containing V-VII group elements), 1993
4	**3**	1.75	Rabbit	RTECS-7gm/m3/1H; Flaccid paralysis without anesthesia (usually neuromuscular blockage), excitement (Journal of Industrial Hygiene and Toxicology
5	**3**	1.75	Cat	RTECS-7gm/m3/1H; Flaccid paralysis without anesthesia (usually neuromuscular blockage), excitement (Journal of Industrial Hygiene and Toxicology

6		TCLo 0.0142 mg/l	Human	RTECS-TCLo=20ppm (Olfaction) - ulcerated nasal septum; (Eye) - conjunctive irritation; Structural or functional change in trachea or bronchi; Archiv fuer Gewerbepathologie und Gewerbehygiene.
7		0,040 mg/l	Human	RTECS- TCLo=40mg/m3 (Eye) - conjunctive irritation; Cough lungs and respiratory depression (Hazardous substances. Inornanic substances containing V-VII group elements), 1993

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive

3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1	**1**	IUCLID

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1	**2**	IUCLID

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C	**C**	Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R		Immunotoxic - I	
Mutagenic - M	**M?**	Photosensitizer - P		Sensitizing - S	

Source/comment:

C: Carcinogenicity

No credible evidence that NH3 can cause cancer. However, exposure to gas in these test systems is difficult.

study no	proposed rating	source / kind of study / animal species
1	Tumor promoter, positive	TOXNET, Rat, Sprague-Dawley/ Male, oral, tumor promotor for N-Methy-N'-Nitro-N-Nitrosoguanidine ; 70-25-7. Stomach Tumor. Cancer Lett. 65(1):15-18, 1992.
2	Tumor promoter, positive	TOXNET, Rat, Sprague-Dawley/Male, oral, tumor promotor for N-Methyl-N'-Nitro-N-Nitrosoguanidine; 70-25-7. Stomach Adenocarcinoma. Carcinogenesis 16(3):563-566, 1995.

M: Mutagenicity

RTECS classified as mutagenic but the two publications on this cannot be found: they are either very old or translated from Russian. M is questionable rating based on IUCLID-data and TOXNET-data. The mutagenic effect is rather weak. No conclusion can be drawn regarding the mutagenicity of NH3.

study no	proposed rating	source / kind of study / animal species
1	All negative	TOXNET, Prokaryotes: Ames test (10 st), Sangyo Igaku 27(6):400-419, 1985.
2	Both negative	TOXNET, Prokaryotes: Tryptophan reverse gene mutation assay (2 st), Sangyo Igaku 27(6):400-419, 1985.
3	Positive?	RTECS, Bacteria - Escherichia coli, Mutation in microorganisms, 1500 ppm/3H, American Naturalist, 1951.
4	Positive?	RTECS,Rodent – rat, Cytogenetic analysis, inhalation, 19800 ug/m3/16W, Biological Journal of Armenia, 1974.

R: Reprotoxicity
RTECS: Lack of reproductive and developmental toxicology studies. IUCLID: No guideline test assessing teratology or reproductive effects in laboratory animals has been reported.
Confirm no R although limited data and lack of studies since it is a common chemical.

S: Sensitization
RTECS: No data, IUCLID: One study with open epicutaneous test indicating no effect. Several repeat dose inhalation with no effects at low concentrations. Confirm no S.

Remarks

ACGIH TLV-TWA 25 ppm; STEL 35 ppm; RTECS several reviews in literature
OEL Denmark: 14 mg/m3 2002
There are a number of entries in IUCLID for inhalation tox. Results similar to RTECS (some are obviously the same studies)
IUCLID: Several repeat dose inhalation. No effects at low concentrations.
IUCLID gives a nice summary on page 108

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date
1	(2)	3	3	3		2009-04

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
Ammonia	7664-41-7	H3-N	RTECS+ IUCLID+ GESAMP+ CPDB- IRIS+ TOXNET+	1	(2)	3	C M?	OEL: 14 mg/m3 Skin: Severely irritating Eye: Severely irritating **C: tumour promoter** **M: Not enough data** **Confirm no R** **Confirm no S** **No further testing needed**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Ammonia/NH3**
Chemical Group	**Ammonium**
CAS Number	**7664-41-7**
LD50 (mg/kg b.w.) (from TOXNET)	350 (Rat oral)
LC50 (from TOXNET)	5,100 mg/cu m/1 hr (Rat inhalation)
Mutagenicity /Carcinogenicity	
Mutagenicity	**Toxnet:** 1) Prokaryotes: Ames test (10 st), TOXNET, Sangyo Igaku 27(6):400-419, 1985 (**all negative**). 2) Prokaryotes: Tryptophan reverse gene mutation assay (2 st), TOXNET, Sangyo Igaku 27(6):400-419, 1985 (**both negative**). **RTECS:** 1) Bacteria - Escherichia coli, Mutation in microorganisms, 1500 ppm/3H, American Naturalist. (**positive?**). 2) Rodent – rat, Cytogenetic analysis, inhalation, 19800 ug/m3/16W, Biological Journal of Armenia. (**positive?**). **HSDB:** The vapor even in low conc is extremely irritating to skin, eyes and respiratory passages. Liquid produces severe burns. Inhalation of high conc causes violent coughing, severe lung irritation, and pulmonary edema. Death can result if rapid escape is not possible. Swallowing liquid is corrosive to mouth, throat, stomach. Not a systemic poison.
Carcinogenicity	**Toxnet:** 1) Rat, Sprague-Dawley/Male, oral, **tumor promoter** for N-Methyl-N'-Nitro-N-Nitrosoguanidine; 70-25-7. **Stomach Tumor**. Cancer Lett. 65(1):15-18, 1992. 2) Rat, SPRAGUE-DAWLEY/MALE, oral, **tumor promoter** for N-Methyl-N'-Nitro-N-Nitosoguanidine; 70-25-7. **Stomach Adenocarcinoma**. Carcionogenesis 16(3):563-566, 1995.
Additional Info	
Conclusions	
RTECS	Classified as a mutagenic, but the two publications on this cannot be found: they are

	either very old or translated from Russian. However, this is questionable rating based on IUCLID-data and TOXNET-data.
TOXNET	All mutagenicity tests are negative. Carcinogenicity results; NH3 is a tumor promoter.
ICLUID	No data
GESAMP	
Current project summary sheet	
Current project conclusion	No conclusion can be drawn regarding the mutagenicity of NH3. No credible evidence that NH3 can cause cancer. However, exposure to gas in these test systems is difficult.
Current project further work	We do not recommend further testing as it Is common chemical and not specific for this project

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Ammonia**
Chemical Group	NH3
CAS Number	7664-41-7
Oral LD50 (mg/kg b.w.)	350 (rat)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** - **IUCLID:** Reduced food intake and weight gain (1)
Fetotoxicity	**RTECS:** - **IUCLID:** Reduced egg production in hens (1)
Fetal development	**RTECS:** - **IUCLID:** -
Additional Info	Substantial doses are formed by gut bacteria: no adverse effects
Conclusions	
RTECS	Lack of reproductive and developmental toxicology studies
IUCLID	No guideline test assessing teratology or reproductive effects in laboratory animals has been reported
GESAMP	No D3 Profile
Current project summary sheet	**C? M (No R Rating)**
Current project conclusion for R	**Confirm no R**
Current project further work for R	**Not required although limited data and lack of studies. Common chemical.**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Ammonia**
Chemical Group	NH3
CAS Number	7664-41-7
Oral LD50 (mg/kg b.w.)	350 (rat)
Sensitization	
Studies	**RTECS:** - **IUCLID:** **Non sensitizing:** Guinea pig: with open epicutaneous test (1)
Additional Info	
Conclusions	
RTECS	No report on S
IUCLID	One study with open epicutaneous test indicating no effect. Several repeat dose inhalation. No effects at low concentrations.
GESAMP	Not classified as S
Current project summary sheet	**C? M**
Current project conclusion for S	**Confirmed not S**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
50-00-0	**Formaldehyde**

EU-Risk phrases	R40; R23/24/25; R34; R43; T; Carc. Cat. 3
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	Yes
RTECS file	LP8925000; Last updated: 200911
IUCLID file	Yes
REACH file	
Other sources	• **CPDB:** hematopoietic system and nasal cancers in rat and mice • **IRIS:** Carcinogenicity and mutagenicity studies • **TOXNET:** Carcinogenicity and mutagenicity studies

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**2**	100	Rat	RTECS- Food and Chemical Toxicology
2	**1**	500	Rat	RTECS- Hazardous substances. Galogen and oxygen containing substances
3	**3**	42	Mice	RTECS- National Technical Information Service
4	**1**	385	Mice	RTECS- Hazardous substances. Galogen and oxygen containing substances)
5	**1**	500	Mice	RTECS- Hazardous substances. Galogen and oxygen containing substances
6	**2**	260	Guinea pig	RTECS- Hazardous substances. Galogen and oxygen containing substances
7			Human (male)	RTECS-TD Lo= 643 mg/kg; Japanese Journal of Toxicology
8			Human (male)	RTECS- TD Lo= 646 mg/kg; Japanese Journal of Toxicology
9			Human (female)	RTECS-TD Lo= 108 mg/kg; Practical Toxicology of Plastics
10			Human (female)	RTECS-TD Lo= 1 mL/Kg; Intensive Care Medicine
11	**2** **3** **2**		Rats Mice Guinea Pig	Several studies in IUCLID with rating (0,1, 2, 4 for rats; 3 for mice and 2 for guinea pig)

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-200 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**2**	270	Rabbit	RTECS-LD50= 270µL/Kg; (Union Carbide Corp. 1967)
2	**2**	270	Rabbit	RTECS-(Hazardous substances. Galogen and oxygen containing substances)
3	**2**	270	Rabbit	Several studies in IUCLID

4			Mice	RTECS- TD Lo=15 mg/kg/3D-intermittent (Contact Dermatitis. Environmental and Occupational Dermatitis, 2001)
5			Rat	RTECS- TD Lo=56.1 mg/kg/3D-intermittent (TXCYAC Toxicology, 1997.)
6			Rat	RTECS- TD Lo=112.5 mg/kg/3D-intermittent (TXCYAC Toxicology, 1997.)
7			Human	RTECS- TC Lo=1 pph/48 H (American Journal of Contact Dermatitis)

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1	**4**	0.203	Rat	RTECS- 203 mg/m3 (Labor Hygiene and Occupational Diseases)
2	**4**	0.154	Rat	RTECS- 308 mg/m3/2H; (Hazardous substances. Galogen and oxygen containing substances)
3	**4**	0.289	Rat	RTECS- 578 mg/m3/2H; (Hazardous substances. Galogen and oxygen containing substances)
4	**4**	0.156	Rat	RTECS- 250 ppm/2H (Hazardous substances. Galogen and oxygen containing substances)
5	**4**	0.312	Rat	RTECS- 250 ppm/4H (Encyclopedia of Toxicology)
6	**4**	0.127	Rat	RTECS- 815 ppm/0.5H (Encyclopedia of Toxicology)
7	**4**	0.454	Mice	RTECS- 454 mg/m3/4H (CUTOEX Current Toxicology, 1993)
8	**4**	0.252	Mice	RTECS- 505 mg/m3/2H (Hazardous substances. Galogen and oxygen containing substances)
9	**4** **4** **4**		Rats Mice Cat	Several studies in IUCLID with rating (3, 4 for rats, mice and cat)
10			Human	TCLo= 17 mg/m3/30M; Journal of the American Medical Association
11			Human	TCLo= 300µg/m3; Labor Hygiene and Occupational Diseases.

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive

3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1	**1**	**Mild** RTECS- (Cutaneous Toxicity, Proceedings of the 3rd Conference)/ Standard Draize test/ human-150µg/3D (intermittent) Unconfirmed data from proceedings of a conference
2	**3**	**Severe** RTECS- Prehled Prumyslove Toxikologie; Organicke Latky/ Standard Draize test/ rabbit- 2mg/24H
3	**1**	**Mild** RTECS-Union Carbide Data Sheet/ Open irritation test/ rabbit 540 mg Unreliable data due to open test method
4	**2**	**Moderate** RTECS- Toxicology and Applied Pharmacology/ Standard Draize test/ rabbit- 50 mg/24 H
5	**3**	**Corrosive** IUCLID, Rabbit (PROTEX S.A LEVALLOIS PERRET)
6	**3**	**Highly Irritating** IUCLID, Rabbit **(**STRATHCLYDE CHEMICAL COMPANY LIMITED JOHNSTONE**)**
7	**3**	**Corrosive** IUCLID, Human ALDER S.p.A. TRIESTE
		Other studies in IUCLID on guinea pig and rabbit valid with restrictions

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1	**1**	**Mild** RTECS- American Industrial Hygiene Association Journal/ Rinsed with water/ human- 1 ppm/6M Comment: Unreliable data due to Rinse with water test
2	**3**	**Severe** RTECS- Prehled Prumyslove Toxikologie; Organicke Latky/ Standard Draize test/ rabbit- 750 µg/24 H
3	**3**	**Severe** RTECS-American Journal of Ophthalmology/ Standard Draize test/ rabbit-750µg
4	**3**	**Severe** RTECS-Toxicology and Applied Pharmacology/ Standard Draize test/ rabbit 10mg
5	**3**	**Severe** RTECS- Journal of Toxicologic Pathology/ Standard Draize test/ rabbit 37%
6	**3**	**Highly irritating** IUCLID- Rabbit draize test, Dose= 0.003 to 0.1 ml
7	**2**	**Irritating** IUCLID- Rabbit, (STRATHCLYDE CHEMICAL COMPANY LIMITED JOHNSTONE)
8	**2**	**Irritating** IUCLID- Human, (ALDER S.p.A. TRIESTE)

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C	**C**	Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R	**R**	Immunotoxic - I	
Mutagenic - M	**M**	Photosensitizer - P		Sensitizing - S	**S**

Source/comment:
Compound description RTECS: Tumorigen, Mutagen, Primary irritant, Reproductive effector

C: Carcinogenicity

RTECS; Gastrointestinal – tumors; IARC Cancer Review: Sufficient evidence in animals and limited evidence in humans; Group 2A
IARC: Group 1
ACGIH TLV: Suspected human carcinogen; Several studies in IUCLID indcating carcinogenic effect.
CPDB: TD50 = 1.35 mg/kg/day (rat, oral).
Classification for carcinogenicity: Class 1=Carcinogenic to humans, 2 = Probably carcinogenic to humans, 3 = Possibly carcinogenic to humans, 4 = Not classifiable as a human carcinogen

Study no	proposed rating	source / kind of study / animal species
1	Positive, Class 1	TOXNET, rat studies (6 positive, 4 negative): Inhalation: Nasal cavity: Squamous cellc carcinoma and papilloma. J. Toxicol. Sci. 22(3):239-254, 1997. Oral exposure: Lymphatic system: Leukemia; Gastrointestinal system: Tumor Toxicol. Ind. Health 5(5):699-730, 1989.

M: Mutagenicity

RTECS, Several Toxicology Reviews (MUREAV Mutation Research; TOLED5 Toxicology Letters); Several (in vitro and in vivo) studies in IUCLID indicating both positive and negative results.

study no	proposed rating	source / kind of study / animal species
1	Positive	TOXNET, Sister-chromatid exchange (SCE) in vitro (Human, 3 st), Mutat Res 141:89-93, 1984.
2	Positive	TOXNET, Lower eukaryotes –reverse gene mutation, Mutat Res 133:87-134,1984.
3	Positive	TOXNET, Prokaryotes - Rec-assay, Mutat Res 87:211-297,1981.
4	Positive	TOXNET, Insects - Sex-linked recessive lethal gene mutation, Mutat Res 123:183-279, 1983.
5	Positive	TOXNET, Lower eukaryotes - Mitotic recombination or gene conversion and Reverse gene mutation (2 st). Mutat Res 133:199-244, 1984.
6	No concl.	TOXNET, Micronucleus test, chromosome aberrations, Mutat Res 90:91-109, 1981.
7	No concl.	TOXNET, Forward gene mutation at the HPRT locus, J Toxicol Environ Health 12:27-38, 1983.
8	No concl.	TOXNET, Unscheduled DNA synthesis (UDS) in vitro, Mutat Res 123:363-410, 1983.
9	Positive?	RTECS, prokaryotes, 16 studies.
10	Positive?	RTECS, insects, 7 studies.

11	Positive?	RTECS, lower eukaryotes, 3 studies.
12	Positive?	RTECS, mammalian cells, 40 studies.
13	Positive?	RTECS, human cells, 10 studies.

R: Reprotoxicity
RTECS data, Toxicology Review (REPTED Reproductive Toxicology, 2003), Two studies in IUCLID on reproduction toxicity indicating small increase in abnormal cells and depression of body weight gain in offsprings. Several studies on reproductive effects in IUCLID indicated fetotoxicity but no statistically significant teratogenic effects except in one study by Strathclyde Chemical Company Limited Johnstone where major malformations were seen in cleft plate and limbs.

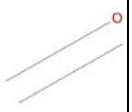
S: Sentization
S: RTECS (Refer Percutaneous toxicity data); GESAMP 2009-04; Seval studies in IUCLID indicating both positive and negative results.

Remarks

ACGIH TLV-CL 0.3 ppm; Several reviews by IARC (1982, 1987, 1995) and several toxicology reviews
OEL-Finland: TWA 0.3 ppm (0.37 mg/m3) 2009
NIOSH Recommended Exposure Level (Rel) -air:8H CA TWA 0.016 ppm; CL 0.1 ppm/15M

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date
2	2	3	3	3	CSM	2009-04

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
Formaldehyde	50-00-0		RTECS+ IUCLID+ GESAMP+ CPDB+ IRIS+ TOXNET+	2	2	4	C M R S	OEL: 0.37 mg/m3 Skin and eye: Severely irritating **Confirm C** **Confirm M** **Confirm R** **Confirm S** **No further testing needed**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Formaldehyde**
Chemical Group	**Aldehyde**
CAS Number	**50-00-0**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**800 (rat oral)** **420 (Rat sc)** **87 (Rat iv)** **Source contains no data on purity of the compound**
Mutagenicity /Carcinogenicity	
Mutagenicity	**Toxnet** 1. Sister-chromatid exchange (SCE) in vitro (3 st) **(positive)** 2. Lower eukaryotes - Reverse gene mutation **(positive)** 3. Lower eukaryotes - Mitotic recombination or gene conversion AND Reverse gene mutation **(both positive).** 4. Prokaryotes - Rec-assay (1 st) **(positive)** 5. Insects - Sex-linked recessive lethal gene mutation (1 st) **(positive)** 6. Micronucleus test, chromosome aberrations **(no concl)** 7. Forward gene mutation at the HPRT locus **(no concl)** 8. Unscheduled DNA synthesis (UDS) in vitro **(no concl)** **RTECS** Human cell assays (10 st)**(positive?)** Mammalian cell assays (40 st)**(positive?)** Lower eukaroyte assays (3 st) **(positive?)** Insect assays (7 st) **(positive?)** Prokaryotic assays (16 st?) **(positive?)** **IUCLID:** Several studies indicating in vitro and in vivo with both positive and negative results. **HSDB** Contact with the skin causes irritation, tanning effect, and allergic sensitization. Contact with eyes causes irritation, itching & lacrimation.
Carcinogenicity	**Toxnet:** Data from rat studies **(6 positive, 4 negative).** Cancerogenic : IARC Group 1 - Carcinogenic to humans. **CPDB:** TD50, mg/kg/day: 1,35 ^{mv}, Rat, oral.

	<u>UCLID:</u> Several studies indicating carcinogenic effect.
Additional Info	
Conclusions	
RTECS	Classified as mutagen and tumorigen.
TOXNET	Mutagenic and carcinogenic.
ICLUID	Mutagenic and carcinogenic.
GESAMP	
Current project summary sheet	
Current project conclusion	Clearly mutagenic (M) and carcinogenic (C). IARC classified: Sufficient evidence in animals and evidence in humans; Group 1, human carcinogen.
Current project further work	We recommend no further studies since M and C are confirmed.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Formaldehyde**
Chemical Group	Aldehydes
CAS Number	50-00-0
Oral LD50 (mg/kg b.w.)	500 (rat); 385 (mice)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** Spermatogenesis (2, 7, 17, 20, 21) Testes, epididymis, sperm duct (10, 11, 19, 21) Prostate, seminal vesicle, Cowper's gland, accessory glands (10) Male fertility index (12) Other effects (7) **IUCLID:** Small non significant increase in no. of abnormal cells. (1) Decrease in food consumption and body weight gain (3, 5) Increased preimplantation deaths (4) Mortality (6, 11) No effect on pregnancy parameters and conceptus (3) No diff in fertility (10) Decrease in pregnancy rate (6) Significant decrease in the male testicular nucleic acid content (10) Maternal toxicity (11) No effect (2, 7)
Fetotoxicity	**RTECS:** Biochemical and metabolic (3, 6, 8, 26) Reduced growth (5, 14, 22, 23) Death (14, 15, 18, 22, 23) Post implantation mortality (18) **IUCLID:** Decrease in body weight (2, 3, 5, 9) Reduced body length and mobility (4) Prenatal death (9) Effective in terminating pregnancy (11) Decrease in number of surviving embryos (11) No effect (7, 8)
Fetal development	**RTECS:** Hepatobiliary system (1, 13, 24, 25) Cytological changes (4) Postnatal measures (5) Musculoskeletal (9, 14)

	Behavioural (9, 26) Craniofacial (14), Urogenital (24, 25), Respiratory system (25) Delayed effects (26) Other (16) **<u>IUCLID:</u>** Decrease in ossification of the pelvic girdle (3) No effect (4, 5, 6, 7, 8) Cleft plate and malformations of the limb (9)
Additional Info	Gonadotropic effects after air or water uptake may be of importance
Conclusions	
RTECS	Classified as Reprotoxic. Toxicology Review (REPTED Reproductive Toxicology, 2003)
IUCLID	Two studies in IUCLID on reproduction toxicity. Several studies on reproductive effects in IUCLID indicated fetotoxicity but no statistically significant teratogenic effects except in one study by Strathclyde Chemical Company Limited Johnstone where major malformations were seen in cleft plate and limbs.
GESAMP	No R rating (CSM)
Current project summary sheet	**CMRS**
Current project conclusion for R	**Mainly fetotoxic effects but may exhibit maternal and developmental toxicity as well**
Current project further work for R	**Confirm R. No reproductive toxicity testing needed.**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Formaldehyde**
Chemical Group	Aldehydes
CAS Number	50-00-0
Oral LD50 (mg/kg b.w.)	500 (rat); 385 (mice)
Sensitization	
Studies	**RTECS:** Inhalation: 3 studies indicating delayed hypersensitivity Percutaneous: toxicity data in mice, rat and human **IUCLID:** **Invalid:** (1, 7, 10, 18, 19, 21, 29, 41, 45, 46, 51) **Sensitizing:** Guinea pig: Buehler test (3), 37% content with Buehler test (4), 37% content with Draize test (8), with Freund's complete adjuvant test (9), with guinea pig maximization test (≥37% aqueous solution-11, 12, 13, 14, 15, 16, 35% content-17, 22, 23, 20% aqueous solution-24, 25, 26, 27, 28, 37% content-30), 40% content with open epicutaneous test (34), 37% content with split adjuvant test (38), with AP2- test (40), with Cumulative contact enhancement test (43), with guinea pig optimisation test (35% content- 47, 37% content- 48), Skin sensitizer in specially designed study (52) Mice: CBA/Ca with mouse local lymphnode assay (32), with cytokine production by draining mouse lymph node cells (44), 37% content in BALB/c with local lymph node assay (49), 37% content in BALB/c with mouse immuno globulin E test (50) **Non sensitizing:** Guinea pig: 37% content with Buehler test (2), with Draize test (5), 37% content with Draize test (6), 37% content with guinea pig maximization test (20), with mouse local lymphnode assay (33), 37% content in guinea pigs with open epicutaneous test (35), 37% content with split adjuvant test (36), No respiratory hypersensitivity in specially designed study (52).

	Mice: CBA/Ca with mouse local lymphnode assay (33) **Ambiguous** Guinea pigs: 37% content with split adjuvant test (37), with Cumulative contact enhancement test (42) Mice: 37% content in Balb/c with mouse ear swelling test (31) Human (39)
Additional Info	
Conclusions	
RTECS	Sensitizer (Refer Percutaneous toxicity data)
IUCLID	Several studies in IUCLID indicating both positive and negative results.
GESAMP	GESAMP classified as sensitizer 2009-04
Current project summary sheet	**CMRS**
Current project conclusion for S	**Confirmed S**
Current project further work for S	**No toxicity testing needed.**

CAS - No.	Name
75-07-0	**Acetaldehyde**

EU-Risk phrases	R12; R40, R36/37; F, Xn, Carc. Cat. 3
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	No
RTECS file	AB1925000; Last updated 200911
IUCLID file	Yes
REACH file	
Other sources	• **CPDB:** nasal cancers in male and female rats; nasal and oral cavity cancers in hamsters. • **IRIS:** Carcinogenicity and mutagenicity studies • **TOXNET:** Carcinogenicity and mutagenicity studies

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			
2			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**1**	661	Rat	RTECS-AGACBH Agents and Actions, A Swiss Journal of Pharmacology
2	**1**	1930	Rat	RTECS- Encyclopedia of Toxicology
3	**1**	1930	Rat	IUCLID- Hoechst AG Frankfurt/ Main
4	**1**	900	Mice	RTECS-Labor Hygiene and Occupational Diseases

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-200 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**0**	3540	Rabbit	RTECS- Union Carbide Data Sheet

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1	**0**	24.41	Rat	RTECS-LC50= 13300 ppm-4H/ National Technical Information Service
2	**0**	24.41	Rat	RTECS-LC50= 13300 ppm-4H/ Encyclopedia of Toxicology
3	**0**	25	Rat	RTECS-LC50=25000 mg/m3/ Farmakologiya i Toksikologiya
4	**0**	42.70	Rat	IUCLID-LC50=20500 ppm/0.5 Hrs/ Hoechst AG Frankfurt/ Main
5	**0**	24.41	Rat	IUCLID-LC50=13300 ppm/4 Hrs/ Hoechst AG Frankfurt/ Main
6			Rat	IUCLID-LC50=37 mg/l -0.5 hrs/ Hoechst AG Frankfurt/ Main
7	**0**	58.73	Rat	IUCLID-LC50=≥16000 ppm -8 hrs/ Celanese GmbH Frankfurt am Main
8	**0**	23	Mice	RTECS-LC50=23gm/m3/4H/CUTOEX Current Toxicology

9	**1**	10.15	Mice	RTECS-LC50=20300mg/m3/2H/ Hazardous substances. Galogen and oxygen containing substances
10	**0**	31.20	Hamster	RTECS-LC50=17000ppm/4H Progress in Experimental Tumor Research., 1979
11	**0**	31.20	Hamster	IUCLID-LC50=17000 ppm -4 hrs/ Hoechst AG Frankfurt/ Main
12	**0**	20.1	Mammal unspecified	RTECS-LC50=20100 mg/m3/ Labor Hygiene and Occupational Diseases
13			Rat	IRIS: LOAEL=400ppm, Short term inhalation studies, Appleman et al., 1986;1982
14			Human	RTECS- TCLo=134 ppm/30M/ Journal of the American Medical Association
15			Human	RTECS- TCLo=100000mg/m3/30M/ Hazardous substances. Galogen and oxygen containing substances

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive

3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1	**1**	**Mild** RTECS-Union Carbide Data Sheet/ Open irritation test/ rabbit/ 500mg Please note that open irritation method is used
2	**1**	**Mild** RTECS- Encyclopedia of Toxicology, Standard Draize test/ rabbit/ 500mg
3	**0**	**Non irritating** IUCLID-rabbit/ OECD 404 /Hoechst AG Frankfurt/ Main
4	**1**	**Slightly irritating** IUCLID-rabbit/ 500 mg/ Hoechst AG Frankfurt/ Main

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1		RTECS- (Journal of Industrial Hygiene and Toxicology)/ Standard Draize Test/ human/ 50 ppm-15M
2	**3**	**Severe** Encyclopedia of Toxicology/ Standard Draize test/ rabbit/ 40mg
3	**3**	**Highly irritating** IUCLID-rabbit/ 40 mg/ Hoechst AG Frankfurt/ Main

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C	**C**	Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R	**R**	Immunotoxic - I	
Mutagenic - M	**M**	Photosensitizer - P		Sensitizing - S	**S**

Source/comment:

C: carcinogenicity

RTECS; Olfaction and lungs, thorax, or respiration- tumors; ACGIH TLV-Confirmed animal carcinogen; IARC: Sufficient evidence in animals and inadequate evidence in humans; IARC Cancer Review: Group 2B, probably carcinogenic to humans; NTP 10th Report on Carcinogens, 2002: Reasonably anticipated to be a human carcinogen; 3 studies in IUCLID.

CPDB: TD50 = 153 mg/kg/day (rat, oral).

study no	proposed rating	source / kind of study / animal species
1	Positive, Class 2B	TOXNET, Rat studies (2 positive): Wistar/Male and Female. Inhalation: Nasal cavity tumor. Toxicology 31(2):123-133, 1984.

M: Mutagenicity

RTECS, Several Toxicology Reviews (Environmental Health Perspectives; Toxicology Letters; Mutation Research; Human Toxicology); EPA GENETOX PROGRAM 1988, Positive: E coli polA without S9; In vitro SCE-nonhuman; EPA GENETOX PROGRAM 1988, Positive/dose response: In vitro SCE-human lymphocytes; EPA GENETOX PROGRAM 1988, Positive/dose response: In vitro SCE-human; Studies in IUCLID: Positive/negative results with Ames test in Escherichia coli/Salmonella typhimurium. Positive results with cytogenetic assay: rat fibroblasts, human lymphocytes, CHO-cells. Positive results in variety of other species with variable tests (both in vitro and in vivo).

study no	proposed rating	source / kind of study / animal species
1	Positive	TOXNET, Sister-chromatid exchange (SCE) in vitro (3 st), Mutat Res 87:17-62, 1981.
2	Positive	TOXNET, Sister-chromatid exchange (SCE) in vivo, Mutat Res 88:389-395, 1981.
3	Positive?	TOXNET, Prokaryotes - Rec-assay, MU.TAT RES 87:211-297, 1981.
4	Positive?	RTECS, prokaryotes, 2 studies
5	Positive?	RTECS, insects, 2 studies
6	Positive?	RTECS, lower eukaryotes, 4 studies
7	Positive?	RTECS, mammalian cells, 23 studies
8	Positive?	RTECS, human cells, 13 studies

R: Reprotoxicity

RTECS data; Toxicology Reviews (REPTED Reproductive Toxicology); IUCLID: Teratogenic and developmental toxic

S: Sensitization

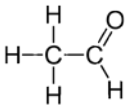
IUCLID data- Several studies indicating sensitizing in humans with patch test

Remarks

ACGIH TLV-CL 25 ppm; MSHA STANDARD-air:TWA 100 ppm (180 mg/m3)
NIOSH Recommended Exposure Level -air:CA (18 ppm LOQ)
OEL-SWEDEN: TWA 25 ppm (45 mg/m3);STEL 50 ppm (90 mg/m3), Carcinogen, JUN2005

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
Acetaldehyde	75-07-0		RTECS+ IUCLID+ GESAMP-CPDB+ IRIS+ TOXNET+	1	0	0	C M R S	OEL: 45 mg/m3 Skin: Mildly irritating Eye: Severely irritating **Confirm C** **Confirm M** **Confirm R** **Confirm S** **No further testing needed**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Acetaldehyde**
Chemical Group	**Aldehyde**
CAS Number	**75-07-0**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**661 (Rat oral)** **640 (Rat sc)**
Mutagenicity /Carcinogenicity	
Mutagenicity	**Toxnet** 1. Sister-chromatid exchange (SCE) in vivo **(positive)** 2.Sister-chromatid exchange (SCE) in vitro (3 st) **(positive)** 3. Prokaryotes - Rec assay **(positive)** **RTECS** Human cell tests, 13 studies **(positive?)** Mammalian cell tests, 23 studies **(positive?)** Lower eukaryote tests, 4 studies **(positive?)** Insects tests, 2 studies **(positive?)** Prokaryote tests, 2 studies **(positive?)** **ICLUID:** Ames test in Escherichia coli/Salmonella typhimurium **(positive).** Cytogenetic assays: rat fibroblasts, human lymphocytes, CHO-cells **(positive).** Variable tests in a variety of other species (both in vitro and in vivo) **(positive).** **HSDB** Corrosive. Causes severe eye and skin burns. Serious health hazard. Iritating to skin, eyes, and respiratory system. Narcosis, nausea, and loss of consciousness may result from exposure to high concentrations of vapor.
Carcinogenicity	**Toxnet:** Data from rat studies **(2 positive**). Cancerogenic : IARC Group 2B - probably carcinogenic to humans. **CPDB:** TD50, mg/kg/day: 153 ^{m}, Rat, oral. **IUCLID:** 3 studies **(positive).**
Additional Info	
Conclusions	
RTECS	Classified as mutagen and tumorigen.
ICLUID	Mutagenic and carcinogenic.
GESAMP	
Current project summary sheet	
Current project conclusion	Clearly mutagenic (M) and cancerogenic (C). IARC classified: Group 2B - probably carcinogenic to humans.
Current project further work	We recommend no further studies since M and C are confirmed.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Acetaldehyde**
Chemical Group	Aldehydes
CAS Number	75-07-0
Oral LD50 (mg/kg b.w.)	661 (rat), 900 (mice)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** - **ICLUID:** No effect (1)
Fetotoxicity	**RTECS:** Post implantation mortality (5, 6, 11, 12) Death (5, 6, 7, 11, 12, 13, 14) Stunted fetus (5, 7, 11, 12, 13, 14) Growth (9) **ICLUID:** Embryotoxic (1, 5) Fetototoxic (1) No embyrotoxic effect (4) No fetotoxic effect (4) Small size (5) Mortality (5, 6)
Fetal development	**RTECS:** Homeostasis (1) Eye/ear (2) Musculoskeletal (2, 4) Craniofacial (3) CNS (6, 8) Respiratory system (7) Hepatobiliary system (7) Endocrine (8) Urogenital (8) Behavioral (10) Extra embryonic structures (11, 13, 14) Other (12) **ICLUID:** Teratogen-oedema, microsephalus, hydrosephalus, exensephalus, micrognathia (1) Delay in ossification and skeletal malformations (2) Stage specific teratogen effects (3) Neuronal malformations (5) Neural tube defects (5) Foetus malformations (6)
Additional Info	
Conclusions	

RTECS	Classfied as reprotoxic; Toxicology Reviews (REPTED Reproductive Toxicology)
ICLUID	No paternal toxicity studies. However, teratogenic and developmental effects are reported.
GESAMP	No Profile
Current project summary sheet	**CMRS**
Current project conclusion for R	**Mainly fetotoxic and developmental effects**
Current project further work for R	**Confirm R. No further testing needed.**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Acetaldehyde**
Chemical Group	Aldehydes
CAS Number	75-07-0
Oral LD50 (mg/kg b.w.)	661 (rat), 900 (mice)
Sensitization	
Studies	**RTECS:** Classified as primary irritant **IUCLID:** **Sensitizing:** Human: with patch test (1, 2, 3, 4, 5,6 ,7)
Additional Info	
Conclusions	
RTECS	Classified as primary irritant
ICLUID	Several studies indicating sensitizing in humans with patch test
GESAMP	No classification
Current project summary sheet	**CMRS**
Current project conclusion for S	**Confirmed S**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
60-35-5	**Acetamide**

EU-Risk phrases	R40, Xn, Carc. Cat. 3
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	No
RTECS file	AB4025000; Last updated 200902
IUCLID file	Chemical found in IUCLID inventory but no chemical data sheet available
REACH file	
Other sources	**CPDB:** liver cancers in male and female rats; hematopoietic cancer in male mice **TOXNET:** Carcinogenecity and mutagenecity studies **Hazmap:** Inhalation toxicity (http://hazmap.nlm.nih.gov)

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**0**	7000	rat	RTECS-LD50=7 gm/Kg/Journal of Reproduction and Fertility
2	**0**	12900	mice	RTECS- Japanese Journal of Pharmacology

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-200 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1				

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1	**0**	39.39	rat	16000ppm- Hazmap (http://hazmap.nlm.nih.gov)

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive
3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1		

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1		

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C	**C**	Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R	**R**	Immunotoxic - I	
Mutagenic - M	**M?**	Photosensitizer - P		Sensitizing - S	

Source/comment:

C: carcinogenicity

RTECS; Liver and blood – tumors, lymphoma, including Hodgkin's disease; IARC: Sufficient evidence in animals and no adequate data in humans; IARC Cancer Review: Group 2B, probably carcinogenic to humans; EPA GENETOX PROGRAM 1988, Positive: Carcinogenicity-mouse/rat; SHE-clonal assay.
CPDB: TD50 = 180 mg/kg/day (rat, oral), target organ: Liver. (TD50 = 3010 mg/kg/day (mouse, oral), target organ: hematopoietic system).
Classification for carcinogenicity: Class 1=Carcinogenic to humans, 2 = Probably carcinogenic to humans, 3 = Possibly carcinogenic to humans, 4 = Not classifiable as a human carcinogen

study no	proposed rating	source / kind of study / animal species
1	Positive	TOXNET, Rat, wistar, male, 1.25; 2.5; 5% in diet fir one year, Liver: Trabecular carcinoma, Adenocarcinoma, Hepatoma. IARC Monographs 1974.

M: Mutagenicity

RTECS data, Several Toxicology Reviews (Mutation Research, Environmental and Molecular Mutagenesis; Critical Reviews in Toxicology; Basic and clinical pharmacology and toxicology; Encyclopaedia of Toxicology); EPA GENETOX PROGRAM 1988, Positive: Cell transform.-mouse embryo, RLV F344 rat embryo; Negative: Host-mediated assay; E coli polA with S9, Histidine reversion-Ames test, Sperm morphology-mouse, S cerevisiae-homozygosis, Inconclusive: E coli polA without S9.

study no	proposed rating	source / kind of study / animal species
1	All Negative	TOXNET, Ames test (5 st), Toxicology 228(1):66-76, 2006.
2	Negative	TOXNET, Micronucleus in vivo, 500, 1000, 2000 mg/kg, De Boek M. et al., Environ. Mol. Mutagen. 46(1):30-42, 2005.
3	Positive	TOXNET, Micronucleus test, chromosome aberrations, Mammalian polychromatic erythrocytes, 3390 µmol/kg, Chieli E. et al. Mutat Res 192:141-143, 1987.
4	All Positive	TOXNET, Cell transformation (3 st), All three in Mutat Res 114:283-385, 1983.
5	Neg / No concl.	TOXNET, Rec assay (2 st), Mutat Res 87:211-297, 1981.
6	Negative	TOXNET, Sperm morphology, mouse, male, Mutat Res 115:1-72, 1983.
7	Negative	TOXNET, Mitotic recombination or gene conversion, Saccharomyces cerevisiae, Mutat Res 133:199-244, 1984.
8	Positive ?	RTECS, Rodent - rat Embryo, Morphological transformation, 5 mg/L, Journal of the National Cancer Institute, 1973.
9	Positive ?	RTECS, Rodent - mouse Ascites tumor, Mutation test systems - not otherwise specified, 10 pph, Japanese Journal of Genetics, 51, 53, 1976.
10	Positive ?	RTECS, Rodent - hamster Embryo, Morphological transformation, 1 mg/L, International Journal of Cancer 1976.
11	Positive?	RTECS, Rodent - mouse Fibroblast, Morphological transformation, 0.01 mg/L/21D, Environmental and Molecular Mutagenesis 1977.
12	Positive?	RTECS, Rodent – mouse, oral, Micronucleus test, Kennedy GL et al., Critical Reviews in Toxicology 31, 139, 2001.
13	Positive	RTECS, Human Mammary gland MCF-7 cells, DNA damage measured by expression of p53R2, 5.8 mg/L/24H, Ohno K. et al., Mutation Research, 2005.
14	Positive	In vivo micronucleus assay, Mavourin KH et al., Mutat Res 239, 1990 (Only a review!)
15	Negative	Ames test, DNA damage and DNA repair of Rat hepatocytes. Dybing et al, Pharmacol Toxicol., 60, 1, 1987.
16	Negative	, Micronucleus test, rat hepatocytes. Mirkova E., Mutat Res 352, 1996.

R: Reprotoxicity
RTECS data; post-implantation mortality (e.g. dead and/or resorbed implants per total number of implants); Specific developmental abnormalities in musculoskeletal system; fetotoxicity.

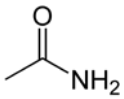
S: Sensitization
No data available

Remarks

OEL-SWEDEN: TWA 10 ppm (25 mg/m3);STEL 25 ppm (60 mg/m3), Carcinogen, JUN2005

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
Acetamide	60-35-5		RTECS+ IUCLIDx GESAMP-CPDB+ IRIS-TOXNET+ Hazmap+	0	-	-	C M? R	OEL: 25 mg/m3 **Confirm C** **M: Need more data** **Confirm R** **S: No data available** **No further testing needed**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Acetamide**
Chemical Group	**Amide**
CAS Number	**60-35-5**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**7000 (Rat oral)** **10,300 (Rat ip)** **10000 (Rat sc)** **12,500 (Rat iv)**
Mutagenicity /Carcinogenicity	
Mutagenicity	**Toxnet** 1. Prokaryotic - Ames (5 st) **(negative).** 2. Prokaryotic - Rec assay (2 st) **(negative, no conclusion).** 3. Micronucleus in vivo **(negative).** 4. Micronucleus test and chromosome aberrations **(positive?)** (Chieli E. et al 1987). 4. Cell transformation (3 st) **(all positive).** 5. Sperm morphology **(negative)** 5. Lower eukaryotes – mitotic recombination and gene conversion **(negative)** **RTECS** Different tests in mammalian cell systems on cytogenicity and DNA damage **(5 positive?)** (Kennedy G.L. et al 2001, Ohno K. et al 2005)**.** **PubMed** Two studies on mammalian cell systems; micronucleus test and DNA damage/repair test **(both negative, and conclude that some previous tests cited in RTECS are wrong)** (Dybing E. Et al 1987 and Mirkova E. 1996)**.** **HSDB** Mild irritant, Low toxicity, causes readily reversible tissue changes which disappear after exposure stops, causes some discomfort.
Carcinogenicity	**Toxnet:** Data from one rat study, 1974 **(positive).** Cancerogenic : IARC Group 2B - probably carcinogenic to humans **CPDB:** TD50, mg/kg/day: 180 m , Rat, oral
Additional Info	
Conclusions	
RTECS	Classified as mutagen and tumorigen.
ICLUID	
GESAMP	
Current project summary sheet	
Current project conclusion	Possibly mutagenic (M?). Clearly carcinogenic (C). IARC classified: Group 2B - probably carcinogenic to humans
Current project further work	We recommend no further studies.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Acetamide**
Chemical Group	Amides
CAS Number	60-35-5
Oral LD50 (mg/kg b.w.)	7000 (rat), 12900 (mice)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** No data available **ICLUID:** -
Fetotoxicity	**RTECS:** Post implantation mortality (1) Death (2) Stunted foetus (2) **ICLUID:** -
Fetal development	**RTECS:** Musculoskeletal (1) **ICLUID:** -
Additional Info	
Conclusions	
RTECS	RTECS data: post-implantation mortality (e.g. dead and/or resorbed implants per total number of implants); Specific developmental abnormalities in musculoskeletal system; fetotoxicity.
ICLUID	Chemical found in ICLUID inventory but no chemical data sheet available.
GESAMP	No Profile
Current project summary sheet	**C M? R**
Current project conclusion for R	**No data on parental toxicity but studies indicate fetotoxic and developmental toxic effect. Confirm R**
Current project further work for R	**Confirm R. No further testing needed.**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Acetamide**
Chemical Group	Amides
CAS Number	60-35-5
Oral LD50 (mg/kg b.w.)	7000 (rat), 12900 (mice)
Sensitization	
Studies	**RTECS:** - **IUCLID:** **-**
Additional Info	
Conclusions	
RTECS	No report on S
ICLUID	Chemical found in IUCLID inventory but no chemical data sheet available
GESAMP	No classification
Current project summary sheet	**C M? R**
Current project conclusion for S	**No data on S**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
74-89-5	**Methylamine**

EU-Risk phrases	**F+; R12, 20, 37/38, 41; Xn**
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	Yes
RTECS file	PF6300000; Last updated 200911
IUCLID file	Yes
REACH file	
Other sources	• **TOXNET: Carcinogenicity and mutagenicity studies**

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**2**	100	Rat	RTECS/ Encyclopedia of Toxicology
2	**1**	689	Rat	IUCLID/ BASF AG Ludwigshafen (40% Loesung)
3	**2**	100-200	Rat	IUCLID/ BASF AG Ludwigshafen (10% Loesung)
4	**0**	1600-3200	Rat	IUCLID/ BASF AG Ludwigshafen (Hydrochloride)
5	**1**	698	Rat	IUCLID/ BASF AG Ludwigshafen (40% Loesung)
6	**1**	375	Rat	IUCLID/ BASF AG Ludwigshafen
7	**2**	80	Rat	IUCLID/ BASF AG Ludwigshafen

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-2000 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1				

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1	**3**	0.97	Rat	2,5 hour exposure. IUCLID
2	**4**	0.362	Rat	RTECS-LC50= 448ppm -2.5H/ Journal of Environmental Biology
3	**4**	0.362	Rat	RTECS-LC50= 448ppm -2.5H/ Encyclopaedia of Toxicology
4	**2**	2.1-2.9	Rat	IUCLID-LC50=2.1-2.9 mg/l -4H/ BASF AG Ludwigshafen
5	**2**	2.9	Rat	IUCLID-LC50=2.9 mg/l-4 H/ BASF AG Ludwigshafen
6	**3**	1.2	Mice	RTECS-LC50= 2400mg/m3/2H/ Encyclopaedia of Toxicology
7	**3**	1.2	Mice	IUCLID-LC50=2.4 mg/l/2H/ BASF AG Ludwigshafen
8	**3**	1.2	Mice	IUCLID-LC50=2.4 mg/l/2H/ BASF AG Ludwigshafen
9	**2**	2.4	Unspecified	RTECS-LC50=2400mg/m3/ Toxicology of New Industrial Chemical Substances.
10	**2**	3.7	Unspecified	RTECS-LC50=3.7gm/m3/ Toxicology of New Industrial Chemical Substances.
11			Human	RTECS-TCLo=0.01 gm/m3/ Toxicology of New Industrial Chemical Substances.

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive
3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1	**3**	**Corrosive** IUCLID- 3 studies in rabbit/ BASF AG Ludwigshafen (40% Loesung)
2	**3**	**Corrosive** IUCLID- 3 studies in guinea pig/ BASF AG Ludwigshafen
3	**3**	**Severe** RTECS- Open irritation test/ guinea pig- 100mg/ Contact Dermatitis. Environmental and Occupational Dermatitis
4	**3**	**Severe** RTECS- Standard Draize test/ rabbit- 40%/ Encyclopedia of Toxicology

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1	**2**	**Irritating** IUCLID- 2 studies in rabbit/ BASF AG Ludwigshafen
2	**3**	**Severe** RTECS- Standard Draize test/ rabbit- 40%/ Encyclopedia of Toxicology
3	**2**	**Mild** RTECS- Standard Draize test/ rabbit- 5%/ Encyclopedia of Toxicology

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C		Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R		Immunotoxic - I	
Mutagenic - M	**M**	Photosensitizer - P		Sensitizing - S	

Source/comment:

Carcinogenicity

RTECS and TOXNET: NA

M:Mutagenicity

RTECS data in rat and mice lymphocytes; Several toxicology reviews (Mutation Research, Chemcial Reviews, Encyclopedia of Toxicology). Several studies in IUCLID: Negative results with Ames test in salmonella and Ecoli (in vitro); Positive results with mice lymphoma assay (in vitro) and dominant lethal assay in rats (in vivo)

study no	proposed rating	source / kind of study / animal species
1	Positive?	RTECS, Rodent – rat, inhalation, 10 ug/m3, Dominant lethal test, Gigiena i Sanitariya.
2	Positive?	RTECS, Rodent - mouse Lymphocyte, 3 mmol/L, Mutation in mammalian somatic cells, Mutation Research.
3	Negative	TOXNET, Ames test (16 st), JAPAN CHEMICAL INDUSTRY ECOLOGY, 1997.
4	Negative	TOXNET, Tryptophan reverse gene mutation assay, (4 st), prokaryotes, JAPAN CHEMICAL INDUSTRY ECOLOGY, 1997.
5	Positive	TOXNET, Forward gene mutation at the thymidine kinase (TK) locus (1st), Mouse, MUTAT. RES. 174(4):285-293, 1986.
6	Positive	IUCLID, rat, dominant lethal assay.

R: Reprotoxicity

ICLUID: Studies on reproduction, developmental and teratogenecity showed negative results on these parameters

S: Sensitization

No data available

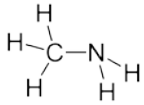
Remarks

ACGIH TLV-TWA 5 ppm; STEL 15 ppm; NIOSH Recommended Exposure Level -air:10H TWA 10 ppm

OEL-Denmark: TWA 5 ppm (6.4 mg/m3), 2002
IUCLID studies on reproduction, developmental and teratogenecity showed negative results on these parameters

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date
2	(2)	3	3	3	M	2009-04

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
Methylamine	74-89-5		RTECS+ IUCLID+ GESAMP+ CPDB- IRIS- TOXNET+	2	(2)	3	M	OEL: 6.4 mg/m3 Skin and eye: Severely irritating Moderate acute toxicity. **C: No data available** **Confirm M** **Confirm no R** **S: No data available** **Need further testing for C**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Methylamine**
Chemical Group	**Akylamine**
CAS Number	**74-89-5**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**80 (Rat oral female adults)** **375 (Rat oral weaning males)**
Mutagenicity /Carcinogenicity	
Mutagenicity	**Toxnet** 1. Ames test (16 st) **(negative)** 2. Forward gene mutation at the thymidine kinase (TK) locus (1 st) **(positive)** 3. Tryptophan reverse gene mutation assay (4 st) **(negative)** **RTECS** 1. Dominat lethal test **(positive?)** 2. Mammalian somatic cell mutation test **(positive?)** **UCLID** 1. Dominat lethal test **(positive)** **HSDB** A severe skin irritant. Irritating to eyes, skin, respiratory tract.
Carcinogenicity	**Toxnet:** No data **RTECS:** No data
Additional Info	
Conclusions	
RTECS	Classified as mutagen.
ICLUID	Mutagenic.
TOXNET	Mutagenic.
GESAMP	
Current project summary sheet	
Current project conclusion	Clearly mutagenic (M).
Current project further work	We recommend further studies on carcinogenicity to confirm C.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Methylamine**
Chemical Group	Alkylamines
CAS Number	74-89-5
Oral LD50 (mg/kg b.w.)	100 (rat)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** **-** **IUCLID:** No effect (1, 2, 3)
Fetotoxicity	**RTECS:** - **IUCLID:** No effect (1, 2, 3) Decrease in average litter size (1)
Fetal development	**RTECS:** **-** **IUCLID:** No effect (3, 4)
Additional Info	
Conclusions	
RTECS	No reprotox study
IUCLID	Studies on reproduction, developmental and teratogenecity showed negative results on these parameters
GESAMP	M (No R Rating)
Current project summary sheet	**M (No R Rating)**
Current project conclusion for R	**No reprotox effects**
Current project further work for R	**Confirm no R. No testing needed.**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Methylamine**
Chemical Group	Alkylamines
CAS Number	74-89-5
Oral LD50 (mg/kg b.w.)	100 (rat)
Sensitization	
Studies	**RTECS:** - **IUCLID:** **-**
Additional Info	
Conclusions	
RTECS	Classified as a primary irritant
ICLUID	No report on S
GESAMP	Not classification as S
Current project summary sheet	**M**
Current project conclusion for S	**No data on S**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
124-40-3	**Dimethylamine**

EU-Risk phrases	**F+, R12, 20, 37/38, 41, Xn**
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	Yes
RTECS file	IP8750000; Last updated 200911
IUCLID file	Yes
REACH file	
Other sources	• **IRIS:** Carcinogenicity and mutagenicity studies • **TOXNET: Carcinogenicity and mutagenicity studies**

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**1**	698	Rat	RTECS/ Hygiene and Sanitation
2	**1**	1000	Rat	IUCLID/ BASF AG Ludwigshafen
3	**1**	698	Rat	IUCLID/ BASF AG Ludwigshafen
4	**0**	8100	Rat	IUCLID/ BASF AG Ludwigshafen
5	**1**	1000	Rat	IUCLID/ BASF AG Ludwigshafen
6	**1**	698	Rat	IUCLID/ BASF AG Ludwigshafen
7	**0**	8100	Rat	IUCLID/ BASF AG Ludwigshafen
8	**1**	316	Mice	RTECS/ Hygiene and Sanitation
9	**1**	316	Mice	IUCLID/ BASF AG Ludwigshafen
10	**0**	8100	Mice	IUCLID/ BASF AG Ludwigshafen
11	**1**	316	Mice	IUCLID/ BASF AG Ludwigshafen
12	**0**	8100	Mice	IUCLID/ BASF AG Ludwigshafen
13	**2**	240	Rabbit	RTECS/ Hygiene and Sanitation
14	**2**	240	Rabbit	IUCLID/ BASF AG Ludwigshafen
15	**1**	1600	Rabbit	IUCLID/ BASF AG Ludwigshafen
16	**2**	240	Rabbit	IUCLID/ BASF AG Ludwigshafen
17	**1**	1600	Rabbit	IUCLID/ BASF AG Ludwigshafen
18	**2**	240	Guinea pig	RTECS/ Hygiene and Sanitation
19	**2**	240	Guinea pig	IUCLID/ BASF AG Ludwigshafen
20	**1**	1070	Guinea pig	IUCLID/ BASF AG Ludwigshafen
21	**2**	240	Guinea pig	IUCLID/ BASF AG Ludwigshafen
22	**1**	1070	Guinea pig	IUCLID/ BASF AG Ludwigshafen

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-2000 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**0**	3900	Rat	IUCLID/ BASF-Tset BASF AG Ludwigshafen (40% aqueous soluble)
2	**0**	3900	Rat	IUCLID/ BASF-Tset BASF AG Ludwigshafen (40% aqueous soluble)

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1	**1**	12.79	Rat	RTECS/ LC50= 4540ppm-6H/ American Industrial Hygiene Association Journal
2	**3**	1.5	Rat	RTECS/ LC50= 3gm/m3/2H/ Toxicology of New Industrial Chemical Substances
3	**1**	12.53	Rat	IUCLID/ LC50= 8.354 mg/l- 6H/ BASF AG Ludwigshafen
4	**2**	≥ 5.8	Rat	IUCLID/ LC50= ≥ 5.8 mg/l- 4H/ BASF AG Ludwigshafen
5	**2**	8.8	Rat	IUCLID/ LC50= 8.8 mg/l- 4H/ BASF AG Ludwigshafen
6	**1**	12.60	Rat	IUCLID/ LC50= 8.4 mg/l- 6H/ BASF AG Ludwigshafen
7	**2**	≥ 5.8	Rat	IUCLID/ LC50= ≥ 5.8 mg/l- 4H/ BASF AG Ludwigshafen
8	**2**	8.8	Rat	IUCLID/ LC50= 8.8 mg/l- 4H/ BASF AG Ludwigshafen
9	**3**	1.15 – 1.2	Rat	IUCLID/ LC50= 2.3 – 2.4 mg/l- 2H/ BASF AG Ludwigshafen
10	**3**	1.85	Rat	IUCLID/ LC50= 3.7 mg/l- 2H/ BASF AG Ludwigshafen
11	**2**	4.44	Mice	RTECS/ LC50= 4725ppm-2H/ American Industrial Hygiene Association Journal
12	**4**	0.035	Mice	RTECS/ LC50= 0.07gm/m3/2H Toxicology of New Industrial Chemical Substances
13	**2**	7.038	Mice	IUCLID/ LC50= 14.076 mg/l- 2H/ BASF AG Ludwigshafen
14	**2**	2.5	Mice	IUCLID/ LC50= 5 mg/l- 2H/ BASF AG Ludwigshafen
15	**2**	7.15	Mice	IUCLID/ LC50= 14.3 mg/l - 2H/ BASF AG Ludwigshafen
16	**2**	3.7	Unspecified	RTECS/ LC50= 3700 mg/m3 Toxicology of New Industrial Chemical Substances.
17	**4**	0.07	Unspecified	RTECS/ LC50= 0.07gm/m3/ Toxicology of New Industrial Chemical Substances.
18	**2**	3.7	Unspecified	IUCLID/ LC50= 3.7 mg/l / BASF AG Ludwigshafen

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive

3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1	**3**	**Corrosive** IUCLID- rabbit/ BASF-Test/ BASF AG Ludwigshafen (40% aqueous solution)
2	**3**	**Corrosive** IUCLID- rabbit/ BASF AG Ludwigshafen (6% aqueous solution)
3	**3**	**Corrosive** IUCLID- rabbit/ BASF Test/ BASF AG Ludwigshafen (40% aqueous solution)
4	**3**	**Corrosive** IUCLID- rabbit/ BASF AG Ludwigshafen (6% aqueous solution)
5	**3**	**Corrosive** IUCLID- mice/ BASF AG Ludwigshafen (tail immersed in 6-20% aqueous solution for 5 min -2 hrs.)
6	**2**	**Primary Irritant** RTECS-rabbit-6pph/ Toxicology of New Industrial Chemical Substances
7	**3**	**Corrosive** RTECS-mice-6pph/2H/ Toxicology of New Industrial Chemical Substances
8	**2**	**Primary Irritant** RTECS-unspecified species-100pph/ Toxicology of New Industrial Chemical Substances
9	**2**	**Primary Irritant** RTECS-rabbit-3pph/5D Toxicology of New Industrial Chemical Substances

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1	**2**	**Irritating** IUCLID/ rabbit/ BASF AG Ludwigshafen (5% aqueous solution)
2	**2**	**Irritating** IUCLID/ rabbit/ BASF AG Ludwigshafen
3	**3**	**Corrosive** IUCLID/ rabbit/ Draize Test/ BASF AG Ludwigshafen (40% aqueous solution)

4	**2**	**Irritating** IUCLID/ rabbit/ BASF AG Ludwigshafen (3% solution)
5	**3**	**Irritating** IUCLID/ rabbit/ BASF AG Ludwigshafen (undiluted solution for 1 min-corneal opacity)
6	**3**	**Irritating** (Risk of serious damage to eyes) IUCLID/ rabbit/ BASF AG Ludwigshafen (40% aqueous solution)
7	**2**	**Irritating** IUCLID/ rabbit/ BASF AG Ludwigshafen
8	**2**	**Irritating** IUCLID/ mice/ BASF AG Ludwigshafen (3% aqueous solution)
9		RTECS/ rabbit- 50mg-5M/ Standard draize test / British Journal of Industrial Medicine.
10	**3**	**Irritation and corneal damage** RTECS/ rabbit-3pph/ Toxicology of New Industrial Chemical Substances
11	**2**	**Conjunctive Irritant** RTECS/ unspecified species-100pph/ Toxicology of New Industrial Chemical Substances

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C		Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R		Immunotoxic - I	
Mutagenic - M	**M**	Photosensitizer - P		Sensitizing - S	**S**

Source/comment:

C: Carcinogenicity

RTECS and TOXNET: NA
ACGIH TLV-Not classifiable as human carcinogen. Several studies in ICUID indicating no carcinogenicity and no increased tumour incidence.

M: Mutagenicity

RTECS data in rat and hamsters with cytogenetic analysis and sister chromatid exchange; Toxicology Review (Chemical Reviews); EPA GENETOX PROGRAM 1988, Negative: Host-mediated assay

Several stuies in IUCLID:
Positive and negative results (In Vitro), Negative: Ames test, Bacillus subtilis recombination assay, Bacterial gene mutation assay, Cytogenetic assay, HGPRT assay, Sister chromatid exchange assay, Unscheduled DNA synsthesis, Yeast mutation assay, Repair tests on bacteria, Host mediated assay; Positive: Ames test, Gene mutation in Saccharomyces cerevisiae, Mutagenecity in plant cells.

Positive and negative results (In Vivo), Negative: Cytogenetic assay, Chromosome analysis, Host mediated assay; Positive: Cytogenetic assay, Chromosome analysis

study no	proposed rating	source / kind of study / animal species
1	Positive?	RTECS, Rodent – rat, Cytogenetic analysis, 50 ug/m3, Gigiena i Sanitariya, 1971, 36 (11).
2	Positive	RTECS, Rodent - hamster Ovary, Cytogenetic analysis, 10 mmol/L, Hsie A.W. et. al., Molecular Toxicology 1987, 1, 217.
3	Positive	RTECS, Rodent - hamster Ovary, Sister chromatid exchange, 500 umol/L, Hsie A.W. et al. Molecular Toxicology 1987, 1, 217.
4	Negative	TOXNET, Ames test, (13 at), MUTAT RES 57:115-121, 1978.
5	Positive	IUCLID, in vitro, Ames test, Gene mutation in Saccharomyces cerevisiae, Mutagenecity in plant cells.
6	Negative	IUCLID,in vitro, Ames test, Bacillus subtilis recombination assay, Bacterial gene mutation assay, Cytogenetic assay, HGPRT assay, Sister chromatid exchange assay, Unscheduled DNA synsthesis, Yeast mutation assay, Repair tests on bacteria, Host mediated assay.
7	Positive	IUCLID, in vivo, Cytogenetic assay, Chromosome analysis.
8	Negative	IUCLID, in vivo, Cytogenetic assay, Chromosome analysis, Host mediated assay.

R: Reprotoxicity

Only one inconclusive study on reproduction toxicity. Several studies in ICUID indicating no developmental toxicity

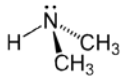
S: Sensitization
IUCLID data indicating positive results with guinea pig maximization test and mouse ear swelling test.

Remarks

ACGIH TLV-TWA 5 ppm;STEL 15 ppm; NIOSH Recommended Exposure Level- air:10H TWA 10 ppm
OEL-NORWAY: TWA 10 ppm (18 mg/m3), JAN1999, Sweden TWA 2 ppm (3.5 mg/m3);STEL 5 ppm (9 mg/m3), JUN2005
Moderate acute toxicity No indication for C or R OEL: 3.5 mg/m3 IRIS file withdrawn ***In presence of nitrous acid may form nitroso-dimethylamine (CAS62-75-9). If such conditions exist then long term serious effects should be anticipated***

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date
2	0	2	3B	3	S	2009-04

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
Dimethylamine	124-40-3		RTECS+ IUCLID+ GESAMP+ CPDB- IRIS+ TOXNET+	2	0	2	M S	OEL: 3.5 mg/m3 Skin and eye: Severely irritating **Confirm no C** **Confirm M** **Confirm no R** **Confirm S** **No further testing needed**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Dimethylamine**
Chemical Group	**Akylamine**
CAS Number	**124-40-3**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**698 (rat)** **1000 (rat)** **8100 (rat)** **316 (mice)** **8100 (mice)** **240 (rabbit)** **1600 (rabbit)** **240 (Guinea pig)** **1070 (Guinea pig)**
Mutagenicity /Carcinogenicity	
Mutagenicity	**Toxnet** Ames test (13 st) **(12 negative, 1 positive)** **RTECS** Cytogenicity analysis (2 st) **(positive)** Sister chromatide exchange (SCE) test **(positive)** **IUCLID** 1. Ames test **(positive).** 2. Ames test, Bacillus subtilis recombination assay, Bacterial gene mutation assay, Cytogenetic assay, HGPRT assay, Sister chromatid exchange assay, Unscheduled DNA synsthesis, Yeast mutation assay, Repair tests on bacteria, Host mediated assay **(negative)** 3. In vivo, Cytogenetic assay, Chromosome analysis **(positive).** 4. In vivo, Cytogenetic assay, Chromosome analysis, Host mediated assay **(negative).** **HSDB** Vapours are eye, skin & respiratory irritants.
Carcinogenicity	**ACGIH TLV:** Not classifiable as human carcinogen. **ICULID:** Several studies indicating no carcinogenicity and no increased tumour incidence.
Additional Info	
Conclusions	
RTECS	Classified as a mutagen.
Toxnet	IARC Group 4 - Not classifiable as a human carcinogen
ICLUID	Mutagenic
GESAMP	
Current project summary sheet	
Current project conclusion	Mutagenic (M). IARC classified: Group 4 – Not classifiable as a human carcinogen.
Current project further work	No further testing recommended.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Dimethylamine**
Chemical Group	Alkylamines
CAS Number	124-40-3
Oral LD50 (mg/kg b.w.)	698 (rat); 316 (mice)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** - **IUCLID:** Degeneration of the seminifereous tubules in rabbit (1) No effect (2, 3, 4)
Fetotoxicity	**RTECS:** - **IUCLID:** No effect (2, 4) Minimization of yolk sac diameter in vitro in mouse whole embryo culture (5) Survival rate in vitro (5)
Fetal development	**RTECS:** - **IUCLID:** No effect in vivo (3, 5) Minimiztion of embryonal DNA, RNA and protein (5)
Additional Info	
Conclusions	
RTECS	No reprotox study
IUCLID	Only one inconclusive study on reproduction toxicity with positive effects. Several studies in IUCLID indicating no developmental toxicity
GESAMP	S (No R Rating)
Current project summary sheet	**MS (No R Rating)**
Current project conclusion for R	**No reprotox effects.**
Current project further work for R	**Confirm no R. No further action.**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Dimethylamine**
Chemical Group	Alkylamines
CAS Number	124-40-3
Oral LD50 (mg/kg b.w.)	698 (rat); 316 (mice)
Sensitization	
Studies	**RTECS:** Pecutaneous: Toxicity data in rabbit and mammals indicating primary irritant **IUCLID:** **Sensitizing** Guinea pig: with guinea pig maximization test (1, 2), mouse ear swelling test (4) **Not assessable** Guinea pig: with guinea pig maximization test (3) Mice: mouse ear swelling test (5)
Additional Info	
Conclusions	
RTECS	Classified as a primary irritant
IUCLID	3 studies reporting sensitization effects in guinea pig
GESAMP	Classified as S
Current project summary sheet	**MS**
Current project conclusion for S	**Confirm S**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
62-75-9	**N-nitrosodimethylamine**

EU-Risk phrases	R45, 26, 25, 48/25, 51/53, 61, T, N, Carc. Cat. 2
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	No
RTECS file	IQ0525000; Last Updated: 20911
IUCLID file	Chemical found in IUCLID inventory but no chemical data sheet available
REACH file	
Other sources	**IRIS**: Carcinogenicity and mutagenicity studies in humans and animals **CPDB**: Carcinogenic in male and female rats and mice **TOXNET:** Carcinogenicity and mutagenicity studies

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**3**	37	Rat	RTECS/ Gigiena i Sanitariya
2	**3**	26	Rat	RTECS/ Encyclopedia of Toxicology
3	**3**	28	Hamster	RTECS/ Journal of the National Cancer Institute
4			Human	RTECS/ Women LDLo= 20 mg/kg/ 2.5 Y/ Oncology

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-200 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	-	15	Rat	RTECS Russian data (Subcutaneous)

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1	**4**	0.24	Rat	RTECS/ LC50= 78 ppm/4H / Archives of Industrial Health
2	**4**	0.24	Rat	RTECS/ LC50= 78 ppm/4H / Encyclopedia of Toxicology
3	**4**	0.18	Mice	RTECS/ LC50= 57 ppm/4H/ Archives of Industrial Health

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive

3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1		

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1		

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C	**C**	Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R	**R**	Immunotoxic - I	
Mutagenic - M	**M**	Photosensitizer - P		Sensitizing - S	

Source/comment:

C: Carcinogenicity RTECS: Several studies, tumorigenic and carcinogenic by RTECS criteria (kidney, liver, lungs, thorax or respiration - bronchiogenic carcinoma, endocrine, GI tumors), transplacental tumorigenesis. ACGIH TLV-Confirmed animal carcinogen. IARC -Group 2A, probably carcinogenic to humans. Sufficient evidence in animals and no adequate data in humans. MSHA STANDARD: skin-SUSPECTED CARCINOGEN. NTP 2004: Reasonably anticipated to be a human carcinogen. IRIS: Humans: Classification –B2, probable human carcinogen; Animals: Liver , kidney, lung tumors in rats after inhalation, transplacental carcinogen

CPDB: Carcinogenic: Male rats: kidney, liver, lung, testis, vascular system cancers; Female rats: liver and vascular system cancers; Male mice: liver and nervous system cancers; Female mice: lung and nervous system cancers; Rheus monkey: Negative; TD50 = 0.0959 mg/kg/day (rat, oral).

Classification for carcinogenicity: Class 1=Carcinogenic to humans, 2 = Probably carcinogenic to humans, 3 = Possibly carcinogenic to humans, 4 = Not classifiable as a human carcinogen

study no	proposed rating	source / kind of study / animal species
1	Positive, 2A	RTECS (39 studies in rodents)
2	Positive, 2A	TOXNET, Rat: (13 pos, Inhalation (2st): Nasal Cavity: Tumor. Oral (3st): Liver: Hepatocellular Carcinoma, Hemangioma; Bile duct: Tumor. s.c.: (2st): Kidney. i.p. (6st): Liver: Hepatocellular Carcinoma, Hepatoma, Cholangioma, Angioma; Kidney: Mesenchymal Tumor, Epithelial Tumor. IV (1 st): Kidney: Mesenchymal Neoplasm; Lung: Tumor), Mouse: (7 pos, 1 neg, oral, liver), Hamster: (4 pos, 1 neg, oral, liver), Duck: (1 pos, oral, liver), Rabbit: (1 pos, oral, liver), Guinea pig: (1 pos, oral, liver), Fish: (1 pos, oral, liver).

M: Mutagenicity

RTECS gives a range of studies; bacteria, yeast, drosophila, mold, protozoa, fish; rats, mice and hamster- (in vitro and in vivo); gerbil and monkey (in vivo); humans (in vitro)

RTECS: Several toxicology reviews.

RTECS: EPA Genotox program. Many positive listings on C and M. Two negative and two inconclusive studies.

IRIS: Positive in yeast, bacteria, drosophila and mammalian cells.

CPDB: Positive in Salmonella.

study no	proposed rating	source / kind of study / animal species
1	Positive	TOXNET, Prokaryotes, Ames test
2	Positive	TOXNET, Mammalian, CHO test
3	Positive	TOXNET, Mammalian, Forward gene mutation at the HPRT locus
4	Positive	TOXNET, Prokaryotes, Tryptophan reverse gene mutation assay
5	Positive	TOXNET, Mammalian, Forward gene mutation at the thymidine kinase (TK) locus
6	Positive	TOXNET, Mammalian, Unscheduled DNA synthesis (UDS) test
7	Positive	TOXNET, Mammalian, In Vitro Micronucleus test
8	Pos and Neg	TOXNET, Mammalian, In Vitro Chromosomal Aberrations
9	Pos and Neg	TOXNET, Mammalian, In Vivo Micronucleus test
10	Pos and Neg	TOXNET, Mammalian, Sister-chromatid exchange (SCE) in vitro
11	No concl.	TOXNET, Mammalian, Cell transformation, clonal assay
12	No concl.	TOXNET,Mammalian, Cell transformation, viral enhanced

13	Negative	TOXNET, Mammalian, Chromosome aberrations in vivo
14	No concl.	TOXNET, Mammalian, Dominant lethal test
15	No concl.	TOXNET, Mammalian, Spot test, gene mutation
16	Positive	TOXNET, Lower eukaryotes-gene mutation (3 st)
17	Positive	TOXNET, Prokaryotes,Rec assay
18	Positive	TOXNET, Insects, Sex-linked recessive lethal gene mutation
19	Negative	TOXNET, Mammalian, Sperm morphology
20	Positive?	RTECS, prokaryotes, 15 studies
21	Positive?	RTECS, insects, 8 studies
22	Positive?	RTECS, lower eukaryotes, 11 studies
23	Positive?	RTECS, mammalian cells, 130 studies
24	Positive?	RTECS, human cells, 31 studies

R: Reprotoxicity

RTECS gives a range of studies; pre and post-implantation mortality, fetotoxicity, fertility, developmental abnormalities.

S: Sensitization

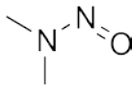
No data available

Remarks

OccExpLevel: several countries: 0,001 mg/m3 carcinogen NIOSH and OSHA analytical methods OEL-SWEDEN: Group B Carcinogen, JUN2005 NIOSH REL TO N-NITROSODIMETHYLAMINE-air:CA use 29 CFR 1910.1016
IRIS: Drinking Water Unit Risk — 1.4E-3 per (µg/L); Inhalation Unit Risk — 1.4E-2 per (µg/cu.m)
Very high acute toxicity Serious long term effects OEL-Switzerland: 0.001 mg/m3 2006

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
N-nitrosodimethylamine	62-75-9		RTECS+ IUCLID+ GESAMP-CPDB+ IRIS+ TOXNET+	3	-	4	C M R	OEL: 0.001 mg/m3 **Confirm C** **Confirm M** **Confirm R** **S: No data available** **No further testing needed**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**N-nitrosodimethylamine**
Chemical Group	**Nitrosamine**
CAS Number	**62-75-9**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**37 (rat)** **26 (rat)** **28 (hamster)** **LD low (human/women): 20 mg/kg/ 2.5 Y/ Oncology**
Mutagenicity / Carcinogenicity	
Mutagenicity	**Toxnet** 1. Ames test **(pos)** 2. CHO test **(pos)** 3. Forward gene mutation at the HPRT locus **(pos)** 4. Tryptophan reverse gene mutation assay **(pos)** 5. Forward gene mutation at the thymidine kinase (TK) locus **(pos)** 6. Unscheduled DNA synthesis (UDS) **(pos)** 7. In vitro micronucleus **(pos)** 8. In vitro chromosomal aberrations **(pos and neg)** 9.In vivo micronucleus **(pos and neg)** 10. Sister-chromatid exchange (SCE) in vitro **(pos)** 11. Cell transformation, clonal assay **(no concl.)** 12. Cell transformation, viral enhanced **(no concl.)** 13. Chromosome aberrations in vivo **(neg)** 14. Dominant lethal test (**no concl)** 15. Spot test, gene mutation (**no concl)** 16. Lower eukaryotes-gene mutation (3st) **(positive)** 17. Rec assay **(positive)** 18. Insects - Sex-linked recessive lethal gene mutation **(positive)** 19. Sperm morphology **(negative)** **RTECS** Prokaryotes (15 studies)**(positive)** Insects (8 studies) **(positive)** Lower eukaryotes (11 studies) **(positive)** Mammalian cells (130 studies) **(positive)** Human cells (31 studies) **(positive)** **HSDB:** The liquid and vapour /may be/ irritating to the skin or eyes.
Carcinogenicity	**Toxnet:** Rat (13 st) (**positive)** Mouse (8st) **(7 positive and 1 negative)** Hamster (5 st)**(4 x positive and 1 x negative)** Duck (1 st)**(positive)** Rabbit (1 st)**(positive)** Guinea pig (1 st) **(positive)**

	<u>RTECS</u> Rodents (39 studies) **(positive)** **CPDB:** TD50, mg/kg/day: 0,0959 mv, Rat, oral **IARC** Group 2A - Probably carcinogenic to humans
Additional Info	
Conclusions	
RTECS	Classified as tumorigen and mutagen.
Toxnet	Mutagenic and carcinogenic. IARC Group 2A - Probably carcinogenic to humans.
ICLUID	
GESAMP	
Current project summary sheet	
Current project conclusion	Clearly carcinogenic (C) and mutagenic (M).
Current project further work	We recommend no further testing to confirm M and C.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**N-nitrosodimethylamine**
Chemical Group	Nitrosamines
CAS Number	62-75-9
Oral LD50 (mg/kg b.w.)	26 (rat)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** Abortion (4) **IUCLID:** -
Fetotoxicity	**RTECS:** Death (1, 2, 3, 5, 6, 7, 9) Stunted fetus (1) Post implantation mortality (3) Pre implantation mortality (4) Stillbirth (10) Viability index (10) Biochemical and metabolic (11) Other (8) **IUCLID:** -
Fetal development	**RTECS:** Other (3) **IUCLID:** -
Additional Info	
Conclusions	
RTECS	Gives a range of studies; pre and post-implantation mortality, fetotoxicity, fertility, developmental abnormalities
IUCLID	Chemical found in IUCLID inventory but no chemical data sheet available
GESAMP	No Profile
Current project summary sheet	**CMR (R Rating)**
Current project conclusion for R	**Confirm R. Mainly fetotoxic but may be developmental and paternal toxic.**
Current project further work for R	**No further testing needed**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**N-nitrosodimethylamine**
Chemical Group	Nitrosamines
CAS Number	62-75-9
Oral LD50 (mg/kg b.w.)	26 (rat)
Sensitization	
Studies	**RTECS:** - **IUCLID:** **-**
Additional Info	
Conclusions	
RTECS	No report on S
IUCLID	Chemical found in ICLUID inventory but no chemical data sheet available
GESAMP	No profile
Current project summary sheet	**CMR**
Current project conclusion for S	**No data available**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
1116-54-7	**N-nitrosodiethanolamine**

EU-Risk phrases	R45, Carc. Cat. 2, T
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	No
RTECS file	KL9550000; Last Updated: 200905
IUCLID file	Chemical found in IUCLID inventory but no chemical data sheet available
REACH file	
Other sources	• **CPDB:** Carcinogenic in male and female rats • **IRIS:** Carcinogenicity studies in humans and animals, mutagenicity studies in bacteria; Risk estimate from drinking water • **TOXNET**

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**0**	7500	Rat	RTECS/ Gigiena i Sanitariya
2				

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-2000 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	-	-	Hamster	LDLo=11000 mg/kg – RTECS (Subcutaneous)
2				

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1				

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive

3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1		

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1		

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C	**C**	Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R		Immunotoxic - I	
Mutagenic - M	**M**	Photosensitizer - P		Sensitizing - S	

Source/comment:

C: Carcinogenicity

RTECS many studies; liver, respiratory; EPA GENETOX PROGRAM 1988, Positive: Carcinogenicity-mouse/rat; NCI Carcinogenesis Studies (oral);clear evidence:rat; NTP 2004:Reasonably anticipated to be a human carcinogen
IARC: Animal sufficient, human inadequate. Group 2B, probably carcinogenic to humans. Classified 2000
CPDB: Carcinogenic in male rats- esophagus, hematopoietic system, kidney, liver, nasal cavity, nervous system, vascular system; female rats- esophagus, kidney, liver, nasal cavity.
IRIS: Classification – B2; probable human carcinogen; human data – inadequate and limited; animal data-hepatocellular carcinoma, cholangiocellar carcinoma and adenoma and neoplastic nodules from drinking water (Lijinsky and Kovatch, 1985); liver and nasal cavity tumors (Preussmann et al., 1982); hepatocellular carcinomas and kidney adenomas (Druckrey et al., 1967), hepatocellular carcinomas and adenocarcinomas of the nasal cavity (Lijinsky et al., 1980; Lijinsky and Reuber, 1984), carcinomas of the nasal cavity and papillomas of the trachea (Hilfrich et al., 1977; Schmeltz et al., 1978; Pour and Wallcave, 1981; Hoffmann et al., 1983). CPDB: TD50= 3.17 mg/kg/day (rat, oral).

study no	proposed rating	source / kind of study / animal species
1	Positive	TOXNET, In vivo carcinogenicity studies, non-human, Nesnow S et al., Mutat Res 185:1-195, 1987.
2	Positive	TOXNET, In vivo carcinogenicity studies, Hamster, sc. Tumor: Liver, injection site, nasal cavity, and trachea. IARC Monographs, p. V17 77 Y78.
3	Positive	TOXNET, In vivo carcinogenicity studies, Rat, oral. Tumors: Kidney, Liver. IARC Monographs, p. V17 77 Y78.
4	Both Positive	TOXNET, In vivo carcinogenicity studies, Syrian Hamsters, sc, 0; 250; 500; 1000 mg/kg in saline 1/week for duration of 41-59 weeks. Tumors: Nasal caity: Adenocarcinoma; Trachea: Papillary polyp; Larynx: Papillary polyp. Pour, P and Wallcave, L. Cancer Lett. 14:23-27, 1981. (2 studies: male and female).
5	Positive	TOXNET, In vivo carcinogenicity studies, Mouse, strain-A, female, oral. 0; 0.2 µmol/ml in drinking water for 10 week (Total dose: 0, 55 µmol) followed by tap water for 20 week (Study duration: 30 week). Tumors: lung. Hecht,SS, Lijinsky,W, et al., Carcinogenesis 10(8): 1475-1477, 1989.
6	Positive	TOXNET, In vivo carcinogenicity studies, Rat/F344, female, oral. 0; 150 mg/L in drinking water 5 Days/week FOR 50 week (Total dose: 0; 5.6 mmol) (Study duration: 124 weeks). Tumors: Liver. Hecht, SS, Lijinsky, W, et al., Carcinogenesis 10(8): 1475-1477, 1989.
7	All Positive	RTECS, Tumorigen classified; (15 studies in rodents: 7 Hamster, 7 Rat, 1 Mouse)

M: Mutagenicity

RTECS many studies- bacteria, yeast, drosophila, rat and mice; Several Toxicology Reviews
IRIS: mutagenic in S. typhimurium (Hesbert et al., 1979; McMahon et al., 1979) and E. coli (McMahon et al., 1979).

study no	proposed rating	source / kind of study / animal species
1	4 Neg, 2 Pos	TOXNET, Ames Test (6 st), 1-20 mg/ plate of purified material, Mori,Y, et al., Mutat. Res. 192(2):91-94, 1987.
2	3 Neg, 4 Pos	TOXNET, Ames Test (17 st), 100 µmol/ plate, Dahl,AR; et al., Mutat. Res. 158(3):141-147, 1985.
3	1 Neg, 11 Pos	TOXNET, Ames Test (11 st), 333-10000 µg/ plate, Zeiger, E, et al., Environ. Mol. Mutagen. 11(SUPPL.12):1-158, 1988.
4	No concl.	TOXNET, Micronucleus test, chromosome aberrations, Mammalian polychromatic erythrocytes, Gilbert P. et al., Mutat Res 89:217-228,1981.

5	Positive?	RTECS, prokaryotes, 2 studies
6	Positive?	RTECS, insects, 1 study
7	Positive?	RTECS, lower eukaryotes, 1 study
8	4 Pos, 1 Neg	RTECS, mammalian cells, 5 studies (Micronucleus test, Cliet I. et al 1993 (pos); Morphological transformation test, Stowers S.J. et al 1988 (neg); DNA damage test, Denkel E. et al 1986 (pos); DNA damage, Bramila G. et al 1987 (pos); DNA damage, Tsuda S. et al 2000 (pos).

R: Reprotoxicity
No data available

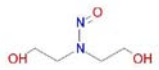
S: Sensitization
No data available

Remarks

OEL: Austria and Switzerland: 0.001-0.0025 mg/m3 (2006)
IRIS: Drinking water unit risk- 8.0E-5/µg/L
No data on reproductive effects

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
N-nitrosodiethanolamine	1116-54-7		RTECS+ IUCLID+ GESAMP-CPDB+ IRIS+	0	(0)	-	C M	OEL: 0.001 mg/m3 Non-toxic by oral or dermal route. **Confirm C** **Confirm M** **R: No data available** **S: No data available** **Need further testing for R**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**N-nitrosodiethanolnitramine**
Chemical Group	**Nitrosamine**
CAS Number	**1116-54-7**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**7500 (rat oral)** **11300 (Syrian Golden Hamster subcutaneous)**
Mutagenicity /Carcinogenicity	
Mutagenicity	**Toxnet** 1. Ames tests **(17 positive and 8 negative)** 2. Micronucleus test, chromosome aberrations **(no concl.)** **RTECS** Procaryotes (2 studies)(**positive?)** Insects (1 study)(**positive?)** Lower eykariotes (1 study)**(positive?)** Mammalian cells (5 studies)**(4 pos, 1 neg)** **IUCLID:** Prokaryote tests (2 st)**(both positive).**
Carcinogenicity	**Toxnet:** In vivo carcinogenicity rodent studies (6st) **(positive)** Tumor promotion study, rat (1 st) **(positive)** **RTECS** Studies in rodents (15 st) **(all positive)** **CPDB:** TD50, mg/kg/day: 3,17 mv , Rat, oral
Additional Info	
Conclusions	
RTECS	Classified as tumorigen and mutagen.
ICLUID	
GESAMP	
TOXNET:	Carcinogenic. IARC Group 2B - Probably carcinogenic to humans.
Current project summary sheet	
Current project conclusion	Clearly carcinogenic (C) and mutagenic (M).
Current project further work	No further testing is recommended for M or C.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**N-nitrosodiethanolamine**
Chemical Group	Nitrosamines
CAS Number	1116-54-7
Oral LD50 (mg/kg b.w.)	7500 (rat)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** - **ICLUID:** -
Fetotoxicity	**RTECS:** - **ICLUID:** -
Fetal development	**RTECS:** - **ICLUID:** -
Additional Info	
Conclusions	
RTECS	No data on reprotoxicity
ICLUID	Chemical found in ICLUID inventory but no chemical data sheet available
GESAMP	No Profile
Current project summary sheet	**CM (No R Rating)**
Current project conclusion for R	**No data available**
Current project further work for R	**Candidate for testing**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**N-nitrosodiethanolamine**
Chemical Group	Nitrosamines
CAS Number	1116-54-7
Oral LD50 (mg/kg b.w.)	7500 (rat)
Sensitization	
Studies	**RTECS:** - **IUCLID:** **-**
Additional Info	
Conclusions	
RTECS	No report on S
IUCLID	Chemical found in ICLUID inventory but no chemical data sheet available
GESAMP	No profile
Current project summary sheet	**CM**
Current project conclusion for S	**No data available**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
59-89-2	**4-nitroso-morpholine**

EU-Risk phrases	
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	No
RTECS file	QE7525000; Last updated: 200908
IUCLID file	No
REACH file	
Other sources	• **CPDB:** Carcinogenic in female rats and hamster, mutagenic in Salmonella • **TOXNET:** Carcinogenicity and mutagenicity studies

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**2**	282	Rat	RTECS/ Naturwissenschaften.
2	**1**	956	Hamster	RTECS/ Cancer Letters

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-200 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	-	170	Rat	RTECS (Subcutaneous dose)
2				

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1	-	-	Mice	RTECS/ LCLo= 1000 mg/m3 for 10 min/ National Defense Research Committee

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive

3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1		

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1		

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C	**C**	Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R		Immunotoxic - I	
Mutagenic - M	**M**	Photosensitizer - P		Sensitizing - S	

Source/comment:

C: Carcinogenicity

RTECS: many reports; liver, kidney, GI, respiratory, ovarian, blood leukaemia, IARC- Animal sufficient, human not adequate. Group 2B, probably carcinogenic to humans. Classified in 1987, EPA GENETOX PROGRAM 1988, Positive: Carcinogenicity-mouse/rat, In vivo cytogenetics-nonhuman and human lymphocyte, Host-mediated assay, Mammalian micronucleus, D melanogaster Sex-linked lethal, In vitro UDS in rat liver, V79 cell culture-gene mutation; Negative: Sperm morphology-mouse; Inconclusive: D melanogaster-reciprocal translocation, Histidine reversion-Ames test, NTP: Reasonably anticipated to be a human carcinogen (2004)
CPDB: Carcinogenic in female rats- liver and vascular system; Male and female hamsters- liver, nasal and oral cavity. TD50 = 0,109 mg/kg/day (rat, oral).
Classification for carcinogenicity: Class 1=Carcinogenic to humans, 2 = Probably carcinogenic to humans, 3 = Possibly carcinogenic to humans, 4 = Not classifiable as a human carcinogen

study no	proposed rating	source / kind of study / animal species
1	Positive, Class2B	TOXNET, In vivo carcinogenicity studies, MUTAT RES 185:1-195,1987
2	Positive, Class2B	RTECS 18 studies, (10 rat, 5 hamster, 3 mouse)

M: Mutagenicity

RTECS many reports in bacteria, yeast, drosophila, mold, human (in vitro), rat, mice and hamster (in vitro and in vivo); Several Toxicology Reviews
CPDB: Mutagenic in Salmonella.

study no	proposed rating	source / kind of study / animal species
1	Positive	TOXNET, Ames test, CANCER RES 37:4572-4579, 1977
2	No concl.	TOXNET, Ames test, MUTAT RES 46:265-268, 1977
3	Positive	TOXNET, CHO test?, Forward gene mutation at the HPRT or ouabain locus, MUTAT RES 87:81-142, 1981.
4	Positive	TOXNET, Unscheduled DNA synthesis (UDS), MUTAT RES 123:363-410, 1983.
5	Positive	TOXNET, In vivo chromosomal aberrations, MUTAT RES 87:143-188, 1981.
6	Both Positive	TOXNET, Sister chromatide exchange (SCE), in vitro, (2 st) PROG MUTAT RES 1:538-550, 1981 and MUTAT RES 157:181-187, 1985.
7	Positive	TOXNET, Micronucleus test, chromosome aberrations, MUTAT RES 123:61-118, 1983.
8	Positive	TOXNET, Sex-linked recessive lethal gene mutation, MUTAT RES 123:183-279, 1983.
9	Negative	TOXNET, Sperm morphology, MUTAT RES 115:1-72, 1983.
10	Positive?	RTECS, prokaryotes, 9 studies
11	Positive?	RTECS, lower eukaryotes, 5 studies
12	Positive?	RTECS, insects, 2 studies
13	Positive?	RTECS, human cells, 8 studies
14	Positive?	RTECS, mammalian cells, 28 studies

R: Reprotoxicity

No data available

S: Sensitization

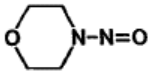
No data available

Remarks

OEL: Switzerland and Austria: 0.001-0,0025 mg/m3 classified in 2006
No data on reproductive effects

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
4-nitroso-morpholine	59-89-2		RTECS+ IUCLID-GESAMP-CPDB+ IRIS-TOXNET+	2	-	-	C M	OEL: 0.001 mg/m3 Moderate toxicity by oral route **Confirm C** **Confirm M** **R: No data available** **S: No data available** **Need further testing for R**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**N-nitrosomorpholine**
Chemical Group	**Nitrosamine**
CAS Number	**59-89-2**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**282 (rat oral)** **956 (hamster)**
Carcinogenicity / mutagenicity	
Mutagenicity	**Toxnet** 1. Ames (**pos and no concl)** 2. CHO test **(positive)** 3. Unscheduled DNA synthesis (UDS) **(positive)** 4.In vitro chromosomal aberrations **(positive)** 5. Sister-chromatid exchange (SCE) in vitro (2st) **(positive)** 6. Micronucleus test, chromosome aberrations **(positive)** 7. Sex-linked recessive lethal gene mutation **(positive)** 8. Sperm morphology **(negative)** **RTECS** Procaryots (9 st) **(positive?)** Lower eukaryotes (5 st) **(positive?)** Insects (2 st) **(positive?)** Human cells (8 st) **(positive?)** Mammalian cells (28st) **(positive?)**
Carcinogenicity	**Toxnet:** Ons study, rat **(positive)** **RTECS:** Rat (10 studies)**(positive)** Hamster (5 studies) **(positive)** Mouse (3 studies) **(positive)** **CPDB:** TD50, mg/kg/day: 0,109 [m] , Rat, oral
Additional Info	
Conclusions	
RTECS	Classified as tumorigen and mutagen.
TOXNET	Mutagenic and carcinogenic. IARC Group 2B - probably carcinogenic to humans.
ICLUID	
GESAMP	
Current project summary sheet	
Current project conclusion	Clearly carcinogenic (C) and mutagenic (M).
Current project further work	M and C are confirmed, no further testing recommended.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**N-nitrosomorpholine**
Chemical Group	Nitrosamines
CAS Number	59-89-2
Oral LD50 (mg/kg b.w.)	282 (rat)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** - **IUCLID:** -
Fetotoxicity	**RTECS:** - **IUCLID:** -
Fetal development	**RTECS:** - **IUCLID:** -
Additional Info	
Conclusions	
RTECS	No data on reprotoxicity
IUCLID	Not found in IUCLID inventory
GESAMP	No Profile
Current project summary sheet	**CM (No R Rating)**
Current project conclusion for R	**No data available**
Current project further work for R	**Candidate for testing**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**N-nitrosomorpholine**
Chemical Group	Nitrosamines
CAS Number	59-89-2
Oral LD50 (mg/kg b.w.)	282 (rat)
Sensitization	
Studies	**RTECS:** - **IUCLID:** **-**
Additional Info	
Conclusions	
RTECS	No report on S
IUCLID	Not found in IUCLID inventory
GESAMP	No profile
Current project summary sheet	**CM**
Current project conclusion for S	**No data available**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
4164-28-7	**Dimethylnitramine**

EU-Risk phrases	
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	-
RTECS file	IQ0450000; Last updated 200711
IUCLID file	-
REACH file	
Other sources	• **Toxnet** Literature references to studies and databases • **CCRIS** Carcinogenicity and mutagenicity studies • **CPDB** Liver and nasal cavity cancers in rats (**http://potency.berkeley.edu/chempages/DIMETHYLNITRAMINE.html)** • **SciFinder** • **Pubmed**

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1	**1**	1095 mg/kg	rat	Reliable data RTECS- Toxicology and Applied Pharmacology, 1975
2	**1**	1095 mg/kg	rat	(Andersen and Lenkins, 1978)
3			rat	LD50 i.v. 600 mg/kg (Andersen and Lenkins, 1978)
4			rat	LD50 i.p. 897 mg/kg (Andersen and Lenkins, 1978)
5			mice	LD50 i.p. 399 mg/kg (Andersen and Lenkins, 1978)

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-2000 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1				

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1				

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive
3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1		

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1		

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C	**C**	Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R		Immunotoxic - I	
Mutagenic - M	**M**	Photosensitizer - P		Sensitizing - S	

Source/comment:

C: Carcinogenicity

C: Carcinogen and tumorigen by RTECS criteria

C: CPDB/ TD50 = 0.547 mg/kg bw/day in both male and female rats.

study no	proposed rating	source / kind of study / animal species
1	Positive	Scherf HR et al., 1989: Positive- tumors in nasal cavity in both male and female rats when administered by gavage, with males being more susceptible. Endpoint: Number of tumors in nasal cavity, spinal cord, spinal and peripheral nerves, pituitary and mammary gland and other; Method: Full post-mortem and histologic examinations of tissues. TDLo - Lowest published toxic dose: 90 mg/kg/2Y (continuous).
2	Positive	Mirvish et al., 1980 / Full post-mortem and histologic examinations of tissues, LD50 determination / Positive- liver and nasal cavity tumors. Endpoint: Survival data, body weight, Number of tumors in respiratory tract, GIT, liver, kidney, nervous system, RES, endocrine and mammary glands, skin, soft tissue and other. Dosage: TD: 20 gm/kg/1Y (continuous).
3	Positive	Andersen and Lenkins, 1978 / Necroscopy / Induction of hemorrhagic foci in the lining of the stomach and intestine after single dose of dimethylnitramine. Endpoint: Gastrointestinal toxicity
4	Positive	Hassel et al. 1987 / Examination of tissues Positive- Aesthesineuroeptheliomas and neurogenic tumours of the lumbar region of the spine in rats. Endpoint: Number of tumors in nasal cavity, spinal cord, spinal and peripheral nerves and other sites
5	Negative	Pala et al., 1982 / light microscopy, histopathology No necrosis in liver. Endpoint: Liver necrosis
6	Positive	Pliss et al., 1982 / Full post-mortem and histologic examinations of tissues. Endpoint: Number of tumors in urinary bladder, liver and kidney in various animal species
7	Positive	Goodal et al, 1976 / The mice developed hepatocellular carcinomas and renal adenocarcinomas. The rats developed hepatocelluar carcinomas, some which metastasized. Statistically significant increases of other tumor types also occurred in mice.
8	Positive	Goodall and Kennedy, 1976/ Reported to cause tumors in liver and nasal cavity. Endpoint: Number of tumors in liver, lung kidney, malignant lymphoma, lung, duodenum, atriocaval; Method: Full post-mortem and histologic examinations of tissues
9	Positive	TD: 40 gm/kg, Oral, Rat (Natway Naturwissenschaften. (Springer-Verlag, Heidelberger Platz 3, D-1000 Berlin 33, Fed. Rep. Ger.) V.1- 1913- Volume(issue)/page/year: 48,134,1961)
10	Positive	TD - Toxic dose (other than lowest). Rodent – rat. Oral. 34 gm/kg/82W (continuous). Tumorigenic - equivocal tumorigenic agent by RTECS criteria Liver - tumors Blood – leukemia. Zekbai Zeitschrift fuer Krebsforschung. (Berlin, Fed. Rep. Ger.) V.1-75, 1903-71. For publisher information, see JCROD7. Volume (issue)/page/year: 69,103,1967.

M: Mutagenicity

M: Mutagen by RTECS criteria

Frei et al., 1986: Negative for DNA single strand breaks in hepatocytes and SV 40-transformed chinsese hamster embryo cell lines.

study no	proposed rating	source / kind of study / animal species
1	Positive	Khudoley et al., 1981/ Mutagenecity assays in TA 1535 and TA 100 (Liquid incubation assays, Host mediated assay) / Salmonella typhimurium TA1530 in a host-mediated assay in rats. Endpoint: Mutagenicity and mutation frequency
2	Negative and Positive	Frei E et al., 1984 / Ames test: Mutagenecity assays in TA 1535 and TA 100 (Plate incorporation assay and preincubation modification assay) / Salmonella typhimurium TA100. Endpoint: In vitro metabolism and mutagenicity
3	Negative	Frei E et al., 1986 / Alkaline elution method- fluorimetrically, Radioactivity-scintillation counting, Enzyme activites-biochemical method / Hepatocytes and SV 40-transformed chinsese hamster embryo cell lines. Endpoint: Induction of single strand breaks, selective DNA amplification and enzyme activities
4	Positive	Pala et al., 1982 / alkaline elution assay / Damage in mice liver DNA. Endpoint: DNA damage in vivo
5	Positive	Pool BL et al., 1984 / Ames test: Positive mutagenic when pre-incubated with bacteria and a complete metabolizing mixture containing 9000 g liver supernatant and NADPH-regenerating cofactors
6	Positive	Pool BL et al., 1986 / DNA single-strand breaks in mammalian cells, amplified DNA sequence in cultured cells, Mutagenecity in S.typhimurium with or without metabolic activation Positive based on literature results from different assay systems. Endpoint: DNA damage, amplified DNA sequence and mutagenicity
7	Positive	Malaveille C. et al., 1983 / Ames test: Mutagenicity assay in TA100 strain/ with and without metabolic activation.

R: Reprotoxicity

No data available

S: Sensitization

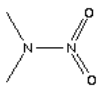
No data available

Remarks

Serious long term effects
Toxicology Review (Mutation Research, 2005)
No data on reproductive effects

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
Dimethylnitramine	4164-28-7		RTECS+ IUCLID- GESAMP- CPDB+ IRIS- Toxnet+ CCRIS+ Scifinder+	1	-	-	C M	**Confirm C** **Confirm M** **R: No data available** **S: No data available** **Need further testing for R**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Dimethylnitramine**
Chemical Group	**Nitramine**
CAS Number	**4164-28-7**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**1095 (rat)** **600 (i.v., rat); 897 (i.p., rat); 399 (i.p., mouse)**
Mutagenicity / Carcinogenicity	
Mutagenicity	**Toxnet** Ames Tests **(negative and positive)** **RTECS** Ames tests (2 st) **(positive)** **PubMed** Ames tests (2 st) **(positive)** DNA damage: SSB in hepatocytes and CHE (2 st) **(negative and positive)** DNA damage in vivo, alkaline elution assay (1 st) **(positive)**
Carcinogenicity	**Toxnet:** Rat study, same as one in RTECS **(1 positive)** **RTECS:** Rat studies **(4 positive):** TD: 40 gm/kg TD: 34 gm/kg/82W (continuous) TD: 20 gm/kg/1Y (continuous) TD: 90 mg/kg/2Y (contiuous) **Pubmed:** Rat studies **(1 negative, 4 positive)** **CPDB:** TD50, mg/kg/day: 0,547 ^{mv} , Rat, oral
Additional Info	
Conclusions	
RTECS	Classified as tumorigen and mutagen.
TOXNET	Mutagenic and carcinogenic.
ICLUID	
GESAMP	
Current project summary sheet	
Current project conclusion	Clearly carcinogenic (C) and mutagenic (M).
Current project further work	We recommend no further testing, M and C are confirmed.

Reproduction Toxicity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Dimethylanitramine**
Chemical Group	Nitramines
CAS Number	4164-28-7
Oral LD50 (mg/kg b.w.)	1095 (rat)
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** - **ICLUID:** -
Fetotoxicity	**RTECS:** - **ICLUID:** -
Fetal development	**RTECS:** - **ICLUID:** -
Additional Info	
Conclusions	
RTECS	No data on reprotoxicity
ICLUID	Not found in ICLUID inventory
GESAMP	No Profile
Current project summary sheet	**CM (No R Rating)**
Current project conclusion for R	**No data available**
Current project further work for R	**Candidate for testing**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Dimethylanitramine**
Chemical Group	Nitramines
CAS Number	4164-28-7
Oral LD50 (mg/kg b.w.)	1095 (rat)
Sensitization	
Studies	**RTECS:** - **IUCLID:** **-**
Additional Info	
Conclusions	
RTECS	No report on S
IUCLID	Not found in IUCLID inventory
GESAMP	No profile
Current project summary sheet	**CM**
Current project conclusion	**No data available**
Current project further work	**No toxicity testing needed**

CAS - No.	Name
74386-82-6	**Ethanolnitramine**

EU-Risk phrases	-
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	-
RTECS file	-
IUCLID file	-
REACH file	
Other sources	

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1				

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-200 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1				

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1				

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive

3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1		

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1		

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C		Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R		Immunotoxic - I	

Mutagenic - M		Photosensitizer - P		Sensitizing - S	

Source/comment:

C: Carcinogenicity
No data available

M: Mutagenicity
No data available

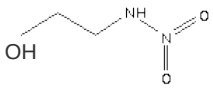
R: Reprotoxicity
No data available

S: Sensitization
No data available

Remarks

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date

Chemical Name	CAS.	Structure	Data bases	Oral	Dermal	Inhal	Long term	Comment on human health hazard
Ethanolnitramine	74386-82-6		RTECS-IUCLID-GESAMP-CPDB-IRIS-					No data available **Need further testing for C, M and R**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Ethanolnitramine**
Chemical Group	Nitramines
CAS Number	74386-82-6
Oral LD50 (mg/kg b.w.)	-
Mutagenicity / Carcinogenicity	
Mutagenicity	**Toxnet** - **RTECS** - **PubMed** -
Carcinogenicity	**Toxnet:** - **RTECS:** - **Pubmed:** -
Additional Info	
Conclusions	
RTECS	Not found in RTECS database
TOXNET	Not found in TOXNET database
ICLUID	Not found in ICLUID inventory
GESAMP	No profile
Current project summary sheet	**No data available**
Current project conclusion	**No data available**
Current project further work	**Candidate for testing for C and M**

Reproduction Toxcity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Ethanolnitramine**
Chemical Group	Nitramines
CAS Number	74386-82-6
Oral LD50 (mg/kg b.w.)	-
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** - **ICLUID:** -
Fetotoxicity	**RTECS:** - **ICLUID:** -
Fetal development	**RTECS:** - **ICLUID:** -
Additional Info	
Conclusions	
RTECS	Not found in RTECS database
ICLUID	Not found in ICLUID inventory
GESAMP	No Profile
Current project summary sheet	**No data available**
Current project conclusion for R	**No data available**
Current project further work for R	**Candidate for testing**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Ethanolnitramine**
Chemical Group	Nitramines
CAS Number	74386-82-6
Oral LD50 (mg/kg b.w.)	-
Sensitization	
Studies	**RTECS:** - **IUCLID:** **-**
Additional Info	
Conclusions	
RTECS	Not found in RTECS database
IUCLID	Not found in IUCLID inventory
GESAMP	No profile
Current project summary sheet	**No data available**
Current project conclusion for S	**No data available**
Current project further work for S	**No toxicity testing needed**

CAS - No.	Name
598-57-2	**Methylnitramine**

EU-Risk phrases	
Comments on chemical	
Comments on evaluation	
GESAMP/EHS file	-
RTECS file	PF8870000; Last updated 200802
IUCLID file	-
REACH file	
Other sources	• **Toxnet** Literature references to studies and databases • **CCRIS** Carcinogenicity and mutagenicity studies • **CPDB:** nervous system cancers **http://potency.berkeley.edu/chempages/METHYLNITRAMINE.html** • **Pubmed**

Evaluation based on (if based on similar chemical)

No.	CAS - No.	chemical name	remark
1			

Column C1: Oral Toxicity

0: >2000 1: 300-2000 2: 50-300 3: 5-50 4: <5 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1				

Column C2: Percutaneous Toxicity

0: >2000 1: 1000-2000 2: 200-1000 3. 50-200 4: <50 mg/kg bw

Study no.	rating based on this study	LD_{50} value	animal species	Source or comment
1		500 (i.p.)	Mice	Pharmaceutical Chemistry Journal 11, 1976.

Column C3: Inhalation Toxicity

0: >20 1: 10-20 2: 2-10 3: 0.5-2 4: <0.5 mg/l (4hrs)

Study no.	rating based on this study	LC_{50} value exp. time	animal species	Details, remarks, please indicate exposure time (hrs)
1				

mg/l = ppm x mw / 24 x 1000

Column D1: Skin Irritation / Corrosion

0: non-irritating 1: Mildly irritating 2: Irritating 3: Severely irritating or corrosive
3A: Corrosive >1 hr-4 hr 3B: Corrosive >3 min < 1 hr 3C: Corrosive < 3min

study no.	proposed rating	source / kind of study / animal species
1		

Column D2: Eye Irritation / Corrosion

0: Not irritating 1: Mildly irritating 2: Irritating 3: Severely irritating with irreversible corneal injury

study no.	proposed rating	source / kind of study / animal species
1		

Column D3: Other long term effects (indicate by appropriate letter in box)

Carcinogenic - C	**C**	Aspiration haz. - A		Neurotoxic - N	
Lung injury - L		Reprotoxic – R		Immunotoxic - I	

Mutagenic - M	**M ?**	Photosensitizer - P		Sensitizing - S	

Source/comment:

C: Carcinogenicity: Carcinogen and tumorigen by RTECS criteria; CPDB/ TD50 = 17.4 mg/kg bw/day in both male and female rats. Reported to cause tumors in nervous system.

study no	proposed rating	source / kind of study / animal species
1	Positive	Scherf HR et al., 1989 / Full post-mortem and histologic examinations of tissues / tumors (neurinoma) in spinal cord and spinal nerve in both male and female rats when administered by gavage, 0.05 and 1 mmol/kg per week, with males being more susceptible. TDLo - Lowest published toxic dose: 76 mg/kg/2Y (continuous).
2	Positive	Hassel M et al. 1987 / Examination of tissues / Neurogenic tumours of the lumbar region of the spine in rats.

M: Mutagenicity Mutagen by RTECS criteria

study no	proposed rating	source / kind of study / animal species
1	Positive for DNA single strand breaks in hepatocytes.	Frei et al., 1986 / Alkaline elution method- fluorimetrically, Radioactivity-scintillation counting, Enzyme activites-biochemical method / Rat hepatocytes and SV 40-transformed Chinese hamster embryo cell lines / Endpoint: Induction of single strand breaks, selective DNA amplification and enzyme activities using hepatocytes and SV 40-transformed Chinese hamster embryo cell lines. Dosage: 12500 nmol/L.
2	Positive based on literature results from different assay systems	Pool et al., 1986 / DNA single-strand breaks in mammalian cells, amplified DNA sequence in cultured cells, Mutagenecity in S.typhimurium with or without metabolic activation /. Endpoint: DNA damage, amplified DNA sequence and mutagenicity.
3	Negative	Frei E et al., 1984 / Ames test: Mutagenecity assays in TA 1535 and TA 100 (Plate incorporation assay and preincubation modification assay) / Salmonella typhimurium TA100. Endpoint: In vitro metabolism and mutagenicity
4	Negative	Pool BL et al., 1984 / Ames test: Mutagenicity assay in TA100 strain / with and without metabolic activation.
5	Negative	Malaveille C. et al., 1983 / Ames test: Mutagenicity assay in TA100 strain/ with and without metabolic activation.

R: Reprotoxicity No data available

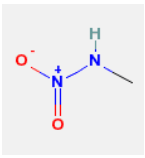
S: Sensitization No data available

Remarks

Serious long term effects
Toxicology Review (Mutation Research, 2005)
No data on reproductive effects

GESAMP/EHS Marine transport

C1	C2	C3	D1	D2	D3	Date

Chemical Name	**CAS.**	**Structure**	**Data bases**	**Oral**	**Dermal**	**Inhal**	**Long term**	**Comment on human health hazard**
Methylnitramine	598-57-2		RTECS+ IUCLID-GESAMP-CPDB+ IRIS-Toxnet+ CCRIS+	-	-	-	C M?	**Confirm C** **M: Need more data** **R: No data available** **S: No data available** **Need further testing for R and M**

Mutagenicity and Carcinogenicity Sheet

Name of the chemical	**Methylnitramine**
Chemical Group	**Nitramine**
CAS Number	**598-57-2 and 11328**
Oral LD50 (mg/kg b.w.) (from TOXNET)	**500 (mouse IP)**
Mutagenicity / Carcinogenicity	
Mutagenicity	**Toxnet:** Ames Tests (1 st) (**negative)** **RTECS:** Ames Tests (1 st) (**negative)** SSB in hepatocytes (1 st) **(positive)** SSB in CHE cells (1 st) **(negative)** **PubMed:** Ames Tests (2 st) (**negative)**
Carcinogenicity	**Toxnet** One study male and female rats, neurinomas **(positive).** TDlow, mg/kg/2Y: 76, Rat, gavage. Examination of tissues in rats. **PubMed:** One study, neurogenic tumors **(positive).** **CPDB:** TD50, mg/kg/day: 17,4, Rat, oral.
Additional Info	
Conclusions	
RTECS	Classified as tumorigen and mutagen.
ICLUID	
TOXNET	Carcinogenic.
GESAMP	
Current project summary sheet	
Current project conclusion	Clearly carcinogenic (C). Probably mutagenic (M?).
Current project further work	We recommend further testing for genotoxicity to confirm M.

Reproduction Toxcity Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Methylnitramine**
Chemical Group	Nitramines
CAS Number	598-57-2
Oral LD50 (mg/kg b.w.)	-
Reproduction Toxicity	
Male/Female Reproduction capacity	**RTECS:** - **IUCLID:** -
Fetotoxicity	**RTECS:** - **IUCLID:** -
Fetal development	**RTECS:** - **IUCLID:** -
Additional Info	
Conclusions	
RTECS	No data
IUCLID	Not found in IUCLID inventory
GESAMP	No Profile
Current project summary sheet	**C M? (No R Rating)**
Current project conclusion for R	**No data available**
Current project further work for R	**Candidate for testing**

Sensitization Sheet

Number in bracket refer to study report sequence in database

Name of the chemical	**Methylnitramine**
Chemical Group	Nitramines
CAS Number	598-57-2
Oral LD50 (mg/kg b.w.)	-
Sensitization	
Studies	**RTECS:** - **IUCLID:** **-**
Additional Info	
Conclusions	
RTECS	No report on S
IUCLID	Not found in IUCLID inventory
GESAMP	No profile
Current project summary sheet	**CM**
Current project conclusion for S	**No data available**
Current project further work for S	**No toxicity testing needed**

Appendix I **Detailed information on Ecotoxicity data for Emission Chemicals**

Ecotoxicological data for potential flue gas components - soil tests

Chemical	Trophical level	Species	Endpoint	No. tests	Test period	Effect conc. (µg/m^3 air-median)	Effect conc range (µg/m^3 air)
Acetaldehyde	No information	--	--	--	--	--	--
Formaldehyde	**Plants**	*P. vulgaris* (common bean)	Increase in shoot growth	1	4 weeks	78-438	
		L. longiflorum (lily)	Pollen tube growth reduced	2	5hrs	1060	**440 -1680**
		M. sativa (alfalfa)	Signs of injury	1	5hrs	840	
		S. oleracea (spinac)	No injury	1	5hrs	840	
		B. vulgaris (beets)	No injury	1	5hrs	840	
		A. sativa (oats)	No injury	1	5hrs	840	
		T. aestivum (wheat, seedling)	no effect	1	40 days	0,18 and 54	
		P. tremuloides (aspen, seedling)	no effect	1	40 days	0,18 and 54	
		B. rapa (rapeseed, seedling)	reduced growth	1	40 days	0,18 and 54	
		P. elliotti (slash pine, seedling)	increased growth	1	40 days	0,18 and 54	
Dimethylamine	No information	--	--	--	--	--	--
Methylamine	No information	--	--	--	--	--	--
Acetamide	No information	--	--	--	--	--	--
Ethanolamine (MEA)	No information	--	--	--	--	--	--
Ammonia, NH3	No information	--	--	--	--	--	--
Dimethylnitramine	No information	--	--	--	--	--	--
Ethanolnitramine	No information	--	--	--	--	--	--
Methylnitramine	No information	--	--	--	--	--	--
N-nitrosodiethanolamine	No information	--	--	--	--	--	--
N-nitrosodimthylamine	No information	--	--	--	--	--	--
N-nitrosomorpholine	No information	--	--	--	--	--	--

Ecotoxicological data for potential flue gas components – aquatic tests

Chemical	Trophical level	Species	Endpoint	No. tests	Test period	Effect conc. (mg/l median)	Effect conc range (mg/l)
Acetaldehyde	Invertebrates	*D. magna*	EC50	2	48hrs	12421	12415-12427
		C. dubia	EC50	1	48hrs	5806.9	
Formaldehyde	Microalgae	*S. quadricauda* (algae)	Reduced cell no., threshold	1	7 days	0.9	
		S. quadricauda (algae)	Growth inhibition, EC-50	1	24hrs	14.7	
	Plants	*L. minor* (Duckweed)	IC50	3	7-21 days	0.15	008-0.18
	Crustacea	*C. vidua* (ostracod)	LC50/EC50	3	96hrs	61.3	54.4-58.6
		Cyclops sp.(copepod)	LC50/EC50	1	96hrs	20	
		Bosmina sp.	LC50/EC50	1	96hrs	20	
		D, magna	LC50/EC50	4	96hrs	13.8	7.6 - 29
		D.pulex	LC50/EC50	9	96hrs	6	1.9-16.8
		C. dubia	LC50/EC50	6	96hrs	11.3	9.5-13.0
		Cypridopsis sp.(Shrimp)	LC50/EC50	1	96hrs	0.36	
		P. hadiakensis (FWprawn)	LC50/EC50	2	96hrs	169.3	160-178.6
	Worms	*T. tubifex* (sludge worm)	LC50	3	7-21 days	0.48	0.39-0.73
	Insects	*Notonecta sp.* (backswimmer)	EC50	1	96 hrs	287	
		Chironomus sp.(midge)	LC50	2	96 hrs	393	337-450
	Fish	*C.auratus* (goldfish)	Cytotox (NR50)	1	--	4	
		Guppies	LC50	1	14 days	0.4	
		R.saxatilis (Striped bass)	LC50	2	96 hrs	6.2	1.8-6.7
		*T. carolinus (*Pampano,)	LC50	1	48hrs	27.3	
		O.mykiss (rainbow trout)	LC50	3	48hrs	68.5	29.3-87
		O.mykiss (rainbow trout)	LC50	14	96hrs	56.3	47.6-98.8
		S. namaycush (lake trout)	LC50	1	96hrs	40.3	
		S. salar	LC50	1	96hrs	69.8	
		M. saxatilis (striped bass)	LC50	13	96hrs	58.5	47.2-98.6
		M- dolomieui (smallmouth bass)	LC50	1	96hrs	54.9	
		M. salmoides (largemouth bass)	LC50	1	96hrs	57.8	
		L..macrochirus (bluegill)	LC50	8	96hrs	51.6	48.8-69.4
		L. cyanellus (green sunfish)	LC50	1	96hrs	69.8	
		I punctatus (channel catfish)	LC50	13	96hrs	25.0	14.1-28.2
		A. rostrata (American eel)	LC50	1	96hrs	31.1	

Chemical	Trophical level	Species	Endpoint	No. tests	Test period	Effect conc. (mg/l median)	Effect conc range (mg/l)
		P. promelas (fathead minnows)	LC50	2	96hrs	16.7	**14.3-19.0**
		A. melas (black bullhead)	LC50	1	96hrs	25.1	
	Molluscs	*Corbicula (bivalve)*	LC50	4	96hrs	44.8	**35.2-50.8**
		Helisoma (snail)	LC50	4	96hrs	48.7	46.7-50.8
	Amphibia	*R. pipiens* (Leopard frog)	LC50	4	24-96 hrs	8.2	8.0-8.7
		R. berlandieri (leopard frog)	LC50	1	24 hrs	13-100	
		R. berlandieri (leopard frog)	NOEC	1	24 hrs	6.0	
		R. catesbeiana	LC50	1	24 hrs	9.5	
		Bullfrog (tadpole)	LC50	3	24-72 hrs	17.9	17.9-29.1
		Bufo sp.(Toad)	LC50	2	72-96 hrs	17.9	17.1-18.6
Dimethylamine	**Crustacea**	*D. magna*	EC50	1	48 hrs	49.4	
Methylamine	**No info**	--	--	--	--	--	--
Acetamide	**Protozoa**	*Entosiphon sulcatum*	IC50	1	?	99	--
	Microalgae	*S. quadricauda*	IC50	1	72 hrs	> 10000	
	Crustacea	Cladocera	EC50	1	??	10	
Ethanolamine (MEA)	**Micralgae (seawater)**	*S. costatum*	EC50	1	72 hrs	100-200	--
		I.galbana	EC50	1	96 hrs	80.0	
		P. tricornutum	EC50	1	72 hrs	24.7	
	Molluscs (seawater)	*C. gigas* (oyster)	EC50	1	24hrs	27.6	
		M. galloprovincialis (mussel)	EC50	1	48hrs	18.2	
		A.fransciscana (brine shrimp)	EC50	1	24hrs	43.0	
		C. crangon (shrimp)	EC50	1	?	100.0	
	Fish	*Brachydanio rerio* (zebra fish fry)	LC50	1	96 hrs	>5000	
Ammonia, NH3	**Rotifers**	*B. rubens*	LC50	2	24 hrs	11.1	3.2-20.4
	Mollusca	*S.novaezelandiae* (fingernail clam)	LC50	1	60 days	3.8 (total $NH4^+$)	
		S.novaezelandiae (fingernail clam)	LC50	1	60 days	0.037 (NH_3)	
		S.novaezelandiae (fingernail clam)	IC50	1	60 days	0.8 (total $NH4^+$)	
		S.novaezelandiae (fingernail clam)	IC50	1	60 days	0.013 (NH_3)	
	Crustacea	*D. magna*	EC50	1	24 hrs	189	
		D. pulex	EC50	1	24 hrs	187	
		Corophium sp. (amphipod)	LC50	1	96 hrs	5.5	
		G.japonica (amphipod)	LC50	1	96 hrs	141	

Chemical	Trophical level	Species	Endpoint	No. tests	Test period	Effect conc. (mg/l median)	Effect conc range (mg/l)
Ammonia, NH_3	**Crustacea**	*A.abdita* (amphipod)	LC50	2	96 hrs	75	50-100
		E. estuarius (amphipod)	LC50	1	96hrs	126	
		L. plumulosus (amphipod)	LC50	2	96hrs	67	44-89
		R. abronius (amphipod)	LC50	1	96hrs	79	
		G. japonica (amphipod)	LC50	1	96hrs	148	
		M. acherusicum (amphipod)	LC50	1	96 hrs	155	
		P.leniusculus (Signal crayfish) (adults)	LC50	1	24-48 hr	8.5	4.1-12.8
	Insects	*Deleatidium sp.* (mayfly juveniles)	EC50	5	29 days	1.605 (total NH4+)	0.69-2.11
		Deleatidium sp. (mayfly juveniles)	EC50	2	29 days	0.136 (NH3)	0.126-0.145
	Fish	*O. mykiss*	Chronic	2	?	0.04	0.03-0.05
		O. mykiss	LC50	1	72 days	0.03	
		H. amarus (silvery minnow)	LC50	2	72-96 hrs	1.065	
		P. promelas (Fathead minnow)	LC50	6	72hrs to 28 days	0.94	0.4-3.44
		Lost river sucker	LC50	1	96hrs	0.34	
		Shortnose sucker	LC50	1	96hrs	0.44	
		Colorado pikeminnow	LC50	1	28 days	0.72	
		Razorback sucker	LC50	1	28 days	0.63	
		Lepomis macrochirus (Bluegill)	Chronic	1	??	0.11	
		Channel catfish)	Chronic	1	??	0.32	
		Lepomis cyanellus (Green sunfish	Chronic	1	??	0.40	
		Smallmouth bass	Chronic	1	??	0.71	
		Galaxias maculatus (Inanga)	LC50	1	96hrs	1.55	
Dimethylnitramine	No info	--	--	--	--	--	--
Ethanolnitramine	No info	--	--	--	--	--	--
Methylnitramine	No info	--	--	--	--	--	--
N-nitrosodiethanolamine	No info	--	--	--	--	--	--
N-nitrosodimthylamine	No info	--	--	--	--	--	--
N-nitrosomorpholine	No info	--	--	--	--	--	--

SINTEF

Appendix J Literature List from SciFinder describing ecotocicity data for emission chemicals

Literature obtained from different sources:

- **Downloaded from publisher or open web sources**
- **Received through library (available as paper copies)**
- **Ordered not yet received from library**
- **Potential interesting reference, obtained through database or open web search**

Aldehydes

Cytotoxicity of anilines and aldehydes to goldfish GFS cells and relationships with 1-octanol/water partition coefficients
Saito, Hotaka; Koyasu, Junko; Shigeoka, Tadayoshi
Chemosphere (1993), 27(8), 1553-1560

Interspecies quantitative structure-activity relationship model for aldehydes: Aquatic toxicity
Dimitrov, Sabcho; Koleva, Yana; Schultz, T. Wayne, et al.
Environmental Toxicology and Chemistry (2004), 23 (2), 463-470

Human and Environmental Impact Assessment of Post-combustion CO2 Capture Focusing on Emissions from Amine-Based Scrubbing Solvents to Air
Veltman, Karin; Singh, Bhawna; Hertwich, Edgar G.
Environmental Science & Technology (2010), 44(4), 1496-1502. Language: English, Database: CAPLUS

Acetaldehyde:
A comparison of ecotoxicological tests
Botsford, James L.
ATLA (Alternatives to Laboratory Animals) (2002), 30, 539-550

Acute Toxicities of Organic Chemicals to Fathead Minnows.
Brooke, L.T.; Call, D.J.; Geiger, D.L.; Northcott, C.E., eds.
University of Wisconsin-Superior, Superior. WI, USA (1984), 1, pp 414

Acute Toxicities of Organic Chemicals to Fathead Minnows.
Geiger, D.L; Brooke, L.T; Call, D.J., eds.
University of Wisconsin-Superior, Superior. WI, USA (1990), 5, pp 332

Biological testing of cyclic acetaldehydes and their oxidation products using mollusks
Kuramshina N G; Kuramshin E M; Gumerova V K
Meditsina truda i promyshlennaia ekologiia (1998), (1), 25-8. Language: Russian, Database: MEDLINE

Formation of toxic aldehydes in cod liver oil after ultraviolet irradiation
Niyati-Shirkhodaee, Fatemeh; Shibamoto, Takayuki
Journal of the American Oil Chemists' Society (1992), 69 (12), 1254-1256

Human and Environmental Impact Assessment of Post-combustion CO2 Capture Focusing on Emissions from Amine-Based Scrubbing Solvents to Air
Veltman, Karin; Singh, Bhawna; Hertwich, Edgar G.
Environmental Science & Technology (2010), 44(4), 1496-1502. Language: English, Database: CAPLUS

Influence of the Energy Relationships of Organic Compounds on Toxicity to the Cladoceran Daphnia magna and the Fish Pimephales promelas
Genoni, Giulio P.
Ecotoxicology and Environmental Safety (1997), 36, 27-37

Quantitative Structure Activity Relationssships in Soil Ecotoxicology
Koch, Rainer; Nagel, Mathias
The Science of the Total Environment (1988), 77, 269-276

Shellfish in biologic testing of cyclic acetaldehydes and their oxidation products
Kuramshina, N. G.; Kuramshin, E. M.; Gumerova, V. K.
Meditsina Truda i Promyshlennaya Ekologiya (1998), 0(1), 25-28
(See same reference, but different translation, above)

The acute toxicity of aldehydes to the guppy.
Deneer, J.W.; Seinen, W.; Hermens, J.L.M
Aquatic toxicology (1988), 12, 185-192

Toxicity on the luminescent bacterium Vibrio fischeri (Beijerinck). I: QSAR equation for narcotics and polar narcotics
Vighi, Marco; Migliorati, Sonia, Monti, Gianna Serafina
Ecotoxicology and Environmental Safety (2009), 72, 154-161.

Formaldehyde:
Acute Toxicities of Organic Chemicals to Fathead Minnows.
Geiger, D.L; Brooke, L.T; Call, D.J., eds.
University of Wisconsin-Superior, Superior. WI, USA (1990), 5, pp 332

Acute toxicity and liver histopathology in larvae of the freshwater fish (Hoplias lacerdae) exposed to an aqueous 10% formaldehyde solution
Da Cruz, Claudinei; Fujimoto, Rodrigo Yudi; Luz, Ronald Kennedy; Portella, Maria Celia; Martins, Mauricio Laterca
Pesticidas (2005), 15, 21-28. Language: Portuguese, Database: CAPLUS

Amphibian micronucleus test(s): a simple and reliable method for evaluating in vivo genotoxic effects of freshwater pollutants and radiations. Initial assessment
Fernandez, Maria; L'Haridon, Jacques; Gauthier, Laury; Zoll-Moreux, Catherine
Mutation Research, Environmental Mutagenesis and Related Subjects (1993), 292(1), 83-99. Language: English, Database: CAPLUS

An ecological risk assessment of formaldehyde
Chenier R
HUMAN AND ECOLOGICAL RISK ASSESSMENT (2003), 9(2), 483-509

An index of the ecological impacts of water toxics emitted to freshwater ecosystems
Papasavva, Stella; Beltramo, Mark A.
Human and Ecological Risk Assessment (2006), 12(3), 476-492. Language: English, Database: CAPLUS

Biological effects of formaldehyde-polluted water
Lin, Taixi; Dong, Baoxian; Liu, Zhusan; Dun, Wanru; Sun, Hekun; Kong, Aixia
Huanjing Kexue Xuebao (1986), 6(1), 107-13. Language: Chinese, Database: CAPLUS

Comparative acute toxicity of organic pollutants and reference values for crustaceans. I. Branchiopoda, Copepoda and Ostracoda
Sanchez-Bayo, Francisco
Environmental Pollution (Amsterdam, Netherlands) (2006), 139(3), 385-420. Language: English, Database: CAPLUS

Comparative assessment of toxicity of phenol, formaldehyde, and industrial wastewater to aquatic organisms
Tisler, Tatjana; Zagorc-Koncan, Jana
Water Air and Soil Pollution (1997), 97(3-4), 315-322

Comparison of the sensitivity of Brachydanio rerio and Leuciscus idus by testing the fish toxicity of chemicals and wastewaters.
Wellens, H.
Z Wasser Abwasser Forsch (1982), 15, 49-52.

Cytotoxicity of anilines and aldehydes to goldfish GFS cells and relationships with 1-octanol/water partition coefficients
Saito, Hotaka; Koyasu, Junko; Shigeoka, Tadayoshi
Chemosphere (1993), 27(8), 1553-1560

Daily physicochemical, microbiological and ecotoxicological fluctuations of a hospital effluent according to technical and care activities
Boillot, C.; Bazin, C.; Tissot-Guerraz, F.; Droguet, J.; Perraud, M.; Cetre, J.C.; Trepo, D. and Perrodin, Y.
Science of the total environment (2008), 403, 113-129

Derivation of ambient water quality criteria for formaldehyde
Hohreiter, David W.; Rigg, David K.
Chemosphere (2001), 45(4-5), 471-486

DETECTION OF SUBLETHAL TOXIC POLLUTION OF BODIES OF WATER BY ESTIMATING ADAPTIVE REACTIONS OF TEST ORGANISMS
VOSKRESENSKII K A; DMITRIEVA N G
Biologicheskie Nauki (Moscow) (1977), 20(10), 132-136

Determination of inhibition kinetics by an automated bacterial luminescence test for the ecotoxicological evaluation of contaminants in wastewater
Schwedt, Georg; Reiter, Christian
Vom Wasser (1997), 89, 353-371. Language: German, Database: CAPLUS

Ecotoxicological Characterisation and Classification of Existing Chemicals Examples from the ICCA HPV Initiative and comparison with other existing chemicals
Licht, Oliver; Weyers, Arnd; Nagel, Roland
Environmental Science and Pollution Research (2004), 11(5), 291-296

Effect of formalin treatment on the Monogenean Pseudorhabdosynochus epinepheli (Monogenea) and the fish Epinephelus coioides
Yang, Ting-bao; Lin, Li; Chen, Yong-zhi; Liu, Sheng-fa
Zhongshan Daxue Xuebao, Ziran Kexueban (2003), 42(4), 59-63. Language: English, Database: CAPLUS

EFFECT OF PYRIDINE AND FORMALDEHYDE ON A MACROPHYTE (LEMNA MINOR L.) AND A SLUDGE WORM (TUBIFEX TUBIFEX MULLER) IN FRESH WATER MICROCOSMS
Singh, B. B.; Chandra, R.; Sharma, Y. K.
Applied Ecology and Environmental Research (2008), 6(2), 21-35

Effects of tributyltin and formaldehyde on the germination and growth of Phyllospora comosa (Labillardiere) C. Agardh (Phaeophyta: Fucales)
Burridge, T. R.; Lavery, L.; Lam, P. K. S.
Bulletin of Environmental Contamination and Toxicology (1995), 55(4), 525-532

Ein neuer Biotest mit der Hefe Saccharomyces cerevisiae auf aquatishe Toxizität
Weber, Jürgen, Plantikow, Anita, Kreutzmann, Jupp
UWSF – Z. Umweltchem. Ökotox. (2000), 12(4), 185-189

Experimental studies of determination of the biological effects of environmental pollutants. (3). Effect of formalin on freshwater planaria and its relation to temperature
Ohyama, Masayuki; Kosaka, Hiroaki; Nogami, Hiroshi; Azuma, Emiko; Kimura, Takae; Uozumi, Mitsuro; Oki, Iwashiro; Tamura, Sachiko
Osaka-furitsu Koshu Eisei Kenkyusho Kenkyu Hokoku, Kogai Eisei Hen (1985), 6, 1-5. Language: Japanese, Database: CAPLUS

Fast examination of water quality using the automatic ecotox based on the movement behavior of a freshwater flagellate
Tahedl, Harald; Hader, Donat-P.
Water Research (1998), Volume Date 1999, 33(2), 426-432. Language: English, Database: CAPLU

Formalin: its toxicity to nontarget aquatic organisms, persistence, and counteraction
Bills, Terry D.; Marking, Leif L.; Chandler, Jack H., Jr.
Investigations in Fish Control (1977), 73 74 75 76, Paper No. 73, 7 pp. Language: English, Database: CAPLUS

Formation of toxic aldehydes in cod liver oil after ultraviolet irradiation
Niyati-Shirkhodaee, Fatemeh; Shibamoto, Takayuki
Journal of the American Oil Chemists' Society (1992), 69(12), 1254-1256

Hazardous properties of paint residues from the furniture industry
Vaajasaari, Kati; Kulovaara, Maaret; Joutti, Anneli; Schultz, Eija; Soljamo, Kari
Journal of Hazardous Materials (2004), 106(2-3), 71-79. Language: English, Database: CAPLUS

Human and Environmental Impact Assessment of Post-combustion CO2 Capture Focusing on Emissions from Amine-Based Scrubbing Solvents to Air
Veltman, Karin; Singh, Bhawna; Hertwich, Edgar G.
Environmental Science & Technology (2010), 44(4), 1496-1502. Language: English, Database: CAPLUS

Lethal toxicity of cyanide and formalin to a freshwater fish Gambusia affinis
Sangli, Asheera Banu; Kanabur, V. V.
Environment and Ecology (2000), 18(2), 362-364. Language: English, Database: CAPLUS

Low sensitivity of ciliary activity in the gills of Anodonta cygnea to some ecotoxicals
Lagerspetz, K. Y. H.; Tiiska, A.; Senius, K. E. O.
Comparative Biochemistry and Physiology, Part C: Pharmacology, Toxicology & Endocrinology (1993), 105C(3), 393-5. Language: English, Database: CAPLUS

Molecular ecotoxicology of plants
Sandermann, Heinrich
Trends in Plant Science (2004), 9(8), 406-413. Language: English, Database: CAPLUS

MUTAGENICITY OF THE COMPONENTS OF OZONATED HUMIC SUBSTANCE
MATSUDA H; OSE Y; NAGASE H, et al.
Science of the Total Environment (1991), 103(2-3), 129-140

Priority assessment of toxic substances in life cycle assessment. Part I: Calculation of toxicity potentials for 181 substances with the nested multi-media fate, exposure and effects model USES-LCA
Huijbregts, M. A. J.; Thissen, U.; Guinee, J. B.; Jager, T.; Kalf, D.; Van de Meent, D.; Ragas, A. M. J.; Sleeswijk, A. Wegener; Reijnders, L.
Chemosphere (2000), 41(4), 541-573. Language: English, Database: CAPLUS

Quantitative study on the biological effect of environmental pollutants. I. Effect of formalin on freshwater planaria
Uozumi, Mitsuro; Kosaka, Hiroaki; Nogami, Hiroshi; Ohyama, Masayuki; Azuma, Emiko; Kimura, Takae; Oki, Iwashiro; Tamura, Sachiko
Osaka-furitsu Koshu Eisei Kenkyusho Kenkyu Hokoku, Kogai Eisei Hen (1983), 4, 1-6. Language: Japanese, Database: CAPLUS

Sensitivity of juvenile striped bass to chemicals used in aquaculture
Bills, T.D.; Marking, L.L.; Howe, G.E.
J Fish Wildl Serv (1993), 192, 11-15

Tolerance to formalin by a fluidized-bed biofilter and rainbow trout Oncorhynchus mykiss in a recirculating culture system
Heinen, John M.; Weber, Amy L.; Noble, Alicia C., et al.
Journal of the World Aquaculture Society (1995), 26(1), 65-71

The acute toxicity of aldehydes to the guppy.
Deneer, J.W.; Seinen, W.; Hermens, J.L.M
Aquatic toxicology (1988), 12, 185-192

Toxicity of agrochemicals to freshwater organism. CIII. Solvents
Nishiuchi, Yasuhiro
Suisan Zoshoku (1984), 32(2), 115-19. Language: Japanese, Database: CAPLUS

Toxicity of laundry detergent components to a freshwater cladoceran and their contribution to detergent toxicity
Warne, M. St. J.; Schifko, A. D.
Ecotoxicology and Environmental Safety (1999), 44(2), 196-206. Language: English, Database: CAPLUS

Toxicity of certain chemicals to juvenile pompano
Birdsong, Charles L.; Avault, James W., Jr.
Progressive Fish-Culturist (1971), 33(2), 76-80. Language: English, Database: CAPLUS

Toxicity of nine therapeutic and herbicidal compounds to striped bass
Wellborn, Thomas L., Jr.
Progressive Fish-Culturist (1969), 31(1), 27-32. Language: English, Database: CAPLUS

Toxicity of pesticides to some freshwater organisms. LIX
Nishiuchi, Yasuhiro; Asano, Kazuye

Suisan Zoshoku (1979), 27(1), 48-55. Language: Japanese, Database: CAPLUS

Toxicity of pesticides to freshwater organisms. LXXX. Differences of the sensitivity of two carp species to pesticides
Nishiuchi, Yasuhiro
Suisan Zoshoku (1982), 30(3), 163-6. Language: Japanese, Database: CAPLUS

Toxicity of pesticides to freshwater organisms. LXXIX. Effects of water temperature on the sensitivity of Daphnia pulex to pesticides
Nishiuchi, Yasuhiro
Suisan Zoshoku (1982), 30(3), 158-62. Language: Japanese, Database: CAPLUS

Toxicity on the luminescent bacterium Vibrio fischeri (Beijerinck). I: QSAR equation for narcotics and polar narcotics
Vighi, Marco; Migliorati, Sonia; Monti, Gianna Serafina
Ecotoxicology and Environmental Safety (2009), 72(1), 154-161. Language: English, Database: CAPLUS

Alkylamines

Dimethylamine:

ACUTE AND LONG-TERM TOXICITY OF WATER-SOLUBLE CATIONIC POLYMERS TO RAINBOW TROUT ONCORHYNCHUS-MYKISS AND THE MODIFICATION OF TOXICITY BY HUMIC ACID
GOODRICH M S; DULAK L H; FRIEDMAN M A, et al.
Environmental Toxicology and Chemistry (1991), 10(4), 509-516

Effects of 2,4-dichlorophenoxyacetic acid on fungal propagules in freshwater ponds
Sherry, James
Environmental Toxicology and Water Quality (1994), 9(3), 209-21. Language: English, Database: CAPLUS

Environmental properties and effects of nonionic surfactant adjuvants in pesticides: a review
Krogh KA, Halling-Sorensen B, Mogensen BB, et al.
CHEMOSPHERE (2003), 50(7), 871-901

HIGH CONCENTRATIONS OF DI METHYLAMINE AND METHYLAMINE IN SQUID AND OCTOPUS AND THEIR IMPLICATIONS IN TUMOR ETIOLOGY
LIN J-K; LEE Y-J; CHANG H W
Food and Chemical Toxicology (1983), 21(2), 143-150

Structural Alerts-A New Classification Model to Discriminate Excess Toxicity from Narcotic Effect Levels of Organic Compounds in the Acute Daphnid Assay
Von Ohe, Peter C.; Kuehne, Ralph; Ebert, Ralf-Uwe; Altenburger, Rolf; Liess, Matthias; Schueuermann, Gerrit
Chemical Research in Toxicology (2005), 18(3), 536-555. Language: English, Database: CAPLUS

STUDY OF DEGRADABILITY OF NITROSAMINES IN THE MARINE ENVIRONMENT
AUBERT J; PETIT L; PUEL D
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Veltman, Karin; Singh, Bhawna; Hertwich, Edgar G.
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Kumar, Ravindar
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de Croux, Parma; Julieta, Maria; Loteste, Alicia
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Water Research (1990), 24(1), 83-90

Long-Term Effects of Ammonia on the Behavioral Activity of the Aquatic Snail Potamopyrgus antipodarum (Hydrobiidae, Mollusca)
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Studies of some biochemical changes in the tissues of Catla catla (Hamilton), Labeo rohita (Hamilton) and Cirrhinus mrigala (Hamilton) exposed to NH3-N, NO2-N and NO3-N
Tilak, K. S.; Veeraiah, K.; Lakshmi, S. Jhansi
Journal of Environmental Biology (2002), 23(4), 377-381

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Williams, Kendall A.; Green, David W. J.; Pascoe, David
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SUBLETHAL EFFECT OF AMMONIA ON CERTAIN BIOCHEMICAL AND HEMATOLOGICAL INDICATORS IN COMMON CARP CYPRINUS-CARPIO
DABROWSKA H; WLASOW T
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The acute lethal toxicity of mixtures of cyanide and ammonia to smolts of salmon, Salmo salar L. at low concentrations of dissolved oxygen
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Journal of Fish Biology (1983), 22(2), 215-22. Language: English, Database: CAPLUS

THE ACUTE TOXICITY OF AMMONIA AND COPPER TO THE GASTROPOD POTAMOPYRGUS-JENKINSI
WATTON A J; HAWKES H A
Environmental Pollution Series A Ecological and Biological (1984), 36(1), 17-30

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Ip, Y. K.; Lau, I. Y.; Wong, W. P., et al.
Physiological and Biochemical Zoology (2005), 78(4) 630-640

The effects of ammonia on freshwater unionid mussels. Comment
Newton, Teresa J.
Environmental Toxicology and Chemistry (2003), 22(11), 2543-2544. Language: English, Database: CAPLUS

The effect of dissolved oxygen and salinity on the toxicity of ammonia to smolts of salmon, Salmo salar
Alabaster, J. S.; Shurben, D. G.; Knowles, G.
From Journal of Fish Biology (1979), 15(6), 705-12. Language: English, Database: CAPLUS

THE GAMMARUS ASELLUS RATIO AS AN INDEX OF ORGANIC POLLUTION
WHITEHURST I T
Water Research (1991), 25(3), 333-340

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GRYGIEREK E; JANUSZKO M; KRUGER D, et al.
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THE INHIBITION BY AMMONIA OF POPULATION GROWTH OF THE ROTIFER BRACHIONUS-RUBENS IN CONTINUOUS CULTURE
SCHLUETER M; GROENEWEG J
Aquaculture (1985), 46(3), 215-220

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Raven JA, Yin ZH
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Tolerance of Physocypria kraepelini (Crustacean, Ostracoda) to water-borne ammonia, phosphate and pH value
Yu, Na; Chen, Shimei; Li, Erchao; Chen, Jiayan; Chen, Liqiao

Journal of Environmental Sciences (Beijing, China) (2009), 21(11), 1575-1580. Language: English, Database: CAPLUS

Toxic effects of unionized ammonia on survival and feeding activity of the freshwater amphipod Eulimnogammarus toletanus
Alonso, A.; Camargo, J. A.
Bulletin of Environmental Contamination and Toxicology (2004), 72(5), 1052-1058. Language: English, Database: CAPLUS

Toxicity and effects of ammonia, nitrite, nitrate and histopathological changes in the gill of freshwater fish Cyprinus carpio
Tilak, K. S.; Veeraiah, K.; Raju, J. Milton Prema
Journal of Ecotoxicology & Environmental Monitoring (2006), 16(6), 527-532. Language: English, Database: CAPLUS

Toxicity assessment of a complex industrial wastewater using aquatic and terrestrial bioassays Daphnia pulex and Lactuca sativa
Sanchez-Meza JC, Pacheco-Salazar VF, Pavon-Silva TB, et al.
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Toxicity estimation of treated coke plant wastewater using the luminescent bacteria assay and the algal growth inhibition test
Peter, Simone; Siersdorfer, Christof; Kaltwasser, Heinrich; Geiger, Markus
Environmental Toxicology and Water Quality (1995), 10(3), 179-84. Language: English, Database: CAPLUS

Toxicity of ammonia to a freshwater fish, Gambusia affinis and its effects on oxygen consumption
Sangli, Asheera Banu; Kanabur, V. V.
Geobios (Jodhpur) (2001), 28(1), 56-58
NB! Ordered, but the library claims the reference to be faulty!

TOXICITY OF AMMONIA TO EARLY LIFE STAGES OF RAINBOW TROUT SALMO-GAIRDNERI
SOLBE J F D; SHURBEN D G
Water Research (1989), 23(1), 127-129

TOXICITY OF AMMONIA TO EARLY LIFE STAGES OF THE SMALLMOUTH BASS AT 4 PH VALUES
BRODERIUS S; DRUMMOND R; FIANDT J, et al.
Environmental Toxicology and Chemistry (1985), 4(1), 87-96

Toxicity of ammonia to nine native New Zealand freshwater invertebrate species
Hickey, C. W.; Vickers, M. L.
Archives of Environmental Contamination and Toxicology (1994), 26(3), 292-8. Language: English, Database: CAPLUS

Toxicity of ammonia to three marine fish and three marine invertebrates
Boardman, Gregory D.; Starbuck, Steven M.; Hudgins, Douglas B.; Li, Xiayoun; Kuhn, David D.
Environmental Toxicology (2004), 19(2), 134-142. Language: English, Database: CAPLUS

TOXICITY OF UN-IONIZED AMMONIA AND HIGH PH TO POST-LARVAL AND JUVENILE FRESHWATER SHRIMP MACROBRACHIUM-ROSENBERGII

STRAUS D L; ROBINETTE H R; HEINEN J M
Journal of the World Aquaculture Society (1991), 22(2) 128-133

Transbranchial ammonia gradients and acid-base responses to high external ammonia concentration in rainbow trout (Oncorhynchus mykiss) acclimated to different salinities
Wilson, R. W.; Taylor, E. W.
Journal of Experimental Biology (1992), 166, 95-112. Language: English, Database: CAPLUS

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Jop, Krzysztof M.; Kendall, Timothy Z.; Askew, Ann M.; Foster, Robert B.
Environmental Toxicology and Chemistry (1991), 10(8), 981-90. Language: English, Database: CAPLUS

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Brack, Werner; Rottler, Horst; Frank, Hartmut
Environmental Toxicology and Chemistry (1998), 17(10), 1982-1991. Language: English, Database: CAPLUS

Water quality guidance for protection of freshwater mussels (Unionidae) from ammonia exposure
Augspurger, Tom; Keller, Anne E.; Black, Marsha C.; Cope, W. Gregory; Dwyer, F. James
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WATER QUALITY REQUIREMENTS FOR 1ST FEEDING IN MARINE FISH LARVAE 1. AMMONIA NITRITE AND NITRATE
BROWNELL C L
Journal of Experimental Marine Biology and Ecology (1980), 44(2-3), 269-284

Nitramines

Ecotoxicity of nitroaromatics to aquatic and terrestrial species at army Superfund sites
Hovatter, Patricia S.; Talmage, Sylvia S.; Opresko, Dennis M.; Ross, Robert H.
From ASTM Special Technical Publication (1997), STP 1317(Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment, (Sixth Volume)), 117-129. Language: English, Database: CAPLUS

Genotoxicity of explosives
Inouye, Laura; Lachance, Bernard; Gong, Ping
Edited by Sunahara, Geoffrey Isao
Ecotoxicology of Explosives (2009), 177-209. Language: English, Database: CAPLUS

Influence of avian reproduction ecotoxicological endpoints in the assessment of plant protection products
Fernandez-Perea, M. T.; Prados, E. Alonso; Villajos, A. Novillo; Prados, J. L. Alonso; Baudin, J. M. Garcia
Journal of Environmental Science and Health, Part B: Pesticides, Food Contaminants, and Agricultural Wastes (2009), 44(2), 106-112. Language: English, Database: CAPLUS

Nitroaromatic munition compounds: environmental effects and screening values
Talmage S S; Opresko D M; Maxwell C J; Welsh C J; Cretella F M; Reno P H; Daniel F B
Reviews of environmental contamination and toxicology (1999), 161, 1-156. Language: English, Database: MEDLINE

Peculiarities of N-nitramines carcinogenic action
Pliss G B; Zabezhinski M A; Petrov A S; Khudoley V V
Archiv fur Geschwulstforschung (1982), 52(8), 629-34. Language: English, Database: MEDLINE

Toxicity and uptake of cyclic nitrarnine explosives in ryegrass Lolium perenne
Rocheleau, Sylvie; Lachance, Bernard; Kuperman, Roman G., et al.
Environmental Pollution (2008), 156(1), 199-206

Toxicity of emerging energetic soil contaminant CL-20 to potworm Enchytraeus crypticus in freshly amended or weathered and aged treatments
Kuperman, Roman G.; Checkai, Ronald T.; Simini, Michael; Phillips, Carlton T.; Anthony, J. Steven; Kolakowski, Jan E.; Davis, Emily A.
Chemosphere (2006), 62(8), 1282-1293. Language: English, Database: CAPLUS

Toxicity of octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine (HMX) to soil microbes
Gong, P.; Hawari, J.; Thiboutot, S.; Ampleman, G.; Sunahara, G. I.
Bulletin of Environmental Contamination and Toxicology (2002), 69(1), 97-103. Language: English, Database: CAPLUS

Toxicological characterization of 2,4,6-trinitrotoluene, its transformation products, and two nitramine explosives
Neuwoehner, Judith; Schofer, Andrea; Erlenkaemper, Bibiane; Steinbach, Klaus; Hund-Rinke, Kerstin; Eisentraeger, Adolf
Environmental Toxicology and Chemistry (2007), 26(6), 1090-1099. Language: English, Database: CAPLUS

Dimethylnitramine:
Peculiarities of N-nitramines carcinogenic action
Pliss G B; Zabezhinski M A; Petrov A S; Khudoley V V
Archiv fur Geschwulstforschung (1982), 52(8), 629-34. Language: English, Database: MEDLINE

Ethanolnitramine:

Methylnitramine:
Genotoxicity of explosives
Inouye, Laura; Lachance, Bernard; Gong, Ping
Edited by Sunahara, Geoffrey Isao
Ecotoxicology of Explosives (2009)

Nitrosamines

An example of interaction between environmental pollutants: modification of thiram toxicity to freshwater organisms by nitrites or nitrates in relation to nitrosamine synthesis
Jouany, J. M.; Truhaut, R.; Vasseur, P.; Klein, D.; Ferard, J. F.; Deschamps, P.
Ecotoxicology and Environmental Safety (1985), 9(3), 327-38. Language: English, Database: CAPLUS

A PRELIMINARY STUDY ON THE MICROBIOLOGICAL BREAKDOWN OF DI METHYL NITROSAMINE
HARADA K; YAMADA K
Journal of National Fisheries University (1977), 26(3), 293-298

STUDY OF DEGRADABILITY OF NITROSAMINES IN THE MARINE ENVIRONMENT
AUBERT J; PETIT L; PUEL D
Revue Internationale d'Oceanographie Medicale (1979), 51-52, RECD.

N-nitrosodiethanolamine:
Amphibian micronucleus test(s): a simple and reliable method for evaluating in vivo genotoxic effects of freshwater pollutants and radiations. Initial assessment
Fernandez, Maria; L'Haridon, Jacques; Gauthier, Laury; Zoll-Moreux, Catherine
Mutation Research, Environmental Mutagenesis and Related Subjects (1993), 292(1), 83-99. Language: English, Database: CAPLUS

N-nitrosodimthylamine:

N-nitrosomorpholine:

SINTEF

Appendix K - Literature List from ISI Web if Science describing biodegradation data for emission chemicals

Ethanolamine

Record 1 of 4
Author(s): Mrklas, O; Chu, A; Lunn, S; Bentley, LR
Title: Biodegradation of monoethanolamine, ethylene glycol and triethylene glycol in laboratory bioreactors
Source: WATER AIR AND SOIL POLLUTION, 159 (1-4): 249-263 NOV 2004
ISSN: 0049-6979

Record 2 of 4
Author(s): Ndegwa, AW; Wong, RCK; Chu, A; Bentley, LR; Lunn, SRD
Title: Degradation of monoethanolamine in soil
Source: JOURNAL OF ENVIRONMENTAL ENGINEERING AND SCIENCE, 3 (2): 137-145 MAR 2004
ISSN: 1496-2551
DOI: 10.1139/S03-074

Record 3 of 4
Author(s): Pietsch, J; Sacher, F; Schmidt, W; Brauch, HJ
Title: Polar nitrogen compounds and their behaviour in the drinking water treatment process
Source: WATER RESEARCH, 35 (15): 3537-3544 OCT 2001
ISSN: 0043-1354

Record 4 of 4
Author(s): Schuch, R; Gensicke, R; Merkel, K; Winter, J
Title: Nitrogen and DOC removal from wastewater streams of the metal-working industry
Source: WATER RESEARCH, 34 (1): 295-303 JAN 2000
ISSN: 0043-1354

Formaldehyde

Author(s): Xu, ZJ (Xu, Zhongjun); Hou, HP (Hou, Haiping)
Title: Formaldehyde Removal from Air by a Biodegradation System
Source: BULLETIN OF ENVIRONMENTAL CONTAMINATION AND TOXICOLOGY, 85 (1): 28-31 JUL 2010
ISSN: 0007-4861
DOI: 10.1007/s00128-010-9975-2

Record 2 of 16
Author(s): Moussavi, G (Moussavi, Gholamreza); Yazdanbakhsh, A (Yazdanbakhsh, Ahmadreza); Heidarizad, M (Heidarizad, Mahdi)
Title: The removal of formaldehyde from concentrated synthetic wastewater using O-3/MgO/H2O2 process integrated with the biological treatment
Source: JOURNAL OF HAZARDOUS MATERIALS, 171 (1-3): 907-913 NOV 15 2009
ISSN: 0304-3894
DOI: 10.1016/j.jhazmat.2009.06.090

Record 3 of 16
Author(s): Eiroa, M (Eiroa, M.); Vilar, A (Vilar, A.); Kennes, C (Kennes, C.); Veiga, MC (Veiga, M. C.)
Title: Formaldehyde biodegradation and its effect on the denitrification process
Source: ENVIRONMENTAL TECHNOLOGY, 28 (9): 1027-1033 SEP 2007
ISSN: 0959-3330

Record 4 of 16
Author(s): Amato, P (Amato, P.); Demeer, F (Demeer, F.); Melaouhi, A (Melaouhi, A.); Fontanella, S (Fontanella, S.); Martin-Biesse, AS (Martin-Biesse, A.-S.); Sancelme, M (Sancelme, M.); Laj, P (Laj, P.); Delort, AM (Delort, A.-M.)
Title: A fate for organic acids, formaldehyde and methanol in cloud water: their biotransformation by micro-organisms
Source: ATMOSPHERIC CHEMISTRY AND PHYSICS, 7 (15): 4159-4169 2007
ISSN: 1680-7316

Record 5 of 16
Author(s): Pedersen, LF (Pedersen, Lars-Flemming); Pedersen, PB (Pedersen, Per Bovbjerg); Sortkjaer, O (Sortkjaer, Ole)
Title: Temperature-dependent and surface specific formaldehyde degradation in submerged biofilters
Source: AQUACULTURAL ENGINEERING, 36 (2): 127-136 MAR 2007
ISSN: 0144-8609
DOI: 10.1016/j.aquaeng.2006.09.004

Record 6 of 16
Author(s): Eiroa, M; Vilar, A; Kennes, C; Veiga, MC
Title: Formaldehyde biodegradation in the presence of methanol under denitrifying conditions
Source: JOURNAL OF CHEMICAL TECHNOLOGY AND BIOTECHNOLOGY, 81 (3): 312-317 MAR 2006
ISSN: 0268-2575
DOI: 10.1002/jctb.1395

Record 7 of 16
Author(s): Mirdamadi, S; Rajabi, A; Khalilzadeh, P; Norozian, D; Akbarzadeh, A; Mohseni, FA
Title: Isolation of bacteria able to metabolize high concentrations of formaldehyde
Source: WORLD JOURNAL OF MICROBIOLOGY & BIOTECHNOLOGY, 21 (6-7): 1299-1301 OCT 2005
ISSN: 0959-3993
DOI: 10.1007/s11274-005-2443-1

Record 8 of 16
Author(s): Eiroa, M; Vilar, A; Amor, L; Kennes, C; Veiga, MC
Title: Biodegradation and effect of formaldehyde and phenol on the denitrification process
Source: WATER RESEARCH, 39 (2-3): 449-455 JAN-FEB 2005
ISSN: 0043-1354
DOI: 10.1016/j.watres.2004.09.017

Record 9 of 16
Author(s): Masters, AL
Title: A review of methods for detoxification and neutralization of formalin in water
Source: NORTH AMERICAN JOURNAL OF AQUACULTURE, 66 (4): 325-333 OCT 2004
ISSN: 1522-2055

Record 10 of 16
Author(s): Bednarik, V; Vondruska, M
Title: Removal of formaldehyde from acrylic acid production wastewater
Source: ENVIRONMENTAL ENGINEERING SCIENCE, 20 (6): 703-707 NOV-DEC 2003
ISSN: 1092-8758

Record 11 of 16
Author(s): Lotfy, HR; Rashed, IG
Title: A method for treating wastewater containing formaldehyde
Source: WATER RESEARCH, 36 (3): 633-637 FEB 2002
ISSN: 0043-1354

Record 12 of 16
Author(s): DiGiano, FA; Singer, PC; Parameswar, C; LeCourt, TD
Title: Biodegradation kinetics of ozonated NOM and aldehydes
Source: JOURNAL AMERICAN WATER WORKS ASSOCIATION, 93 (8): 92-104 AUG 2001
ISSN: 0003-150X

Record 13 of 16
Author(s): Garrido, JM; Mendez, R; Lema, JM
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Author(s): Vidal, G; Jiang, ZP; Omil, F; Thalasso, F; Mendez, R; Lema, JM
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Author(s): Qu, MB; Bhattacharya, SK
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Acetaldehyde

Record 1 of 4
Author(s): Chen, LJ (Chen, Li-Jung); Bangs, KM (Bangs, Katherine M.); Kinney, KA (Kinney, Kerry A.); Katz, LE (Katz, Lynn E.); Seibert, AF (Seibert, A. Frank)
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Acetamide

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Author(s): Li, T (Li, Tinggang); Liu, J (Liu, Junxin); Bai, R (Bai, Renbi); Ohandja, DG (Ohandja, Dieudonne-Guy); Wong, FS (Wong, Fook-Sin)
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Source: WATER RESEARCH, 41 (15): 3465-3473 AUG 2007
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Methylamine

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Author(s): Doronina, NV; Ezhov, VA; Trotsenko, YA
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N-nitrosodimethylamine

Record 1 of 10
Author(s): Kong, LL (Kong Lulu); Guo, XY (Guo Xiaoyan); Zhou, QX (Zhou Qixing); Li, QL (Li Qilin); Hu, WL (Hu Wanli); Lu, JF (Lu Jinfeng)
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Author(s): Patterson, BM (Patterson, B. M.); Shackleton, M (Shackleton, M.); Furness, AJ (Furness, A. J.); Pearce, J (Pearce, J.); Descourvieres, C (Descourvieres, C.); Linge, KL (Linge, K. L.); Busetti, F (Busetti, F.); Spadek, T (Spadek, T.)
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Source: WATER RESEARCH, 44 (5): 1471-1481 MAR 2010
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Author(s): Jahan, K (Jahan, K.); Smith, R (Smith, R.); Scrivani, D (Scrivani, D.); Giacobbe, D (Giacobbe, D.); McDonough, J (McDonough, J.); Addu, A (Addu, A.)
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Conference Date: 2008
Conference Location: Granada, SPAIN
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Title: Bio-reduction of N-nitrosodimethylamine (NDMA) using a hydrogen-based membrane biofilm reactor
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Author(s): Sharp, JO (Sharp, Jonathan O.); Sales, CM (Sales, Christopher M.); LeBlanc, JC (LeBlanc, Justin C.); Liu, J (Liu, Jie); Wood, TK (Wood, Thomas K.); Eltis, LD (Eltis, Lindsay D.); Mohn, WW (Mohn, William W.); Alvarez-Cohen, L (Alvarez-Cohen, Lisa)
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Author(s): Sharp, JO; Wood, TK; Alvarez-Cohen, L
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Nitrosomorpholine

Record 1 of 1
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