European Science Foundation

Standing Committee for Life, Earth and Environmental Sciences (LESC)

ESF LESC - SETAC EXPLORATORY WORKSHOP

New Improvements in the Aquatic Ecological Risk Assessment of Fungicidal Pesticides and Biocides

Wageningen, Netherlands, 6-9 November 2005

Convened by:

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Co-sponsored by

The Dutch Ministry of Agriculture, Nature and Food Safety

Contents

1 Summary

Protection of non-target organisms from the potential effects of agricultural pesticides is based on a risk assessment procedure that has become increasingly harmonised and sophisticated. For the aquatic environment, fate models are used to predict exposure via spray drift, surface runoff and drainflow. Baseline laboratory experiments measure toxicity in indicator species under standardised conditions and there are established criteria and procedures for undertaking more sophisticated studies like mesocosm experiments. A similar approach is chosen for the risk assessment of biocides, although the exposure methodology is different and higher tiers in the risk assessment are less well defined. Since, depending on its use, one active ingredient may be evaluated by both directives a comparison of the two regulations is needed.

The tiers of the current risk assessment schemes for pesticides (Directive 91/414/EEC) and biocides (Directive 98/8/EC) are suitable for the risk assessment of chemicals with an insecticidal and herbicidal mode of action but may ignore endpoints important for the risk assessment of fungicidal pesticides and biocides. There are few published community-level studies (i.e. micro/mesocosm) with fungicidal pesticides and biocides and there is a general lack of information on the effects of these compounds on aquatic ecosystems. Antifouling paints containing boosters biocides like tributyltin (TBT) have been studied, but this research is principally concerned with marine environments. Even less is known about the effect of fungicides on freshwater microbial communities. This is despite the fact that micro-organisms, in particular fungi, play a crucial role in key ecosystem processes such as litter breakdown and nutrient cycling.

In the light of the above the workshop highlighted and discussed the differences in risk assessment approaches supporting the pesticide and biocide directives and the implications of studying microbial endpoints for the risk assessment of fungicidal pesticides and biocides in terms of functional redundancy, sensitivity and trophic interactions. The workshop also aimed to bring together the state of the science on microbial ecology and approaches for studying microbial systems.

During the workshop no evidence was put forward that fungicidal pesticides approved under 91/414 and applied using GAP have large unacceptable impacts on aquatic microbial processes. However, only a few studies have investigated the effects of fungicidal pesticides and biocides on aquatic microbial processes (degradation, decomposition). Decomposition was sometimes found to be affected but was not found to be the most sensitive endpoint in any of the examples considered. Very little information is available on the effects of fungicidal pesticides and biocides on the structure and composition of aquatic microbial communities. More studies are available on the impact of fungicides on soil microbial processes (C and N mineralization, litter decomposition), since such studies are data requirements under 91/414/EEC. The studies submitted under 91/414/EEC show limited evidence of adverse effects, but one should keep in mind that they focus on function rather than structure and that effects of < 25 % on C and N mineralization or recovery to < 25 % within 100 days are considered as non-adverse effects. The same trigger for adverse effects may not be applicable for aquatic ecosystems. It is also questionable whether one can read across from soil to aquatic systems, since similar species and functions are present for bacteria in these two matrices, but not for fungi. It was concluded that microbes have high capacity for rapid recovery and adaptation, but there may be functional bottlenecks when groups of species are at risk. There is a wealth of literature that community structure and function are related, but there are many functions and it is difficult to define bacterial diversity. There are major differences in the importance of toxicant-induced effects on heterotrophic microbes in different aquatic systems, so for the risk assessment it is important whether streams, lakes, ponds or ditches are of concern. The group also identified a potential for indirect effects on invertenbrate consumers of microbes. The workshop ended by identifying research and policy recommendations.

2 Introduction

2.1 Workshop Background:

Protection of non-target organisms from the potential effects of agricultural pesticides is based on a risk assessment procedure that has become increasingly harmonised and sophisticated (EU, 1997). For the aquatic environment, fate models are used to predict exposure via spray drift, surface runoff and drainflow (FOCUS, 2001). Baseline laboratory experiments measure toxicity in indicator species under standardised conditions and there are established criteria and procedures for undertaking more sophisticated studies like mesocosm experiments (Gidding et al., 2002). A similar approach is chosen for the risk assessment of biocides, although the exposure methodology is different and higher tiers in the risk assessment are less well defined (EU, 1998).

The tiers of the current risk assessment schemes for pesticides (Directive 91/414/EEC) and biocides (Directive 98/8/EC) are suitable for the risk assessment of insecticides and herbicides but might ignore endpoints important for the risk assessment of fungicidal pesticides and biocides. In particular, the lower tiers ignore effects on microbial assemblages and potentially important non-standard test species like micro-organisms and non-arthropod invertebrates. In addition, when considering the toxic mode-of-action of fungicidal pesticides and biocides, potential effects on functional endpoints indicative of the metabolic activities of micro-organisms in ecosystems cannot be ignored.

There are few published community-level studies (i.e. micro/mesocosm) with fungicidal pesticides and biocides and there is a general lack of information on the effects of these compounds on aquatic ecosystems. Antifouling paints containing boosters biocides like tributyltin (TBT) have been studied, but this research is principally concerned with marine environments (Konstantinou and Albanis, 2004). These studies indicate that endocrine disrupting effects (i.e. imposex) may occur (Mensink et al., 2002), although the extent to which this is of concern in the freshwater environment, which is the main target of concern for the registration procedure, is not known. Even less is known about the effect of fungicides on freshwater microbial communities. This is despite the fact that micro-organisms, in particular fungi, play a crucial role in key ecosystem processes such as litter breakdown and nutrient cycling (Maltby, 1992a; Wong et al., 1998; Hieber and Gessner, 2002). It may be argued, however, that the adaptation and functional redundancy within microorganisms is high, although this has hardly been studied in relation to fungicide exposure.

It is recognised that many key ecosystem processes are driven by microbial activities. However, the lack of techniques that enable the diversity and functioning of microbial communities to be studied, have hampered investigation of the effects of pesticides and biocides on these organisms. Recent developments in molecular biology have resulted in species-specific tools for studying structural changes in the microbial community in natural ecosystems. These include the use of monoclonal antibodies for identifying and quantifying fungal mycelia (Bermingham et al, 1997) and the use of gene sequences to identify species and quantify diversity (Burnett, 2003; Nikolcheva et al. 2003). Kersting (1994) reviewed the use of functional endpoints in semi-field testing. Functional endpoints include measures of ecosystem processes such as production, decomposition, nutrient cycling and energy transfer. They are seen as indicators of ecosystem health since they integrate the effects on lower levels of biological organization and indicate the severity of a perturbation. Although advocated (Giddings et al., 2002), functional endpoints are hardly studied, with the exception of measures indicative of photosynthesis like DO and pH. Other functional endpoints describing microbial activity such as phototrophic carbon assimilation and heterotrophic bacterial productivity are relatively new (Downing et al., 2004) and not incorporated into protocols. The ecological significance of these novel approaches for the freshwater environment and their use in ecological risk assessment, need to be evaluated.

Important questions when assessing the risk that fungicidal pesticides and biocides pose to aquatic ecosystems are:

- which taxonomic groups of organisms are affected?
- to what extent are these effects related to the toxic mode-of-action of these chemicals?

This information may be important when constructing Species Sensitivity Distribution curves (SSD). Recently it was demonstrated for insecticides that the taxonomic composition of the species assemblage used to construct the SSD does have a significant influence on the assessment of hazard (Maltby et al. 2005). When assessing the hazards of pesticides with a specific toxic mode-of-action it is common practise to construct the SSD with species from the sensitive taxonomic group (e.g. arthropods in the case of insecticides and primary producers in the case of herbicides). The influence of taxonomy in predicting hazards of fungicidal pesticides and biocides in aquatic ecosystems on basis of the SSD approach needs to be evaluated.

Indirect or secondary effects of a pesticide in an ecosystem are those that result from a reduction or elimination of biological populations due to direct toxic effects. In other words, a decrease in activity or reduction in population size of pesticide-susceptible species may result in shifts in interactions between species not directly affected by the pesticide. This again may result in pronounced shifts in ecosystem processes. Indirect effects are to be expected in particular when direct toxic effects result in the removal of key species (e.g. macrophytes, important grazers or top-predators that control community structure). From a literature review, it appeared that indirect effects differed in the different types of community, at least when evaluated on the basis of those species that showed responses. However, types of indirect effect in different types of community were broadly similar when evaluated on the basis of functional groups of freshwater organisms (e.g. carnivores, herbivores, detritivores). The types of indirect effect most frequently observed, and fairly well predictable at the level of functional groups, are those that result from the removal of competition and that involve two adjacent trophic levels. Since fungicidal pesticides and biocides have different effects on the ecosystem compared to herbicides and insecticides also their indirect effect and food-chain effects will be different. Gammarus for instance feeds on microbial assemblages and detritus, which may be influenced qualitatively and quantitatively by fungicidal pesticides and biocides. There is a considerable body of evidence to suggest that detritivores are selective feeders and that their feeding behaviour is mediated by the microflora colonizing the detritus on which they feed. Any direct effects on the composition or activity of this microflora could therefore have indirect effects on detritivore feeding and hence detritus processing and nutrient cycling (e.g. Maltby, 1992b). A complete understanding of the possible indirect effects of fungicidal pesticides and biocides on aquatic food-web will be more important, so that their effects on ecosystem function can be assessed.

2.2 Workshop objectives:

In the light of the above the workshop had the following objectives:

- To highlight and discuss the differences in risk assessment approaches supporting the pesticide and biocide directives
- To bring together the state of the science on microbial ecology and approaches for studying microbial systems
- Discuss the implications of studying microbial endpoints for the risk assessment of fungicidal pesticides and biocides in terms of functional redundancy, sensitivity and trophic interactions

3 Scientific content of the event

3.1 Fungicidal mode of action

Fungicides cover a wide range of chemicals, from inorganic simple molecules (e.g. sulphur) to organic complex molecules (e.g. triazoles and strobilurins). They have very diverse mode of actions targeting different cellular processes in the fungal cell; it may be a respiratory inhibitor interfering with the electron transport and oxidative phosphorylation in mithochondria; it may affect cell wall/membrane production through inhibition of the sterol (ergosterol) synthesis; it may interfere with the cell division or intra cellular movement by disturbing the formation of microtubule; it may interfere with RNA and DNA synthesis; it may

be a multi-site inhibitor and for some fungicides the specific mode of action is not even known. Due to this large variation in mode of action it is very difficult to foresee potential effect in the environment or even to predict which group of non-target organisms is likely to be most sensitive. Therefore a comprehensive risk assessment covering all non-target groups of organisms that may be exposed is essential for preventing adverse effects in the environment.

3.2 Regulatory background and risk assessment approaches

3.2.1 Regulation of pesticides and biocides according to European Union legislation

(Lina Wendt-Rasch and Floor Peeters)

Regulation of fungicidal chemicals in EU

The AERA workshop focused on issues relating to aquatic environmental risk assessment and in order to provide a background to the discussion an introductory account of the data requirements and risk assessment procedures for the aquatic compartment is given below.
The same fungicidal chemical can be used as both an agricultural pesticide and a biocide, and whether a product is regarded as a biocide or an agricultural pesticide is not governed by inherent properties of the chemical but rather by its intended use. A plant protection product is by definition used to protect plants or plant products against harmful organisms or to prevent the action of such organisms (Directive 91/414/EEC). A biocidal product is intended to destroy, deter, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means, in order to protect other kinds of products than those used for plant protection (Directive 98/8/EC). In addition to the biocidal and pesticidal use fungicides are also used for veterinary and human medicine and are for these uses regulated by national and European legislation not further described in this document.

Regulation of plant protection products

Plant protection products are in the European Union (EU) regulated by the 'Council Directive 91/414/EEC of 15 July 1991 concerning the placing of plant protection products on the market'. The main objective of the directive is to harmonise the authorisation of plant protection products within the EU. In order to achieve this the Directive 91/414/EEC establishes a positive list of active substances, the so called Annex I, which includes substances that have been evaluated to be safe for humans and which do not present an unacceptable risk to the environment according to the criteria stipulated in the directive.
The national product authorisation remains the responsibility of individual member states, however the directive establish harmonised data requirements and a harmonised risk assessment procedure and criteria for member states to use when considering the safety of plant protection products. Hence, also the procedure of authorisation of plant protection products should be harmonised amongst member states, and member states are only permitted to authorise marketing and use of plant protection products where the active substances are listed in Annex I, except where transitional arrangements apply. The goal set out in the Directive is that there should be a complete harmonisation in 2008, i.e. only active substances which are included in Annex I should by then be used in the EU.
The procedure by which an active substance is considered for Annex I inclusion first involves an initial expression of interest in supporting the active substance by a notifier (e.g. a pesticide producing/supplying company), followed by submission of a dossier conforming to the data requirements of the Directive (stipulated in Annex II and III, described further below). A Rapporteur Member State then conducts an evaluation of the data submitted and a risk assessment which is summarised in a Draft Assessment Report (also known as draft monograph). The Draft Assessment Report (DAR) is then considered via a technical peer review process in which other Member States and the European Food Safety Authority (EFSA) jointly review the outcome of the evaluation and the risk assessment conducted by the Rapporteur. Following the peer review EFSA produces a report on the conclusion on the peer review of the substance and the evaluation is then discussed in a European

Commission Evaluation Group meeting at which all Member States have an opportunity to consider whether all issues have been satisfactorily addressed. When the scientific issues have been resolved a vote based on qualified majority will be taken by the Standing Committee on Food Chain and Animal Health (SCFA). The outcome of the vote is then reflected in a Decision which is adopted and then published by the European Commission in the Official Journal.

Regulation of Biocidal products

Biocidal products are regulated by the 'Directive 98/8/EC of the European parliament and of the Council of 16 February 1998 concerning the placing of biocidal products on the market'. The main objective of the directive is to harmonise the authorisation of Biocidal products within the EU. To attain this aim, Directive 98/8/EC establishes a list of active substances, Annex I which have been evaluated according to the criteria for inclusion in Biocidal Products (criteria mentioned in Annex VI of the Directive and the TNsG on annex I inclusion).
To give an impression of the wide range of different Biocidal product types an overview is given in Table 1 as referred in Annex V of the Directive 98/8. Fungicidal chemicals can be found in several product types like 4, 6-13 and 20-22.

Table 1. Grouping of biocidal products used in the EC

Main group 1: Disinfectants and general biocidal products
Product type 1: Human hygiene biocidal products
Product type 2: Private area and public health area disinfectants and other biocidal products
Product type 3: Veterinary hygiene biocidal products
Product type 4: Food and feed area disinfectants
Product type 5: Drinking water disinfectants
Main group 2: Preservatives
Product type 6: In-can preservatives
Product type 7: Film preservatives
Product type 8: Wood preservatives
Product type 9: Fibre, leather, rubber and polymerised materials preservatives
Product type 10: Masonry preservatives
Product type 11: Preservatives for liquid-cooling and processing systems
Product type 12: Slimicides
Product type 13: Metalworking-fluid preservatives
Main group 3: Pest control
Product type 14: Rodenticides
Product type 15: Avicides
Product type 16: Molluscicides
Product type 17: Piscicides
Product type 18: Insecticides, acaricides and products to control other arthropods
Product type 19: Repellents and attractants
Main Group 4: Other biocidal products
Product type 20: Preservatives for food or feedstocks
Product type 21: Antifouling products
Product type 22: Embalming and taxidermist fluids
Product type 23: Control of other vertebrates

Similar to plant protection products the national product authorisation remain the responsibility of the national authorities but with the risk assessment procedure and criteria established in the Directive 98/8/EC.
The goal set out in the Directive is that a complete harmonisation of the authorisation of biocidal products should be achieved around 2010, when only biocidal products listed in Annex I should be authorised in EU member states.
The procedure by which an active substance is considered for Annex I inclusion is generally the same as for plant protection products. After the active substance is supported by a notifier and the dossier is submitted the Rapporteur Member State will summarise and evaluate the data. The evaluation and a risk assessment will be presented in a concept Competent Authority Report (CAR). Thereafter all Member States can give their opinions on the draft CAR which will be considered by the Rapporteur Member State and thereafter the CAR will be discussed at a scientific level at the Technical Meeting (TM). When the member states have agreed on the scientific issues of the risk assessment the CAR will be discussed

in the Competent Authority meeting where representatives of relevant ministries of all Member States will discuss the CAR from a policy view point. When all issues have been resolved a vote based on qualified majority will be taken by the Standing Committee on Biocides (SCB). The final Decision based on the vote will be adopted and then published by the European Commission in the Official Journal.

Risk assessment of fungicides for the aquatic compartment according to EU legislation

According to directive 91/414/EEC the following areas should be considered for the assessment of an active substance or plant protection product:, identity, physical and chemical properties; methods of analysis (e.g. for residues in food products and in the environment); toxicological issues (i.e. relating to human risk assessment); residues in or on treated products, food and feed; fate and behaviour in the environment and ecotoxicological issues. For plant protection products the efficacy is considered at a national level. The same areas are also considered for the risk assessment of biocides under Directive 98/8/EC with an exception for efficacy which is also considered at EU level.

Protection goal

In order to focus a risk assessment on the relevant issues the protection aims needs to be defined. According to the 'Guidance document on aquatic Ecotoxicology in the context of Directive 91/414/EEC' (SANCO 3268/2001) it is in general the sustainability of populations of non-target organisms that should be ensured, and during the risk assessment structural and functional endpoints should be regarded as of equal importance. For biocides the protection aims are not clearly mentioned.

Exposure assessment

In order to estimate the concentration (i.e. Predicted Environmental Concentration - PEC) of the active substance, or other substances of concern present in the product, which may contaminate aquatic ecosystems following the proposed use of plant protection products or biocides an exposure assessment is needed. The exposure assessment is a tiered approach with the possibility for the notifier to submit higher tier data, e.g. monitoring data or higher tier modelling, if the risk assessments at a lower tier indicate risks above the established criteria.

Plant protection products
During the first stages of the revision of existing active substances for their potential inclusion in Annex I, aquatic exposure assessment was based on loadings to surface water only through spray drift. However, for many uses drift is not a concern and other routes of water contamination may be more relevant (e.g. run off and drainage). This was one of the motives for the Commission to initiate the FOCUS Surface Water Working Group that has developed FOCUS surface water scenarios which are used for computer simulations to assess the potential contamination of surface water with active substances and metabolites of plant protection products.
The FOCUS methodology is a tiered approach with four levels of assessment. The first step (FOCUS Step 1) is a relatively simple calculation based on a maximal loading and a fixed scenario, while Step 2 is more complex and allows multiple applications and regional variation between South and North Europe. In the third step of the approach exposure is simulated in several different scenarios. These FOCUS surface water scenarios are ten standard combinations of climate, soil, cropping data and water body characteristics, which collectively represent the agriculture within the EU. In a fourth step an even more detailed site-specific approach can be developed on a case by case basis (e.g. the implementation of no-spray buffer-zones). To minimise the influence of the user on the outcome of the simulation (i.e. the PEC) as many as possible of the input variables have been fixed, leaving only the dossier data (i.e. fate and behaviour data of the pesticide e.g. DT50 and Koc) as main input data. The scenarios and their derivation are described in detail in the report 'FOCUS surface water scenarios in the EU evaluation process under 91/414/EEC' (SANCO/4802/2001).

Biocidal products

The development of emission scenarios for biocides started in 1993 and is still ongoing. There are 23 product types and for most of them Emission Scenario Documents (ESD) have been prepared (one ESD can contain one or more scenarios). However, there are still several product types for which relevant emission scenarios are lacking. Only for some product types (i.e. 8 and 14) there is some experience in using the ESDs and discussions between the Member States about the content of these scenarios are still going on. For the other product types there is only limited experience. In the next years the products of these product types will be evaluated at EU level and so will the ESDs. The scenarios available so far are described in detail in the different ESDs which can be found at the ECB website (http://ecb.jrc.it/biocides/)

The variation between all the different ESD's and their scenarios is very large. Biocide exposure of water (and sediment) can be direct, e.g. for wood preservatives and antifoulings or indirect via a sewage treatment plant (STP), e.g. for disinfectants. Most biocidal products are used in such a way that the exposure of aquatic ecosystems can be regarded as chronic. One difference between biocides and pesticide exposure assessment is that the risk has to be evaluated for the whole life cycle (application phase, use phase and waste phase) including the application phase, service life and waste treatment phase for biocides while for pesticides only the exposure resulting from the application is considered. Similar to the procedure for pesticides the input variables to be used in the models to generate PECs for biocides are fixed as much as possible and only the dossier data (i.e. fate and behaviour data of the pesticide e.g. DT50 and Koc) are left as main input data.

Effect assessment for the aquatic compartment

Similar to the exposure assessment also the ecotoxicological effect assessment is a tiered approach with the possibility for the notifier to submit higher tier data if the risk assessment indicates high risks at a lower tier.

Plant protection products
The first tier data generally consists of acute LD50 / EC50 values (the amount of a chemical that is Lethal to /has an Effect on half of the experimental organism being exposed) estimated from laboratory tests as well as NOECs (No Observed Effect Concentration for reproductive or other sublethal endpoints) from long term laboratory tests (Table 2).

Table 2. First tier tests required to fulfil the data requirements of Annex II of the Dir. 91/414/EEC

Always required	If the exposure is prolonged or repeated	If log K_{ow} > 3, i.e. bioaccumulation can be suspected	If the substance may end up in the sediments	For herbicides
96-h LC_{50} for rainbow trout and one warm water fish	Long term test on fish (chronic test on juvenile fish, early life stage test or fish full lifecycle test)	BCF (Bio Concentration Factor) for fish	Toxicity to sediment living organisms (*Chironomus)*	96-h EC_{50} for a diatom or blue green algae
48-h EC_{50} for daphnia	Long term test on daphnia			EC_{50} for macrophytes (*Lemna)*
96-h EC_{50} for one species of green algae				

The data requirements for higher tier tests are not as strictly regulated in the directive and can be decided case by case. For aquatic ecosystems they may consist of additional single species tests, tests with modified exposure and multiple species tests (e.g. microcosm and mesocosm tests).

Biocidal products

The data requirements for the biocidal products are divided into three groups/categories (see also Technical Notes for Guidance on data requirements in support of Dir. 98/8/EC concerning the placing of biocidal products on the market):

- core data which are required for all product types;
- product type specific data which are standard requirements for some product types;
- additional data which are required when higher tier risk assessment is needed (if PEC/ PNEC >1,see risk assessment).

The first tier data generally consist of acute LD50 / EC50 data estimated from laboratory tests as well as NOECs from long term laboratory tests (Table 3).

Table 3. Data requirements of the Directive 98/8/EC

Core data, for all product types, always required	Product type specific data	Additional data for aquatic organisms:	Additional data for sediment organisms:
96-h LC_{50} fish for one or two species	Long term test on fish	Long term test on fish [(A)]	Toxicity to sediment living organisms (*Chironomus)*[(E)]
48-h EC_{50} invertebrate species for one or two species	Long term test on invertebrate species	Long term test on invertebrate species[(A)]	
96-h EC_{50} algae for one or two species	Test on aquatic plants	Test on aquatic plants[(B)]	
BCF on basis of partioning coefficient n-octanol-water		BCF for fish[(C)]	
		Field data or model ecosystem [(D, F)]	Field data or model ecosystem [(D, F)]
		More data to use Species Sensitivity Distribution (SSD)[(F)]	More data to use Species Sensitivity Distribution (SSD)[(F)]

[(A)]: These additional data are required if: there is long-term exposure, or; log Kow > 3 and/ or BCF > 100, or; PEC local > 1/100th water solubility, or; there is risk on basis of acute data (see Technical Guidance Document)
[(B)] Case-by- case
[(C)] If bioaccumulation can be suspected according to the TGD (e.g. if a substance: has a log Kow ≥ 3, or; is high adsorptive etc.) and if there is a risk for predators by secondary poisoning
[(D)] not further defined
[(E)] Product type specific data for 1 product type. Required as additional data if: log Kow > 3, or; there is a risk using equilibrium partitioning method.
[(F)] These data are not mentioned in the TNsG on data requirements. These data are mentioned in the Technical Guidance Document.

The table above shows that there are core data, product types specific data and additional data for aquatic organisms. For sediment organisms there are only additional data.
For aquatic organisms the predicted no-effect concentration (PNEC) will be calculated from the data on effects by applying an assessment factor to the values resulting from tests on organisms, e.g. acute LD50 / EC50 or NOECs. An assessment factor is an expression of the degree of uncertainty in extraplolation from test data on a limited number of species to the real environment (see also below where the assessment factors used under 98/8/EC is compared to the factors used under 91/414/EEC). Therefore, in general, the more extensive the data and the longer the duration of the tests, the smaller is the degree of uncertainty and the size of the assessment factor. For sediment organisms the PNEC will be calculated by using the equilibrium partitioning method or by applying an assessment factor to the values resulting from tests on organisms (for more details see the Technical Guidance Document (TGD).

Risk assessment

Plant protection products

Under 91/414/EEC the risk of adverse effects is estimated through the calculation of a Toxicity to Exposure Ratio (TER) which is the toxic effect value (LD50, EC50 or NOEC) divided by the Predicted Environmental Concentration (PEC, obtained from FOCUS simulations as described above). The higher this ratio is the larger is the margin between the concentration that is predicted to occur in the environment and the concentration at which effects are observed during testing. In the Uniforming Principles (Annex VI of the directive) trigger values are given for when the margin is considered large enough for the risk to be sufficiently low for product registration by national authorities. The same criteria are also used to take decisions on an inclusion of the active substance in Annex I. It is stated that "Where there is a possibility of aquatic organisms being exposed, no authorisation should be granted:

- if the toxicity/exposure ratio for fish and Daphnia is less than 100 for acute exposure and less than 10 for long-term exposure,
- or the algal growth inhibition/exposure ratio is less than 10,
- or the maximum bio-concentration factor (BCF) is greater than 1000 for plant protection products containing active substances which are readily biodegradable or greater than 100 for those which are not readily biodegradable,
- unless it is clearly established through an appropriate risk assessment that under field conditions no unacceptable impact on the viability of exposed species (predators) occurs - directly or indirectly - after use of the plant protection product according to the proposed conditions of use."

For groups of organisms not specifically mentioned in Annex VI, the appropriate TER trigger values for related groups for acute and chronic risk assessments are used. For example, assessments using data on insects (including Chironomus sp.) should use the trigger values specified for Daphnia. (Guidance document on aquatic Ecotoxicology in the context of Diretive 91/414/EEC (SANCO 3268/2001)). For higher tier studies no trigger values are specified in the Uniforming Principle instead these are agreed on by member states on a case-by-case basis.

The trigger values given in Annex VI are equivalent to what often also is called assessment factors and are supposed to account for several uncertainties inherent in the risk assessment procedure, for example they should cover variation between individuals; extrapolation of effect data on one or a few species to all exposed species; laboratory to field extrapolation of effect data. The use of a assessment factor is therefore a key part of the risk assessment. It should however be noted that the scientific basis of their derivation has not been clearly described and they are more based on pragmatism and tradition than on firm scientific grounds. The assessment factors used for the risk assessment for plant protection products differs slightly from the factors used for risk assessment of biocidal products under Dir. 98/8/EEC, see Table 4.

Table 4. Assessment factors for aquatic risk assessment used under Dir. 98/8/EEC and 91/414/EEC.

Available data	Assessment factor under 98/8/EC[1]	Assessment factor under 91/414/EEC[2]
At least one short-term L(E)C50 from each of three trophic levels of the base set (fish, Daphnia and algae)	1000	100
One long-term NOEC (either fish or Daphnia)	100	Both fish and daphnia required if long term exposure can be anticipated
Two long-term NOECs from species representing two trophic levels (fish and/or Daphnia and/or algae)	50	10 (when long term NOEC for fish and daphnia are available)
Long-term NOECs from at least three species (normally fish, Daphnia and algae) representing three trophic levels	10	10
Species sensitivity distribution (SSD) method	5-1 (to be fully justified case by case)	Reviewed on a case by case basis
Field data or model ecosystems	Reviewed on a case	Reviewed on a case

	by case basis	by case basis

[1] Technical Guidance document
[2] Uniforming Principles (Annex VI of the Dir. 91/414/EEC)

Biocidal products
Under Directive 98/8/EC the risk of adverse effects is estimated through the calculation of a PEC/PNEC-ratio (i.e. the Predicted Environmental Concentration (PEC, obtained from the emission scenario documents as described above) divided by the predicted no-effect concentration (PNEC)). This calculation is done both for the first tier and the higher tier approaches.
In the directive it is stated that "The Member State shall not authorise a biocidal product where there is a reasonably foreseeable possibility of aquatic organisms including marine and estuarine organisms being exposed to the biocidal product if for any active substance or substance of concern in it:

- the PEC/PNEC is above 1 unless it is clearly established in the risk assessment that under field conditions the viability of aquatic organisms is not threatened by the biocidal product according to the proposed conditions of use, or
- the bioconcentration factor (BCF) is greater than 1000 for substances which are readily biodegradable or greater than 100 for those which are not readily biodegradable, unless it is clearly established in the risk assessment that under field conditions no unacceptable impact, either directly or indirectly, occurs on the viability of exposed organisms including marine and estuarine organisms after use of the biocidal product according to the proposed conditions of use (Common Principles, Annex VI of the Directive)."

Are microbial end-points covered by the current risk assessment schemes?

When considering risk assessment of fungicides one important question from a regulatory point of view is whether the current risk assessment schemes for agricultural pesticides (Directive 91/414/EEC) and biocides (Directive 98/8/EC) do include all endpoints important for the risk assessment of these chemicals. As can be seen from the account given above no tests or trigger values are specified for the aquatic microbial community in neither of the two directives. It is however possible that the risk to microbial communities nevertheless are covered by the tests already included in the scheme, e.g. if the microbial community in general is less sensitive than the standard test species. If not, it is possible that new regulatory endpoints are needed in order to cover possible effects on this group of organisms.

3.2.2 Effect assessment of fungicidal pesticides and biocides : a case study

On the first day of the AERA workshop a case study was presented to discuss the differences in risk assessment methodologies between the pesticide and biocide directives. Basic physico-chemical and first tier toxicity data was collected for two chemicals (fungicide A and B), as well as some higher tier effect data. For both chemicals a use as a pesticide as well as a biocide was identified, resulting in 4 breakout groups. The data sheets that the different groups received as well as their composition is given in Appendix 1.

All groups were asked to answer questions on the basis of the provided information :

Considering only the tier 1 data (fate scenario, PEC and toxicity data)
Questions:

1. Which risks are not identified for chemicals with a fungicidal MoA considering the data available in the standard aquatic first tier data package?
2. Would it be possible to use any of the Annex II/III first tier data for other organisms groups (e.g. soil nitrogen mineralization-, earthworm-, STP – tests, see further in the directive available in breakout rooms) in order to solve risks identified in a)?
3. If the risks identified in a) cannot be solved/identified by the use of any of the Annex II/III first tier data for other organisms groups are any testing methods available that can be used?

4. If risks identified in a) cannot be solved either by methods identified in b) or c) should MoA alone be a trigger for higher tier testing?

Considering higher tier data (further species tests or a microcosm study)
Questions:

5. Are the risks identified in a) above solved by these higher tier data?
6. Are potential risks particular for fungicidal MoA identified by these "standard" higher tier risk assessment methods or are some risks still unidentified?
7. What special considerations are needed when designing higher tier risk assessment methods for fungicidal MoA?

The answers of the four groups are provided in Appendix 2, below a synthesis is provided for fungicide A and B seperately.

Fungicide A

The standard data for the use as a a pesticide are available. In addition, information on a large number of fish species and further higher tier data (mesocosms) has been provided. Further to information on aquatic systems also data on terrestrial systems have been given. Several data are missing with respect to biocidal effect assessment such as a third chronic NOEC (algal NOEC lacking) and information on other marine invertebrates (two).

For the pesticidal use a range of PECini values are calculated depending on GAP and distance to surface waters (0.62 – 110 µg/L) as a result of multiple application. For biocidal use different values according to scenarios (0.02 – 1.4 µg/L) are calculated due to chronic concentrations.

Fish (rainbow trout) provide to be the most sensitive endpoint. The risk assessment for the pesticide result in the loss of two GAPs and significant mitigation required for the remaining one, For the biocide a treshold value of 0.2 µg/L is calculated for freshwater, one of 0.02 µg/L for the marine system. This results in no registration.

Higher tier risk asssessment for the pesticide uses take into account information on SSD for fish (most sensitive group), acute to chronic ratios and the trout study that was performed under realistic worst case exposure conditions. The lowest realistic endpoint is the NOEC of 60 µg/L and if an assessment factor of 3 is used (instead of 10 because of available info on SSD and acute to chronic ratio), all PECini lower than 20 µg/L are considered not to cause unacceptable risk. Using this assessment all GAPs are possible with appropriate mitigation measures.

The HC5 approach according to the biocide TGD may not be appropriate because this relates to chronic tests, moreover the taxa asked for under the biocide TGD have not been covered.There is no information on further marine invertebrates so no registration can be provided. From a scientific perspective, the use of HC5 based on the most sensitive groups seems appropriate. The acute / chronic ratio is low (2-5) and information on three chronic endpoints assumed to be available. Information from mesocosm on other taxonomic groups is available but it is questionable whether this is this relevant for marine species. A factor of 20 on the acute HC5 appears to be sufficiently protective: 33/20 = 1.6 µg/L so registration seems possible from a scientific perspective.

Compiled answers to the questions (see above)

1. Group 1 (biocide) had identified several data GAPs for which additional information would be needed. Main risk to fish has been identified, however, no specific major risks or need for additional data due to the fungicidal mode of action. Group 2 considered there may be a lack of information with respect to effects on microbial communities, fungi.
2. The information on terrestrial soil functional properties (C / N – mineralization) and on STP under high contamination levels did not provide any indication of disturbance of processes related to microbioal functions. Accordingly it is rather unlikely that such effects would occur at much lower levels of contamination.

3. There are guidelines or at least guidances available to test marine species. Although there are no standard methods it seems also possible to adapt methods for assessing decomposition in aquatic ecosystems (like leaf litter bag). Tests which report the effects on pH and oxygen levels of the pesticide/biocide in aquatic systems could provide indications on disturbances of microbial functions.
4. No
5. Pesticides: yes (elaborated guidance documents provide relevant and accepted routes for higher tier assessments). Biocides: no (limited accepted routes for higher tier assessments).
6. No indication of risk to whole ecosystems although microbial endpoints were not studied specifically.
7. For biocides it would be appropriate to run tests also under more constant chronic exposure conditions. In general, the potential MoA should be considered and information on species sensitivities if deciding whether to run studies addressing a complex aquatic community (e.g. in a mesocosm) or whether to concentrate just on the most sensitive group (e.g. SSD). But these statements are more general and not specific for fungicidal MoA.

Fungicide B

Exposure profiles are different

Crop protection and biocide uses of fungicides are likely to result in substantially different exposure profiles of the compounds in the environment. For crop protection, broadly speaking, exposure is likely to be limited temporally (i.e. applications are only made during spring and summer), and inputs are most likely to result from spray drift, and to a lesser extent runoff. Use rates of fungicides on an area basis for crop protection are generally likely to be higher than those used in biocide applications. The water bodies assessed under 91/414 are generally small edge-of-field water bodies. For biocide uses, generally speaking for fungicide applications, exposure is likely to be localized (associated e.g. with industrial facilities) but the inputs of the compound into the environment are more likely to be temporally consistent (albeit recognizing that there will be fluctuations due to differences in discharge and dilution). The water bodies assessed under 98/8 tend to be larger than those assessed under 91/414. Consequently, crop protection uses are likely to be characterized by relative high, pulsed exposures. Biocide uses are likely to be characterized by relatively low, long-lasting exposures.

Effects data and profiles should be similar

Fungicides that have both crop protection and biocide uses should in principlehave similar available effects data. Considering that the lower tier data do not modify exposure (all studies should be conducted with maintained exposure), the assessment of the predicted no effect concentration (from a scientific perspective) should in essence be the same at the lower tier for both directives. This is not the case however because 98/8 in several cases has increased application factors for the laboratory data. It is not clear why such differences exist. The difference in exposure might be captured using an approach that is considered under the Water Framework Directive (2000/60) in which short-term and long-term acceptable concentrations are considered separately (the maximum acceptable concentration and the annual average environmental quality standard, respectively).

“Read-across” from soil microbial studies, and soil and water-sediment fate package

For biocide fungicides which also have an application in crop protection, there will be studies to look at the effects on soil microbial function (carbon mineralization and nitrification), which can provide some insight into potential issues with aquatic microbial systems. Fate data in soil and water-sediment systems (which concentrate on microbial breakdown processes) will also provide some information about the possible microbial breakdown rate. If breakdown is occurring, active microbe populations must be present, although insight into whether this is fungal or bacterial is not provided. Generally said, if these studies show no effects on microbial function, and degradation is occurring, then it might be concluded that microbial function is unlikely to be impeded (although the relative importance of fungal and microbial

degradation should be kept in mind). For biocides that do not have a crop protection use, this may not be the case, so the potential for using 'read-across' approaches is more limited.

Are standard Tier 1 studies representative for fungicides?

Initially there seemed to be some concerns that perhaps due to the variety of chemistry and mode-of-action of fungicides, perhaps the standard fish, *Daphnia* and algae tests might not identify species that might be particularly sensitive to fungicides. However, the analysis conducted by Maltby and Van den Brink (Maltby pers. comm..). provides some reassurance that this is not in fact the case and that Tier 1 data are generally protective in case of fungicides. Further analysis of data on additional species would be welcome to further confirm this conclusion.

Phytotoxicity

Despite the generally ubiquitous/conserved nature of the mode of action of fungicides, fungicides that are developed for use in crop protection are unlikely to be substantially phytotoxic since this would probably limit their commercial viability. For fungicides that are only used as biocides, this would need to be established.

Guidance for higher-tier studies and assessments

For assessment under 98/8, the Technical Guidance Document is used to provide the assessment framework for higher-tier studies. The descriptions of how to conduct and interpret higher-tier studies in the TGD is somewhat limited. Under 91/414, the higher-tier risk assessment process has an extensive discussion of approaches to higher-tier studies and assessment in the Guidance Document on Aquatic Ecotoxicology (3268.2002) which is further supported by detailed outputs from SETAC workshops (e.g. EWOFFT, HARAP, CLASSIC). Approaches to refine exposure assessment are also discussed in detail in the FOCUS landscape and mitigation report. Similar approaches do not seem to have been considered in detail in the TGD, and there could perhaps be some benefit from making use of the learning under 91/414 for the process under 98/8. Higher-tier assessments should be focused on the concerns identified in the lower tier assessment. Lower tier data can be used to identify the taxa of potential concern with reasonable confidence (see above) and also identify whether the potential concern is of an acute or chronic nature. A number of testing and assessment approaches are then available. Further work is needed to develop refined exposure assessment under 98/8 as has been done to some extent under 91/414. Where SSDs are identified as a potential approach for higher-tiers (risks to invertebrates), it would be appropriate to include non-arthropods in the testing. Furthermore, some measure of decomposition (e.g. leaf litter bags) should be considered for inclusion if micro- or mesocosm studies are conducted.

3.2.3 Discussion

<u>Exposure profiles of biocides and pesticides</u>

Crop protection and biocide uses of fungicides are likely to result in substantially different exposure profiles of the compounds in the environment. For crop protection exposure usually tends to be limited temporally (i.e. applications are performed from spring to autumn), and inputs result from spray drift, runoff or drain. Use rates of fungicides on an area basis for crop protection are generally likely to be higher than those used in biocide applications, and the water bodies assessed under 91/414 are generally small edge-of-field water bodies.
For biocide uses, generally speaking for fungicide applications, exposure is likely to be localized (associated e.g. with industrial facilities) but the inputs of the compound into the environment are more likely to be temporally consistent (albeit recognizing that there will be fluctuations due to differences in discharge and dilution). The water bodies assessed under 98/8 tend to be larger than those assessed under 91/414.
Consequently, crop protection uses are likely to be characterized by a relative high exposure with a tendency of a pulsed exposure pattern. Biocide uses are likely to be characterized by

relatively low, long-lasting exposures. This, however, may differ from case to case because of the many different use patterns for biocides.

Ecotoxicological effects profiles of biocides and pesticides

In principle for fungicides that have both crop protection and biocide uses, the available effects data should be similar. Considering that the tier 1 data do not modify exposure (all aquatic studies are usually to be conducted under constant exposure conditions), the assessment of the predicted no effect concentration should - from a scientific perspective - in essence be the same at the lower tier for both directives. However this is not the case because 98/8 in several cases has increased assessment factors for the laboratory data as compared to 91/414.
The reasons for these differences are unclear to Workshop participants but don't fall under objectives of the AERA Workshop. A potential approach to this difference in exposure might be the Water Framework Directive (2000/60) in which short-term and long-term acceptable concentrations are considered separately (the maximum acceptable concentration and the annual average environmental quality standard, respectively).

Availability of data on effects on micro organisms

For biocide fungicides which also are used in crop protection, studies on the effects on soil microbial function (carbon mineralization and nitrification) are available next to studies on the effects on sewage sludge, and for more persistent molecules also the effects on the Soil Litter Degradation under field conditions. (Dehydrogenase activity – not a standard test for pesticides and biocides – was not considered as a suitable endpoint to analyse effects on fungi.) These studies can also provide some insight into potential issues with aquatic microbial systems. Fate data in soil and water-sediment systems (which concentrate on microbial breakdown processes) will also provide some information about the possible microbial breakdown rate: if breakdown occurs active microbial populations must be present, although the study results do not provide insight into whether the degradation is caused by fungal or bacterial activities. However, biodegradation studies do not investigate whether degradation is hampered by toxic effects on micro organisms. In general it was concluded that the microbial function is unlikely to be impeded if these studies show no effects on microbial function and degradation is observed, although the relative importance of fungal and microbial degradation remains open. It was considered rather unlikely that such effects would occur at much lower levels of contamination in aquatic systems as compared to residues of pesticides in agricultural soils. For biocides that are not used in crop protection, the situation may be different.

Risks not identified by the standard first tier data package

Studies on soil micro organisms, sewage, degradation in soil under laboratory and field conditions, fate in water-sediment (laboratory), and - for persistent molecules – field studies on the breakdown of organic materials in soil give some reassurance about the potential effects on functional endpoints. In addition, the data from the efficacy dossier might indicate which fungal taxa might be expected to be especially sensitive for the product. Although the workshop participants felt reassured about functional effects as being addressed appropriately, structural endpoints of micro organism populations are not covered which may lead to a more resilient micro organism community.
Some workshop participants identified data gaps in the ecotoxicological toxicity profile for the biocide use of both fungicides as presented in this workshop. For example, no data for sediment species and aquatic macrophytes have been provided. Since the data for both molecules had been developed following Directive 91/414 for pesticide registration, some data which might be useful for the biocide registration because of differences in the exposure pattern are not available. This, however, will be considered by the risk assessment factor which is selected for the final risk assessment as a biocide on the basis of the available data base according to 98/8. The appropriate assessment factor is also considered to cover uncertainties due to differences in species sensitivity.
No information is provided about effects on aquatic organisms like bacteria and especially fungi which might be sensitive due to the mode of action of the molecules. However, no specific major risks or need for additional data due to the fungicidal mode of action was

identified by all working groups. Nevertheless, it remains partly unclear how important these organisms are to the aquatic ecosystem. Several fundamental questions had been discussed, as e.g. the natural variability, the potential for recovery and the redundancy of ecosystems.
A need to differentiate between lakes and streams was discussed in detail. There is a lot of information available for lakes and the importance of reed areas, of species, processes, nutritional requirements, growth rates and recovery to natural pressures. The fungi present above the water on the reeds are important for the breakdown of the foliage when the plant dies at the end of the season. However, the persistence of a fungicide and its residues in plant material at the end of the season is to be considered. In addition, the level from spray drift will be much reduced on the reeds as compared to agricultural fields where potential effects on the breakdown of agricultural plants have to be considered anyway.

Further test methods if risks cannot be solved by first tier data

Next to standard tier I studies, special ecotoxicological results might be available (e.g. further chronic studies on standard species, marine organisms, molluscs, worms, bioaccumulation), which however are not considered as important to address concerns on effects of fungicides to micro organisms.
However, studies which report the effects on pH and oxygen levels of the pesticide/biocide in aquatic systems can provide indications on disturbances of microbial functions. Although there are no standard methods it seems also possible to adapt methods for assessing decomposition in aquatic ecosystems e.g. Populus leaves in mesocosm studies - like the terrestrial Soil Litter Degradation (leaf litter bag) study. Litter degradation is a critical measure to decide whether the functionality of the ecosystem is affected and this is an accepted approach for the terrestrial environment.
The main question, however, is whether a functional endpoint as degradation can be considered as sufficient or a possibly more sensitive structural endpoint is necessary to address potential concerns. Studies on the effects on the fungicide community structure are hardly available and affordable. The concern for fungi is really focused on functional endpoints as the breakdown of leaf litter. If the evidence to data demonstrates that function is not affected, no unacceptable effects are considered to be expected, although some uncertainty remains on the robustness of the function and the extrapolation from terrestrial to aquatic systems.

Relevance of the “mode of action” for the ecotoxicological risk assessment

The working groups agreed that the mode of action alone should not trigger further higher tier studies in addition to those studies mentioned above, but might be relevant in combination with the problems not solved so far to trigger higher tier testing.

Are the risks sufficiently addressed for both fungicides by the higher tier data?

The ecotoxicological data base for both fungicides had been mainly evaluated for the pesticide registration. The relevance of the data base for a biocide registration is therefore partly limited mainly because of major differences in exposure patterns.
Potential effects of the pesticidal use of fungicide A on fungal populations are considered as being covered by the field mesocosm study, which also includes effects on the aquatic community (fish, invertebrates, phytoplankton and macrophytes). For a biocide use the acute HC_5 was considered as most relevant endpoint being more than 20 times higher than the maximum PECs (chronic) and thus significantly above a suggested trigger of 1-5. However, this conclusion is based on fish data only and thus not really according to the Technical Guidance Document.
Daphnia are amongst the most sensitive organisms for fungicide B. The higher tier data do not address concerns about fungal communities. The SSD – based on vertebrates and invertebrates separated or combined – are considered as relevant for the pesticide registration of this fungicide. Contrary, a biocide registration does not seem possible for this molecule since only acute data are provided: applying an acute-chronic ratio of 100 to the HC_5 of 17 µg/L for the whole data set or 26 µg/L for the invertebrates only, the PEC/PNEC

ratio is still higher than 1. Some uncertainty has also been identified about the sensitivity of non-arthropod invertebrates since only one mollusc species was tested.

Identification of potential risks by higher tier risk assessments

No indication was identified from the standard higher tier risk assessment methods of both fungicides for a risk to whole ecosystems although microbial endpoints were not studied specifically. Some uncertainty however remains about the role of fungi and bacteria and potential effects of fungicides on these organisms.

Special needs when designing higher tier risk assessment methods for fungicides

Molecule A was a multi-site fungicide therefore the approach taken with a full range of species was correct. However, for a fungicide with a specific mode of action mesocosm studies should include at least those species which are thought to be affected. The mesocosm studies simulated the rapid degradation of the a.s. and may therefore not sufficiently cover chronic risk of the biocide exposure. However, it provides information on which groups of organisms might be particularly sensitive and thus whether the data available are adequate to address the risk. For biocides studies under more constant chronic exposure conditions seem usually to be more appropriate. There is no indication in this case that another taxonomic invertebrate group was substantially more sensitive than aquatic the standard test species (e.g. daphnia). However, algae were affected in the mesocosm at significantly lower levels than expected from standard tests.
For fungicide B a mesocosm study was recommended and perhaps decomposition leaf litter bags might be included with particular attention to the detritivores in the study. As an alternative a Gammarus population study to look at leaf litter bags was proposed. The pesticide risk assessment should also consider the mode of action carefully, which in this case is clearly a chronic concern to fish and invertebrates. For a biocide registration, several options were discussed, including testing a broad range of non-arthropods, SSD based on NOECs, more detailed analysis of destruents in micro-/mesocosms (as e.g. bacterial / fungal biomasses, detritivorous abundance and function), microbial bioassays with sediment taken from the mesocosms).
Higher tier studies as presented for e.g. fungicide A should in general consider the specific mode of action. The information on species sensitivities should be considered to decide whether studies addressing a complex aquatic community (e.g. in a mesocosm) are suitable or whether to concentrate just on the most sensitive group (e.g. SSD). But these statements are more general and not specific for a fungicidal mode of action only.

3.3 Role of microbial communities in aquatic ecosystems

3.3.1 Streams

(Felix Bärlocher)

Nitrification and denitrification

Traditionally, the main role of microorganisms has been considered to be mineralization, i.e., the release of inorganic nutrients from dead organic matter. In soils, this is referred to as nutrient cycling. In streams, released ions are typically displaced downstream before they are recaptured by plants, algae or microorganisms. Stream ecologists therefore talk about “nutrient spiralling” (Allan 1995). Nitrification or ‘ammonium oxidation’ is a two-step respiratory process occurring in sediment (benthic nitrification) or the water column (pelagic nitrification). This process is dominated by oxygen-consuming, lithotrophic bacteria. Various groups of heterotrophic bacteria can also carry out nitrification, though typically at lower rates. In vitro, nitrification by fungi has been observed, but it seems doubtful that they make a major contribution in soils or relatively clean streams (Wainwright 1992). However, their contribution in wastewater might be considerable (Guest & Smith 2002). Fungi complete nitrification in a single step. Ammonia can also be catabolized anaerobically by Brocadia and related organisms in a process called anammox (conversion of ammonia to dinitrogen gas; Madigan & Martinko 2006). The final product of nitrification is nitrate (or dinitrogen gases in

anammox), which rapidly leaches out of soil. In anaerobic conditions, it is used by nitrifying bacteria instead of oxygen; the endproduct is dinitrogen gas. Some fungi can carry out denitrification (e.g., Uchimura et al. 2002), but signficant fungal contribution to the process in nature is rare. Nitrification and denitrification represent a sink and result in a net loss of nitrogen to the system. Ammonia is released during decomposition of organic nitrogen compounds. Typically, it is rapidly and preferentially taken up by plants and microbes and incorporated back to amino acids. During the decomposition of plant detritus in streams, nitrogen (and phosphorus) are often limiting factors, and the effect of pesticides and biocides on these processes may be more relevant than their effects on nitrification/denitrification (Allan 1995).

Importance of vascular plant remains in streams

Before human intervention, the banks of most streams and rivers were densely covered with terrestrial vegetation (Hynes 1971). Small streams and the littoral zones of larger rivers were effectively shielded from direct sunlight by riparian vegeation. Running waters therefore made a poor habitat for autotrophic organisms. On the other hand, the adjacent terrestrial vegetation supplied the water with substantial amounts of dead organic material, especially in the form of leaves, needles, branches and twigs. These conditions favoured a stream community that largely depends on allochthonous (imported) organic material for its food supply. This was pointed out as early as 1912 by Thienemann: springs in beech forests ("Buchenlaubquellen") lacked plant growth but nevertheless had a characteristic fauna, existing on decaying beech leaves which accumulated in great masses in the spring. Egglishaw (1964) demonstrated a positive correlation between the distribution of detritus, mostly of terrestrial origin, and the occurrence of many species of invertebrates, obviously attracted by the available food substances. Gut analyses of a large number of animals by several authors have shown that allochthonous material, especially leaves, serve as food for many members of almost all important groups of aquatic organisms (Hynes 1971, Allan 1995), in many cases providing the bulk of their diet. The limited importance of aquatic plants in the food chain of streams also becomes obvious when the ratio between gross primary production and community metabolism is determined. It is almost always less than 1, indicating that streams are essentially heterotrophic (reviews in Hynes 1971, Webster & Benfield 1986, Allan 1995). Several studies in the late 60s and early 70s estimated the contribution by allochthonous organic material to the total energy available to stream organisms to be between 50 – 99 %.
These early studies concentrated on relatively pristine, low-order streams, where the impact of terrestrial plant debris is likely to be high. A more differentiated view was introduced with the River Continuum Concept (RCC; Vannote et al. 1980). The RCC predicts that the relative contribution of imported plant materials will decline as we move downstream and the stream order increases – this allows more light to reach the water, and autochthonous production increases. The ecosystem may remain predominantly heterotrophic, however, when water turbidity limits photosynthesis, or, when dissolved organic compounds (natural compounds, introduced via groundwater, or, runoff from sewage or industries) make a major contribution. Other important exceptions may include streams above the tree limit, in deserts, or streams where the natural vegetation has been removed from the banks. One important fact to keep in mind is that no stream section can be considered in isolation. Both import from upstream and export to downstream reaches have to be included for realistic budgets.
A comprehensive summary of organic matter budgets in 27 streams was published by Webser & Meyer (1997). There was a clear North American, temperate zone bias to the studies. By and large, they confirmed predictions by the RCC. In an essentially undisturbed stream, Hubbard Brook, production in the stream, primarily by epiphytes, contributed 0.5 % to the total energy budget, and litter over 85 %. The remaining 13.7 % derived from dissolved organic matter (DOM) introduced via the groundwater. Similarly, in three subarctic streams, allochthonous material (leaves, conifer needles, wood) accounted for 95 % of total energy input. In a stream running through forest mixed with open grasslands, autochthonous production was greater (31 %) than imports from terrestrial plants (27 %), but both were exceeded by DOM (42 %) introduced via groundwater. In the cold desert region of southeastern Washington, primary production in Rattlesnake Springs contributed 96 % and litterfall the remaining 4 %; similar values were found in an Arizona desert stream (98 % autochthonous, 2 % imported). Overall, primary production in the streams varied by over 4 orders of magnitude, ranging from 3.5 – 5400 g m-2. It was highest in arid regions and

showed no relationship with latitude. By comparison, litter input from terrestrial vegetation varied between 0 (open stream in the Canadian Antarctic) to 843 g m-2 in a mixed deciduous forest in Georgia. In the available data set, there was no correlation between litter input and stream order, indicating that local conditions such as steepness of slopes are more important. However, litterfall increased with precipitation and decreased with latitude.
Attached algae, bryophytes and submerged or emergent vascular plants contribute to primary production in streams, while true planktonic organisms are absent except in very large rivers or in quiet bays and side arms of smaller rivers and streams (Hynes 1971, Allan 1995).

Decomposition of vascular plant remains

Only a small fraction of the energy contained in leaves, needles or wood can be directly exploited by animals. Usually more than 60 % and often as much as 80-90 % of ingested leaf litter is returned in the forn of faeces. Baier (1935) assumed that in lakes most detritus-feeders are nourished by detritus-decomposing bacteria rather than by the actual detritus they appear to be feeding on. This suggests that a crucial function of bacteria and fungi is the synthesis of microbial proteins and lipids, which will be eaten and digested by invertebrates. Kaushik & Hynes (1971) were the first to study leaf decomposition in streams. Their key findings, which have since been confirmed by many others, are:

- fungi are more important than bacteria in controlling mass loss in leaves (shown by comparing the effects of antifungal and antibacterial antibiotics).
- during the early stages of decay, nitrogen and protein levels of decaying leaves often increase. Again, this only happens when fungi are present and active.
- leaf-shredding invertebrates prefer leaves colonized by fungi (conditioned leaves).

Kaushik & Hynes (1971) suggested that the leaf is essentially a substrate, and invertebrates derive most of their nutrition from the associated fungal biomass. Various feeding experiments have shown that several invertebrates (amphipods, isopods, caddisfly and stonefly larvae) can differentiate among different fungal species, even if these grow on the same leaf or leaf disk (reviews in Bärlocher 1987, Suberkropp 1992). Their survival and growth rates are higher on conditioned than on freshly fallen leaves. Mycelia of pure fungal cultures as sole sources of food can vary from being toxic to highly nutritious.
In early studies, fungal biomass was estimated by direct counts of hyphal lengths in bleached and stained leaves. This was replaced by extracting and measuring ATP from decaying leaves. ATP is restricted to living cells, but occurs in both fungal and bacterial cells. Today, the indicator molecule of choice is ergosterol, which is essentially restricted to membranes of true fungi (Gessner et al. 2003). Since its ratio to total fungal biomass is reasonably constant, measuring ergosterol allows estimates of fungal biomass. Based on a large number of studies, the maximum fungal biomass in decaying leaves can be as high as 18 % of total detrital mass. Ergosterol levels, and therefore fungal biomass, are considerably lower on fine organic particles (e.g., faeces, sloughed-off periphytes, etc.). Incorporation of C14-acetate into ergosterol has been used to estimate fungal production (Suberkropp 1997). By combining this with measuring thymidine and leucine incorporation for bacterial production, studies have consistently shown that fungi outperform bacteria by a factor of at least 10:1 on coarse particulate organic matter such as leaves (e.g., Pascoal & Cassio 2004). On an annual basis and taking into account all compartments of forested stream ecosystems, fungal, bacterial and invertebrate productions fall within similar orders of magnitude (Suberkropp 1997).
The most striking fungi on decaying leaves are aquatic hyphomycetes, a heterogeneous group of fungi which typically disperse by tetraradiate or sigmoid spores (Bärlocher 1992a-c). Phylogenetically, most of them belong to Ascomycetes and a few to the Basidiomycetes (Belliveau & Bärlocher 2005).
Traditional methods (microscopic observations, isolation of pure cultures) suggest that aquatic hyphomycetes dominate leaf decay, however, molecular techniques such as PCR with selective primers, followed by T-RFLP or DGGE, suggest the presence of other groups, such as Chytrids, Oomycota and Zygomycota (Nikolcheva & Bärlocher 2004).
Little is known about the role of aquatic hyphomycetes or other fungi in streams with reduced inputs of leaf and other terrestrial plant litter. However, aquatic hyphomycetes have been reported from bryophytes (Sridhar et al. 2000) and from a number of submerged macrophytes such as *Ranunculus penicillatus* and *Nasturtium officinale* (Kirby et al. 1990). Maximum fungal biomass on these substrates was estimated to be 1.5 %. It is unlikely

(though untested) that fungi play a major role in streams where energy flow is dominated by periphytic algae. Heavy metal contamination tends to depress fungal diversity, but at low levels appears to have little effect on fungal biomass and production (Pascoal & Cassio 2004). Eutrophication (N, P) may initially increase both fungal diversity and production. At intermediate level, diversity tends to decline. Most laboratory experiments have found a small diversity effect in aquatic hyphomycetes (i.e., increased diversity tends to increase leaf decay and fungal production; Bärlocher & Corkum 2003, Treton et al. 2004, Duarte et al. 2005, Dang et al. 2005). Field observations have not revealed significant correlations between fungal diversity and function (Bärlocher & Graça 2002).

3.3.2 Lakes

(Roger Pickup)

The aim of this document is to briefly describe the bacterial activity in lakes in relation to their physical and chemical cycles and to reinforce the presentation made at the ESF workshop that molecular analyses have limitations for testing pesticide activities.

Limitations to this report:

Lakes world wide comprise a range of productivity from low nutrient (oligotrophic) lakes through mesotrophic to eutrophic waters, this range being exceeded by the hypereutrophic water bodies such as Priest pot, UK. They also vary in volume and depth, underlying and surrounding geology and local geographic conditions. Although biogeochemical processes are well characterised, their intimate nature (the chemical drivers e.g. electron acceptors/substrates) is defined by local conditions. Therefore is difficult to produce a generic assessment of lakes per se. However some generalities are possible and these are discussed, particularly with eutrophic temperate lakes that do not experience climatic extremes.

Lake cycles (from Wetzel, 2001)

It is important to note that, although intimately linked, the lakes comprise two compartments namely the sediments and overlying water. The depth of overlying water defines the sediment as benthic (deep) or littoral (shallow). Conditions in the two compartments change with the seasons as the main drivers for activity (oxygen and temperature) undergo predictable changes. Under winter conditions lakes are isothermal and well mixed with oxygen present throughout. The sediments surface will be exposed to oxygen and anaerobic conditions will occur at about 1-5 cm depending on depth of overlying water and the type of sediment. The water body undergoes thermal stratification as ambient temperatures rise resulting in oxygen and thermal gradients appearing which maximise the stratification of the system. A stratified lake comprises overlying water (the epilimnion), a mixed more turbulent layer of water which sits over the bulk of deoxygenated cold water of the hypolimnion. The two compartments do not mix and are separated by the metalimnion which is a region of thermal discontinuity and contains both the thermocline and oxycline. These gradients both show a steep decline. The hypolimnion changes little during summer stratification. The sediments under stratified conditions become anoxic until the breakdown of stratification in autumn/winter conditions (decreasing temperature/high winds) when the lake mixes and becomes isothermal. Oxygen becomes well distributed and the sediment receives oxygen and the surfaces become aerobic. Scale is very important: Windermere England's largest lake (depth 60m) has a thermo/oxycline of approximately 7m where as Priest Pot (depth 3.5m) is occurs over 20-30cm and is very tightly defined.

Activities in lakes: bacterioplankton

Most decomposition of particulate and dissolved organic matter in lakes occurs by means of planktonic bacteria in aerobic pelagic waters prior to sedimentation of detritus. The rates of degradation are determined by many conditions including quality of substrates and physical and chemical parameters. The numbers, the biomass and the productivity of bacteria increases with increasing photosynthetic productivity of freshwater, so biomass of bacteria is closely correlated with phytoplankton.In thermally stratified lakes, bacterial

biomass/productivity is highest in the epilimnion, decreases to a minimum at the metalimnion/upper hypolimnion interface and increases in the lower hypolimnion. Seasonal and vertical distribution of bacteria may change rapidly. Bacteria are predominantly free-living, however where suspended particles are numerous attached bacteria will predominate. Bacterial numbers are controlled by a number of factors:

- Growth is positively correlated with temperature particularly around 10-15oC above that other factors lay a more significant role.
- Restricted nutrients (particularly phosphate) limit growth.

Bacterial decomposition of organic matter is governed by the complexity of the substrate (simple compounds are mineralised faster than the more complex soluble organic compounds). Bacterial growth is generally balanced by mortality and other losses, but both the physiological bacterial death as the entry into dormancy under physiological stress are poorly understood. Viral parasitism may account for 25% bacterial mortality and upto 50% bacterial mortality results from predation by protests. Decomposition of particulate organic detritus initially conforms to first order kinetics during initial stages but the decline as recalcitrant compounds accumulate. Measurements of productivity of bacterial communities are few but productivity is generally less than annual production of phytoplankton and relies heavily on organic carbon from allochtohonous and littoral sources. Bacterial productivity increases in deeper lakes where retention times for particulate degradation are greater and allochtohonous and littoral sources have less influence. Chemosynthesis in bacteria (CO_2 utilisation in the presence of dissolved organics substrates) is significant only in areas of steep redox gradients (metalimnion).

Sediments (From Jones , 1985)

Microbial populations and metabolic activity in surface sediments are orders of magnitude greater than the overlying water. Bacterial activity rapidly produces anaoxic conditions. Buried sediments are generally characterized by the absence of oxygen and so microbial processes will be dominated by the activity of anaerobic bacteria. Carbon turnover in the presence of electron acceptors therefore involves interaction of the carbon, nitrogen and sulphur cycles. In the absence of electron acceptors other methods of energy conservation become significant. Interspecies H2-transfer permits the use of otherwise energetically unfavourable reactions. Conservation of the energy in polyphosphate bonds and reduction of Fe(III) and Mn(IV) to produce more energetically favourable end-products are other mechanisms available to benthic bacteria. The reduction of CO_2 to acetate may require more serious consideration as a hydrogen sink in certain sediments. The list of substrates known to be susceptible to attack by anaerobic bacteria has grown rapidly in recent years and estimates are now available for the turnover of refractory components such as lignin. Finally, bacteria are considered as producers of biomass, particularly of specific cell components that may be used as biomarkers to identify zones of activity.

The microbial population and the processes mediated by microbes in aquatic sediments, as in the water body, are subject to several controlling factors. The decomposition and modification of organic matter is affected by:

- the nature and source of the organic matter, for example, whether it is freshly sedimented algal material or much processed soil organic matter transported from the catchment,
- temperature,
- the presence of electron acceptors; general thermodynamic considerations suggest that potential electron acceptors might be used in the order 02; Mn(IV) ; nitrate ; Fe(III) ; sulphate; carbonate,
- bioturbation (sediment mixing by animals) and finally
- the presence of particular decomposer microbes in the sediment.

It is important to note that the presence of oxygen does not exclude the organisms that predominate in redox gradients (e.g. sulphur reducing bacteria exhibit high oxygen tolerance and can exhibit activity in anaerobic microniches of aerobic sediments. Similarly nitrate reduction may occur on large particles in shallow oxygenated lake sediments. Decomposition can also occur in the absence of electron acceptors by non-redox dependent fermentors and proton reducing bacteria usually in syntrophic associations with hydrogen consuming bacteria. Catabolism of low molecular weight compounds may yield acetate and hydrogen with the hydrogen being scavenged and consumed by sulphate reducing bacteria

and methanogens. Some of alternative strategies include sulphate reducing bacteria that conserve energy from pyrophosphate. Microbes act as decomposers but also contribute to sediment matter (act as producers) as they contain components within their biomass that are effective biomarkers thus identifying zones where particular organisms are active.

Example of seasonal lake activity: Priest Pot, Cumbria UK (from Finlay and Maberley, 2000).

The one hectare pond, priest pot, gives one of the best examples of the range of microbially mediated processes that occur in succession driven by seasonal drivers such as light penetration and temperature. As it is located at a latitude where physical factors such as incoming radiation and air temperature vary markedly over a year. This, in combination with its small volume, high rates of biological activity, and present-day shelter from wind, produces a system where ecological conditions vary markedly with season and also with depth when the pond stratifies. This range of ecological conditions results from reciprocal interactions among the biota and their environment: altered conditions produce new niches that can be exploited by new species and these, in turn, alter the environment to produce different niches. Because of the dominating importance of micro-organisms, with generation times of hours or days, changes in conditions and the development of different microbial communities can occur very rapidly. Similarly, the strong stratification that develops in summer, coupled to the high rates of biological activity, produce steep depth-gradients in chemical and physical conditions and so create a large number of potential niches with depth.

During winter, the water column is unstratified and aerated, with few if any depth gradients, apart from light. Consequently, the number of niches within the water column is low. The low biological demand for resources, caused by low temperature and light, results in high concentrations of nutrients. In spring, increasing daylength, surface insolation and water temperature, cause an increase in the rate of biological activity. In the sediment, increased rates of respiration eventually raise the rate of demand for oxygen above the rate of supply, particularly as the incipient stratification reduces rates of water movement at depth and restricts the transfer of oxygen from the atmosphere to the sediment. As these processes intensify, the sediment surface eventually becomes anoxic, with profound consequences for the biology of the pond. Aerobic microbes are forced to migrate out of the sediment and the altered redox conditions release phosphorus that was previously bound to ferric ions. This phosphorus, along with ammonium and carbon dioxide produced by respiration within the sediment, is released into the water column.

As the process of stratification progresses and oxygen becomes depleted in the bottom water, the pond develops into three interlinked layers: an oxygenated epilimnion, a micro-aerobic mid-lake region around the oxycline, and an anoxic hypolimnion. In the epilimnion, increasing temperature and availability of light, further promoted by the development of stratification, allows phytoplankton populations to develop in the high nutrient conditions. Continued algal growth and microbial transformation leads to nutrient depletion in the surface waters. Conversely, at depth, decomposition leads to nutrient regeneration. Consequently, steep concentration gradients exist within the water column, producing high flux rates of nutrients from depth. These upward fluxes of nutrients intersect with downward fluxes of oxygen and light. One consequence of this is the development in the metalimnion of a diverse and dense community of prokaryotes, algae, protozoa and other microfauna (especially rotifers and gastrotrichs). Meanwhile, in the anoxic hypolimnion, a transient niche opens for nitrate reducers as oxygen is no longer available as an electron acceptor, but these soon exhaust the nitrate and are replaced by sulphate-reducing bacteria. At the same time, fermenting bacteria in the sediment continue to degrade organic matter, releasing nutrients and carbon dioxide. Syntrophic consortia become active, especially those incorporating H_2 evolving and H_2-consuming organisms (e.g. anaerobic protozoa and methanogenic bacteria respectively). With continuing activity of the sulphate reducers in deep water and in the sediment, sulphide increases in concentration. This, in conjunction with the low levels of mainly long-wavelength light that penetrates the water column and the layer of algae at the metalimnion, opens a niche for photosynthetic anaerobic bacteria that use sulphide as an electron-donor. These typically form a thick, slow-growing layer about 30 cm above the sediment where they consume nutrients including ammonium that would otherwise pass to the overlying metalimnetic community.

By the beginning of September, decreasing solar radiation and air temperature leads to cooling of the surface water and a degradation of stratification, which allows oxygen to penetrate deeper in the water column. The renewed co-occurence of oxygen, carbon dioxide and ammonium throughout the water column promotes an intense burst of chemotrophic activity, causing a rapid increase in the concentration of nitrate and a reduction in dissolved oxygen, usually to about 50% of the air-saturation value. The return of inorganic nitrogen to the surface water may cause a temporary increase in algal biomass before stratification breaks down completely and surface light and water temperature decrease to winter values.

The changes in species composition with space and time can occur with large amplitude and high frequency depending on the interactions among species and their environment, and the impact of environmental perturbation caused by prolonged dry weather, or sudden heavy rainfall. It is the reciprocal interaction between this spatial and temporal variation and the biology of the pond that leads to the potential for high microbial diversity.

Microbial community: constraints and limitations

The processes by which biogeochemical cycling in lake water and sediments occurs are clearly definable as are some of the types of bacteria that carry out these processes. Less clear are the identities of the actual 'players', however, evidence is available that the same or similar organisms carry out defined processes in freshwater, marine and soil environments. Molecular techniques allow us to attempt to dissect microbial communities and describe components not revealed by culture (Head et al. 1998). Evidence from culture and molecular techniques show, for example, that ammonia oxidation is carried out by *Nitrosospira* spp. which are present both in freshwater and soil, however, the significance of their contribution to ammonia oxidation remains unresolved (Stephen et al., 1998; Hiorns et al., 1995). Purkhold et al. (2000) examined the phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoA sequence analysis. They showed that that within the limits of their analyses their target site did not contain any sequences that unequivocally suggested the existence of previously unrecognized species in the wastewater treatment environments examined. Therefore within this particular process, common species are responsible for this activity in a number of environments (Head et al., 1998). Similarly, common genera/species of sulphate reducing bacteria, such as *Desulfobulbus* sp., appear in freshwater sediments, freshwater, soil and marine environments. Regularly new, previously undescribed functional bacterial are described at the sequence level (Earl et al., 2005). However, Curtis et al (2002 and 2004) noted that the numbers of acquisitions of new sequence data is reducing as the small number of more abundant taxa obscure the larger number of moderately rare or very rare species. Therefore numerically dominant species may not be the ecologically significant species that drive that processes in specific environments, and these remain undescribed due to the limitation imposed by sampling and subsequent analyses. At present we cannot describe the entire bacterial community or sometimes the significant players in any particular environment; therefore, testing sensitivities of processes at a microbial community level will be difficult. We may receive a sense of false security by using type cultures to test sensitivities, for example, *Nitrosomonas europaea* has long been recognised as the type strain for ammonia oxidation (Head et al., 1998). Subsequent evidence has shown it to be culture-dominant and but not ecologically significant and therefore not truly representative of the ecologically significant ammonia oxidisers (Head et al., 1998). Its behaviour is not necessarily representative of that of the community. At present this would lead to the assumption that testing process sensitivity is better at the process level (where measurement can be made) rather than at the microbial community level where 'measurement' (e.g. changes in community structured) is more difficult to achieve without extensive statistical mediation (Hong et al., 2006).

3.3.3 Lentic macrophyte-dominated freshwater ecosystems

(Theo C.M. Brock)

Introduction

Micro-organisms can be found in high numbers in all environmental compartments (water, sediment, biota) of wetlands and shallow, lentic freshwater ecosystems. Planktonic bacteria are suspended freely in the water as single cells or small colonies, and commonly are associated with dead organic particles or other organisms. Compared to water a thousand fold concentration of micro-organisms can be found in the upper layers of the sediments where organic matter accumulates. In macrophyte-dominated systems, the plant surfaces (living or dead) may become colonized by dense communities of algae and micro-organisms. In all these habitats bacteria and fungi play an essential role in the mineralization of autochtonous and allochtonous organic matter, including the degradation of organic pollutants (e.g. pesticides), but also as symbionts and pathogens.

In freshwater ecosystems, pesticide and biocide exposure at realistic concentrations are reported to induce shifts in the dominance of certain groups of microbes in microbial communities (e.g. Widenfalk et al. 2004; DeLorenzo et al. 2001; Van Beelen and Doelman, 1997). These toxicant-induced structural changes, however, are not necessarily accompanied by functional changes, because of functional redundancy among populations of micro-organisms. In addition, rapid development of tolerance and adaptation of micro-organisms to pesticides and biocides may play a role. In recent years, Pollution-Induced Community Tolerance (PICT) has received significant attention, particularly when applied to communities of algae and micro-organisms in the periphyton and plankton. Overall these organisms have short generation times that offer opportunuties for rapid adaptations. The temporal resolution of disappearance of PICT is reported to depend on the succession rate of the community after the exposure to the contaminant has disappeared. This succession rate may be high in microbial communities. To date, mainly metals, pesticides and biocides have been evaluated in PICT studies (Blanck, 2002)

Bacteria and fungi as symbionts and pathogens

In freshwater ecosystems, bacteria and fungi may be important symbionts or pathogens, in this way influencing the physiological performance and wax and wane of populations of other freshwater organisms. For example, symbiosis of methanogenic bacteria and sapropelic protozoa is reported to play an important role in degradation processes in anoxic environments (Van Bruggen et al. 1983; Schweikert and Meijer, 2001). In addition, symbiotic bacteria in digestive tracks of aquatic (in)vertebrates facilitate the utilization of digested food. Bacteria even exist as symbionts inside mycorrhizal fungi (Bianciotto and Bonfante, 2002). Vesicular-abuscular mycorrhizal fungi that infect the roots of aquatic vascular plants may be beneficial for these plants in that they increase the uptake of essential nutrients. The occurrence of these mycorrhizal fungi are reported in particular on and in the roots of emergent shoreline plants that grow in habitats characterized by fluctuating water, nutrient and oxygen conditions (Cooke and Lefor, 1998). Also certain soft-water macrophytes that grow submerged in sediments with a relatively high redox potential (e.g. *Litorella uniflora*) may be infected by mycorrhizal fungi. In contrast, submerged macrophytes that root in sediments with a low redox potential (< 250 mV) usually are not infected by mycorrhizal fungi (Beck-Nielsen and Vindbaek Madsen, 2001). These data suggest that symbiotic relationships between aquatic vascular plants and mycorrizal fungi in particular may be important for emergent macrophytes. To date, no information could be found in the scientific literature that specifically addressed the impact of fungicides and biocides on symbiotic micro-organisms in freshwater habitats.

Infection of algae by chytrid fungi (e.g. *Zygorhizium planktonicum*) is fairly common in freshwater ecosystems, and selective fungal parasitism on certain algal species will favour the development of other algae and, in this way, can be one of the factors influencing seasonal succession (Van Donk, 1983). Pathogenic fungi are also reported for aquatic vascular plants and several of them are host-specific. Pathogenic bacteria and fungi may even be used in the biological control of invasive aquatic weeds (e.g. Shabana and Charudattan, 1996) and arthropods. The observation that the biomass of the submerged macrophyte *Elodea nuttallii* increased in freshwater microcosms treated with the fungicide carbendazim relative to control microcosms might be explained by the suppression of phytopathogens by this fungicide (Van den Brink et al. (2000).

Bacteria and fungi as saprotrophs

As stated already a very important ecological function of micro-organisms is the fundamental role they play in the biogeochemical cycling of organic matter and nutrients. Specific processes (e.g. nitrification and methane oxidation) are carried out by a limited portion of the microbial community, while other more general processes (e.g. the mineralization of organic matter) may be carried out by a wide range of micro-organisms (Griffiths et al. 2000). It is evident that the consequences of inhibition of specific processes by fungicides and biocides may be greated than that of general processes, the latter because of functional redundancy.

Although in all freshwater habitats the degradation of organic matter for a large part is performed by bacteria and fungi, their relative contribution to these processes may differ between lotic and lentic systems and between shallow macrophyte-dominated systems (e.g. ditches, ponds) and deeper lakes. This report will focus on the role of bacteria and fungi in the decomposition of aquatic vascular plant material in shallow, lentic freshwater habitats. In the agricultural landscape, relatively small and shallow freshwater ecosystems, whether natural (e.g. oxbow lakes) or man-made (e.g. drainage ditches), commonly occur. These systems are at least potentially dominated by aquatic vascular plants, and may also be subject to contamination by fungicides and biocides. Also in the littoral zones of deeper lakes emergent and submerged macrophytes may form the bulk of the biomass and annual organic matter production. Emergent aquatic plant communities are among the most productive, per unit area, of all the worlds vegetation types, while vegetation dominated by floating-leaved and submerged macrophytes is reported to be more productive than plankton (Westlake, 1982; Brock, 1985). In addition, the physical framework and specific physico-chemical conditions prevalent in macrophyte beds give rise to suitable circumstances for many other organisms, so that the biodiversity of these habitats is potentially high (Den Hartog and Van der Velde, 1988).

The fate of organic matter produced by aquatic macrophytes

The flow of organic matter from aquatic vascular plants to other trophic levels takes place by secretion, by direct herbivore grazing and by decomposition of senescent and dead plant parts. Generally, the organic matter losses from aquatic macrophytes due to secretion constitute only a few per cent of the total organic matter production, although higher percentages (up to 20%) are reported (e.g. Søndergaard, 1981). The dissolved organic matter released by intact macrophytes may be directly utilized by bacteria and algae occurring in the periphyton and plankton and by micro-organisms in the rhizosphere (Allen, 1971). Subsequently, these micro-organisms may enter the grazer food-chain (e.g. snails that graze periphyton and cladocerans that graze bacterioplankton).

Herbivores and phytopathogenic micro-organisms colonize aquatic macrophytes during their development. This can inflict considerable damage, either on the growing or senescent tissues (Kok et al. 1990). Consequently, production and decomposition of aquatic vascular plants (particularly that of floating-leaved and submerged macrophytes) may be a continuous process. Most herbivores of aquatic vascular plants are terrestrial or semi-terrestrial organisms. Relatively few animals which complete their whole life-cycle in the water are able to consume the living tissues of these plants (Gaevskaya, 1966). Overall, only a small part of the total annual organic matter production by aquatic vascular plants may enter the grazer food-chain. For example, herbivorous animals are reported to be responsible for the disappearance of ca. 22% of the leaves produced annually by the floating-leaved macrophyte *Nymphoides peltata* (Van der Velde et al. 1982). This percentage is, however, the combined effect of consumption and damage succeeded by microbial decay. Actual grazing was estimated to take away no more than 5% of its annual production.

The major part of the organic matter produced annually by emergent and submerged macrophytes enters the detritus food-chain. Decomposition of aquatic macrophyte tissues by bacteria and fungi consist of a complex series of interacting processes. Often different stages of the decomposition process can be found on one plant or even one leaf. Before the death of plant material, the plant tissue usually goes through the senescence phase. Senescent material is more attractive for facultative detritivorous macrofauna than vital tissue, due to the loss of tissue structure and the colonization of these tissues by bacteria and fungi (Kok, 1993). Contamination of macrophyte-dominated ecosystems with high concentrations of

herbicides may suddenly cause the dying-off of macrophyte vegetation. In herbicide-stressed freshwater systems an increase in bacteria and detritivorous macro-invertebrates has been reported, due to an increase in dissolved and particulate organic matter (e.g. Murphy and Barrett, 1990).

Decomposition of aquatic macrophyte tissues

The decomposition process of aquatic macrophytes, starting at cell death, can be divided into two phases. The first stage is the debris phase. During the debris phase, the original structure of the plant material is still recognizable. At cell death, the integrity of the cell membrane is lost and small molecules can leach out of the tissue. A consequence of the loss of membrane integrity at cell death is the loss of the inner structure of the cell. Submerged and floating-leaved macrophytes usually have a lower structural fiber content compared to emergent macrophytes and terrestrial plants, as the former generally lack supporting tissues. Webster and Benfield (1986) rank plant types according to their decomposition rates in aquatic ecosystems: submergent and floating-leaved macrophytes > helophytes and terrestrial herbacceas plants > woody plants.

The relative contribution of bacteria and fungi in the decomposition of macrophyte material may be dependent on the chemical quality and fiber content of this material, as well as on the physico-chemical properties of the surroundings. For example in an eutrophic, alkaline environment and during the initial phase of decomposition, the leaves of the floating-leaved macrophyte *Nymphoides peltata* were predominantly processed by bacteria (Brock 1984), while in acidified water the role of fungi in the initial decomposition process of floating leaves of *Nymphaea alba* was relatively large (Kok et al. 1992). Gaur et al. (1992) report that bacteria were the predominant degraders of the tropical floating macrophyte *Eichornia crassipes*, whereas fungi degraded smaller quantities of the litter of this macrophyte characterised by a low structural fiber content in its tissues. Mason (1976) showed that in eutrophic water, bacteria were as important as fungi in both the weight loss and microbial respiration in dead leaves of the emergent macrophyte *Phragmites*. In contrast, Komínková et al. (2000) demonstrated that the microbial biomass associated with both leaf and stem litter in an oligotrophic lake was predominantly fungal, even though bacterial biomass increased and fungal biomass decreased or remained constant as litter decay proceeded. Significant differences in fungal and bacterial biomass were observed between leaf (lower fiber content) and stem (higher fiber content) material, with leaves often having 5 times higher values than corresponding stems. However, according to Hieber and Gessner (2002), bacteria turnover times can be considerably shorter than for fungi, and hence their contribution to decomposition may be larger than implied by their biomass.

Antagonistic interactions between bacteria and fungi may be an important controlling factor for microbial colonization and growth on aquatic plant litter (Mille-Lindblom and Tranvik, 2003). They demonstrated in microcosm experiments that fungal biomass accumulation on *Phragmites* leaves was approximately 12 times higher in the absence than in the presence of bacteria. Bacterial biomass accumulation on decomposing *Phragmites* was about double in te absence of fungi compared to when fungi were present. However, despite the great difference in biomass development between the treatments, the carbon metabolism was similar regardless of whether fungi and/or bacteria were present or in co-existence (Mille-Lindblom and Tranvik, 2003). This again demonstrates the phenomenon of functional redundancy in the microbial community associated with decomposing macrophyte litter.

In freshwater ecosystems the debris (dead plant material and associated micro-organisms in the initial phase of decomposition) is used as a food source by detritivores of the functional groups of shredders and grazers/scrapers. The resource quality of the debris for these detritivores is enhanced by microbial colonization (Bärlocher and Kendrick, 1975). In concert, the microbial colonization of the debris may be stimulated by the activities of detritivores (Harrison, 1989). The action of detritovores and of cell-wall degrading micro-organisms decreases the particle size of the decomposing macrophyte material. Several model ecosystem studies with pesticides demonstrated that the breakdown of macrophyte litter may be slowed down when shredders (e.g. *Gammarus, Asellus*) are eliminated by the pesticide-

treatment (e.g. Cuppen et al. 1995). This again may indirectly impact the activities of the microbial community associated with the plant litter.

The next phase is the decomposition is the detritus phase, in which the original structure of the decomposing macrophyte material is lost. The material becomes increasingly recalcitrant, due to the loss of easily degradable components and the progressing humification (Webster and Benfield, 1986). In this stage the detrital particles are further degraded by specialised aerobic or anaerobic micro-organisms. The decomposition process of aquatic macrophytes as a whole and the activity of associated micro-organisms is strongly influenced by environmental factors such as pH, nutrient content, temperature and oxygen content of the surrounding medium (e.g. Godshalk and Wetzel, 1978; Brock et al. 1985). Under acid and anoxic conditions the inhibition of micro-organisms may be so complete that the decomposition of macrophyte material is more or less stopped, resulting in peat formation.

3.4 Break out group discussions on functional redundancy, sensitivity and trophic interaction

Tuesday's breakout groups focussed on sensitivity, functional redundancy and trophic interactions in microbial communities.

Microorganisms (algae, bacteria and fungi) form the base of aquatic food webs and drive key ecosystem processes such as decomposition, nutrient cycling, contaminant degradation and primary production. Although aquatic risk assessments consider the impact of chemicals on algae, little attention if any, is given to potential impacts on heterotrophic microorganisms. This may be of little concern if heterotrophic microbes are less sensitive than, or of equal sensitivity to, those taxa used in the risk assessment. If they are more sensitive, then the ecological implications of underprotecting heterotrophic microbes will depend on the degree of functional redundancy in microbial communities and on the specificity of trophic interactions.

Understanding the consequences of species loss on ecosystem functioning is a very current and controversial topic in ecology. Hypotheses proposed to explain the relationship between biodiversity and ecosystem function can be placed into one of three groups:

1. Species are primarily singular and hence make unique contributions to ecosystem functioning. This results in a linear biodiversity-function relationship.
2. Species are primarily redundant and hence their ecological role can be performed by other species. This results in a curvilinear biodiversity-function relationship. Remaining species compensate for species loss and therefore there is little impact on function until a threshold level is reached after which species loss results in loss of function.
3. Species impacts are context-dependent and therefore idiosyncratic or unpredictable.

Bacteria and fungi influence energy flow in ecosystems both as a food source and as modifiers of organic material. For instance, fungal colonisation of detritus is essential for its utilization by many detritivores, which exhibit preferences for particular fungal x leaf combinations. A significant proportion of carbon fixed by primary producers may be released as dissolved organic carbon or particulate organic carbon. This carbon is utilized by bacteria and fungi, which are consumed by flagellates and ciliates in what is termed the 'microbial loop'.

To address the topics above three breakout groups were formed around the topics sensitivity, functional redundancy and trophic interactions. All three groups were asked to answers posed by the steering committee. In the three paragraphs below a summary of the ansers of the three groups can be found.

3.4.1 Sensitivity

1. <u>What are the effects of fungicidal biocides and pesticides on microorganisms?</u>

- Microorganisms are a very diverse group and therefore, with the current state of knowledge, it is difficult to make a general statement as to the effects of fungicidal biocides and pesticides on microorganisms.
- Effects are almost inevitable when considering such a large and diverse group. However, the important question is what level of effect will be unacceptable structurally or functionally?
- Microbial communities exhibit an enormous capacity to adapt or recover.

Extrapolating from terrestrial to aquatic systems

- Limited effects on terrestrial microorganisms have been observed in experiments looking at carbon mineralisation and nitrogen transformation on terrestrial systems.
- On-crop effects may be observed in soil communities although recovery is typically rapid. Recovery of microbial functional endpoints in mesocosms also tends to be rapid.
- Can we extrapolate this information to aquatic systems? Probably yes for bacteria communities but probably not for fungal communities. In streams, aquatic hyphomycetes (predominantly with Ascomycete affinities) dominate, and Basidiomycetes, which are common in soils, are relatively rare. In general, Basidiomycetes are better able to degrade lignins, which may give them an inherent advantage when dealing with certain types of fungicidal pesticides and biocides. There is evidence from mesocosm studies that some fungicidal pesticides do not affect aquatic hyphomycete function (i.e. leaf decomposition) at predicted environmental concentrations.
- There may be different taxonomic groups present depending on the prevalence of aerobic/ anaerobic habitats in terrestrial and aquatic systems.
- In extrapolating from terrestrial to aquatic systems we make the assumption that structure and function are related. One of the main functions of microbial communities is nutrient cycling, but there may be significant differences in the structure of the community carrying out this function (as there are with invertebrate communities in freshwater, marine and terrestrial habitats). In addition, microbial production plays a huge role in sustaining soil and aquatic invertebrates.
- Do case studies presented give us confidence to extrapolate to other scenarios? There is a lack of knowledge, but the information we have suggests there are some effects but there is no evidence on major effects on microbial communities compared to other taxonomic groups.
- However, molecular methods cannot currently quantify the abundance and activity of species. Increases in abundance may be measured but the methods are not suited to measuring decrease in community. For example, phospholipid fatty acids (PLFA) analysis can be used as a measure of total microbial biomass, and there are also certain PLFA biomarkers for fungi, making it possible to estimate the fungal biomass in a sample. Fungal biomass can also be estimated by analysing ergosterol, although this may not distinguish between live and dead mycelium as it degrades slowly (Mille-Lindblom et al., 2004). New molecular techniques are being developed using mRNA as an indicator, and this would give a picture of only the active cells in a microbial community.
- Many microorganisms in aquatic systems live in biofilms, which may render them less sensitive to environmental stress than their terrestrial counterparts (Decho 1990).

Duration of effects

- PICT – low concentrations may cause shift in microbial community tolerance. When the stressor is removed recovery is often observed within a short time.
- This is not relevant for continuous exposure (e.g. some biocides)
- However, if recovery is rapid after exposure then how important is the observed effect?

Community adaptation

- In permanent agricultural cultures, and with some biocidal product exposures, microbial populations may adapt quickly to exposure to the fungicide, utilising the molecules as a food source. This results in accelerated decomposition of the fungicide. This process may be predominantly driven by bacterial microbes that can respond very rapidly. For instance, soil bacteria have been observed to increase in numbers after application of the fungicide captan (Martinez-Toledo et al. 1998).
- Aquatic hyphomycetes grow more slowly than most bacteria and most other fungi and may be less likely to be part of the initial opportunistic assemblages.
- There was a discussion about extrapolating from pathogenic fungi (adaptation and recovery/recolonisation) to non-pathogenic fungi. Some of our knowledge of effects on fungi is based on efficacy data. The question was raised whether pathogenic fungi are representative of the sensitivity on non-pathogenic fungi. However, it was noted that there was no known evidence of fungi demonstrating a long term effect.
- Is recolonisation in terrestrial habitat faster than in temporary aquatic systems? Not known.
- Research need: Effects of adaptation on community functioning (and structure?) in aquatic systems

Keystone species/functions

- There do not appear to be keystone fungal species (Bärlocher 1992c). Are there microbial keystone species groups (bottleneck processess?
- Some terrestrial functions carried out by small number of taxa (e.g. nitrification, ammonia oxidizers).
- There is evidence that some of these bacteria may be sensitive (e.g. STP studies, through these are designed to evaluate "catastrophic" impacts like spillages etc).
- Although there is some evidence that nitrification is a sensitive process in aquatic systems (Petersen et al. 2004), knowledge of whether there are any keystone species groups or processes conducted by few species in aquatic systems are missing. This is a research need. What are the process and functions and what is required to ensure they continue.
- Decomposition of natural substrates in pristine streams is dominated by a keystone fungal group, the aquatic hyphomycetes. While there appears to be some differentiation between early and late colonizers, there is no convincing evidence that certain species play a “key role” and are therefore indispensable (Bärlocher 1992b,c 2005).
- Research need: Which taxonomic groups are responsible for ‘key processes’ in aquatic systems, and are they sensitive to fungicides? There is no doubt that fungi are sensitive to fungicides; in fact, the importance of fungal activity in leaf decomposition was in part established by measuring decomposition in the presence of antifungal antibiotics (Kaushik & Hynes 1971). What is unknown is whether this will be significant at levels of agricultural fungicides that might occur in the field.

2. <u>Are microorganisms more sensitive than other taxonomic groups used in the risk assessment of fungicidal biocides and pesticides?</u>

- Many fungicidal biocides and pesticides affect a wide range of taxa and therefore the potential effects all microorganisms should be considered (Widenfalk et al., 2004, DeLorenzo et al. 2001).
- Where there is a general mode of action microorganisms are not necessarily the most sensitive taxonomic group.
- There is evidence from presentations shown that other taxonomic groups may be more sensitive or that microbes are better at recovering.
- Standard aquatic first tier tests with AF – considered to be protective of fungi or bacteria. Evidence from SSD vs. microbial functional endpoints in mesocosms showed the microbial endpoint was not most sensitive endpoint. (may be low sensitivity or rapid

recovery) although discussions around recolonisation rates suggested recovery of fungal assemblages my take weeks, therefore effects would be captured in mesocosm sampling)
- Rapid adaptation makes it difficult to define a population or even a species.
- Existing data set together with standard assessment factors appears to be protective of microbial communities.

3. Are bacteria more or less sensitive than fungi to fungicidal biocides and pesticides?

- Bacteria may be more or less sensitive than fungi to fungicidal biocides and pesticides – depend on life cycle, MOA etc.
- Recent unpublished studies have indicated that fungi are slightly more sensitive to fungicide than bacteria, but there was surprisingly little difference in response. It also appeared as if different bacteria had different sensitivity (A. Widenfalk, unpublished).
- Fungi normally have longer life cycle than bacteria although some bacteria also have very long life cycles.
- Bacterial toxicity tests are part of standard procedures in testing of chemicals, waste waters, pharmaceuticals and plant protection products (Table 5).
- There is evidence that bacteria are generally less sensitive compared to other test organisms used for risk evaluation (i.e. algae, *Daphnia*, fish), although there are notable exception (e.g. pharmaceuticals such as antibiotics). Existing ecotoxicological data for at least some fungicides could be reviewed to compare the sensitivity of microbes relative to algae, daphnia and fish data.
- There are differences in sensitivity among bacteria. Gram-positive bacteria are considered more stress-tolerant, for example to toxic chemicals (Stainer et al., 1977). Nitrifying bacteria are characterized as being gram-negative (Chandler, 1997) which would render these organisms and thus the nitrification process more sensitive than many other processes. It has been demonstrated that gram-negative cells are susceptible to the high membrane activity of pyrithiones (Al Adham, 1998).
- Bacteria, if affected, may have a high (functional) recovery potential because of their:
    - high reproduction rates
    - potentiality of “fast” genetic adaptation to environmental changes (e.g. by mutation)
    - ubiquitous distribution of a lot of bacteria
    - high functional redundancy
    - fast recolonisation potential
- Bacteria may be more or less sensitive than fungi to fungicidal biocides and pesticides

4. Research needs

- Which taxonomic groups are responsible for ‘key processes’ in aquatic systems, and are they sensitive to fungicides?
- Effects of adaptation on community functioning (and structure?) in aquatic systems

Table 5: Examples of ecotoxicological methods that are used to determine effects of chemicals towards Bacteria

Guidelines	Parameter	Test system	Use	Determination of
88/302/EEC – C.11 Biodegradation: activated sludge respiration inhibition test Corresponds to OECD TG 209 ISO 8192	Inhibition of respiration	Activated sludge	Testing of chemicals	Toxicity towards bacteria
92/69/EEC – C.4 A to F Biodegradation: determination of the "ready" Biodegradability Corresponds to OECD TG 301!	Degradation Toxicity control demonstrates if the test item is toxic towards bacteria	Activated sludge	Testing of chemicals	Biological degradation Including a toxicity control!
DIN EN ISO 10712 Water Quality –*Pseudomonas putida* growth inhibition test (Pseudomonas cell multiplication inhibition test)	Turbidimetry of growth	*Pseudomonas putida*	Waste water, "Chemicals"	Toxicity towards the bacterium *Pseudomonas putida*
DIN EN ISO 9509 Water Quality – Method for assessing the inhibition of nitrification of activated sludge micro-organisms by chemicals and waste water	Determination of nitrite and nitrate which result of the oxidisation process of ammonia	Activated sludge	Sewages, "Chemicals"	Toxicity towards nitrifying bacteria
DIN 38415-3 Bestimmung des erbgutverändernden Potentials von Wasser mit dem umu Test German standard methods for the examination of water, waste water and sludge-Sub animal testing (group T)-Part 3: Determination of the genotype potential of water with the umu test (T3)	Induction of the umuC-gene – measurement via determination of the ß-galactosidase activity. & Turbidemetry of growth	Genetically engineered *Salmonella typhimurium* TA 1535/pSK 1002	Waste water, ("Chemicals" possible to do)	Toxicity towards Salmonella typhimurium and induction of umuC-gene
German standard methods for the examination of water, waste water and sludge-bio-assays (group L); determination of the inhibitory effect of waste water on the oxygen consumption of *Pseudomonas putida*	Inhibition of respiration	*Pseudomonas putida*	Waste water	Toxicity towards the bacterium *Pseudomonas putida*
DIN EN ISO 11348-2 Water quality – Determination of the inhibitory effect of water samples on the light emission of *Vibrio fischeri* (Luminescent bacteria test) –Part 2: Methodd using liquid-dried bacteria	Inhibition of light emission	*Vibrio fischeri* NRRL B-11777	Waste water, ("Chemicals" possible to do)	Toxicity towards the bacterium *Vibrio fischeri*

3.4.2 Functional redundancy

1. What is the temporal and spatial variation in the structure and functioning of microbial communities?

- There is high seasonal variation in microbial communities (Finlay et al., 1997, Fernandez et al., 1999). There is also annual variation in structure, but less in function. (Kent et al., 2004). Inter-year variation is stable over long time periods, as judged at a process level (Finaly et al., 1997).
- Variability depends on the substrate, temperature and nutrient regime, and on succession (Gray et al., 2004, Muylaert et al., 2004).
- In lake systems, planktonic microbes (free or particle associated) ultimately sink to the bottom of the lake where their activity is determined by redox gradients.
- In rivers, there are no true planktonic microbes except in very large rivers or in stagnant side-arms. However, there are microbes associated with surfaces such as macrophytes, detrital material and biofilms.
- There is considerable phylogentic and functional overlap between soil and aquatic bacteria. (see Bergey's Manual of Systematic Bacteriology), but one characteristic that has become clear is that prokaryote diversity in aquatic environments is orders of magnitude less than in sediments and soils (Torsvik et al., 2002)).
- In contrast, soil and aquatic fungi are phylogenetically and functionally distinct. In streams, leaf decomposition is dominated by aquatic hyphomycetes whereas wood decomposition is primarily by aquatic Ascomycetes. Leaves and other plant detritus at the bottom of stagnant ponds are readily colonized by aero-aquatic fungi. Basidiomycetes, common in soils and on wood decomposing in terrestrial habitats, are relatively rare in streams and lakes (Bärlocher 1992a, c).
- Spatial variation in stream fungal assemblages is large, especially as one moves down a stream system. However, we understand why this is so (we think!) – substrate, stream order, nutrients – so interpretation of this factor could be taken on board in regulatory studies. (Allan 1995).

2. What evidence is there of functional redundancy in microbial communities.

- Functional redundancy is defined as the potential of one species to replace another whilst maintaining the overall functional process in the system.
- In order to assess the extent of functional redundancy in freshwater microbial assemblages, we need to**:**
  - define environments for assessment
  - define functions to be monitored
  - differentiate between plant protection and biocide usage, and link assessments to the appropriate exposure and response timescales.
- We know that, although it is possible to measure several microbially-mediated ecosystem functions, microbial diversity is more difficult to measure.
- Functional redundancy is often taken at a specific process level not at an overall process level (as it is not defined). Current focus on a process (leaf litter) but that may not be relevant to all assessments.
- In general, function is related to structure, but is more specifically related to expression.
- Microbes:
  - are multifunctional (see Bergey's Manual of Systematic Bacteriology).
  - have genetic plasticity (Ochman & Santos, 2003),
  - are metabolically flexible and are adaptable (Torsvik et al., 1996)
  - are able to switch to new functions which are expressed as conditions dictate (Lovely, 1991)
  - are able to acquire new functions which are expressed as conditions dictate .(Yin & Stotsky, 1997)

  - often habitat drives function (Gray et al., 2004).
- Bacterial populations (species) should not be linked to one process as many microbes are multifunctional and changes in environmental conditions will allow them to switch function without an overall change in biodiversity.
- Temporal spatial structural and function changes can be large and are related to the local (physical/chemical and the rest) environments (see above). In response, overall biodiversity may not change but numbers of individual members are dynamic (Finlay et al 1997). Field studies suggest that there appears to be high functional redundancy in terms of leaf weight loss (Bärlocher & Graça 2002), but lab studies suggest otherwise (e.g., Bärlocher & Corkum 2003, Duarte et al. 2005, Treton et al. 2004, Dang et al. 2005). The contradiction may be due to the much larger numbers of species in the field, or because of confounding factors in the field
- Because of specific fungal-consumer interactions, ecological function may be preserved but there could still be adverse effects on consumers.
- There do not appear to be keystone fungal species (Bärlocher 1992c)
- Metabolic flexibility within microbes appears to be enormous although there are 'bottleneck' processes where a small number of taxa fulfil the role.
- Where fewer species are able to fulfil the same function then the species may be more adapted to survive in fluctuating environmental conditions (e.g. ammonia oxidisers; Avrahami et al., 2003). Conversely, some groups could exhibit sensitivity to a high degree of perturbation with loss of function as they are highly vulnerable to toxic shock (methanogens to oxygen (Hall et al., 1996); ammonia oxidisers to high level pollutants in sewage treatment (Hallin et al., 2005).
- Functional redundancy should be discussed within specific spatial or temporal scale. Are all micro-organisms ubiquitous or selected by the environment? This represents a knowledge gap.

3. <u>How important is it to maintain microbial diversity in order to protect function against future perturbations?</u>

- Less diverse ecosystems (e.g. desert streams, polluted streams) maybe at greater risk because of lower redundancy and because functional stability generally increases with diversity (portfolio effects; e.g., Dang et al. 2005; Kinzig et al. 2001).
- However, applying ecological principles derived for macroorganisms is not always relevant at a microbial scale (Curtis et al., 2002).
- The importance of biodiversity is difficult to assess in a system where it is unknown, undefined or not understood, which is a specific issue with bacterial assemblages as they are largely unexplored (Curtis et al., 2002)
- Precaution suggests we should attempt to preserve fungal diversity, since we know that aquatic fungi differ from terrestrial fungi in terms of physiology, ecology and phylogeny.
- In agricultural streams, fungal diversity is generally reduced due to high nutrients etc. (Bärlocher 1992c). Therefore the potential for functional redundancy may be less, and it becomes more important to protect the remaining diversity (perhaps by reducing nutrient inputs).
- It is also important to preserve the microbial diversity of more pristine sites.
- Microbial diversity rather than function is not the most sensitive measure as it may change as a result of secondary effects rather than primary toxicity. (Fahy et al., 2004).
- Microbial parameters (function) appear very useful in monitoring pollution (e.g. heavy metal pollution), but no single microbial parameter can be used universally (Brookes, 1995).
- Niyogi et al. (2002) showed that biodiversity of stream fungal communities were sensitive to anthropogenic stress, whereas biomass and function were sustained at low to moderate stress levels and decline only when stress is very high.

3.4.3 Trophic interaction

1. What is the relative importance of heterotrophic microbes as a food source and as modifiers of organic material?

There are major differences in organic matter degradation between different types of water body, principally streams/rivers, slow-flowing ditches, ponds and lakes, and these were treated separately:-

Streams/rivers

- In pristine streams, coarse particulate organic matter (CPOM, > 1 mm) of terrestrial origin dominates energy input – up to 99 % of the food available to stream communities is based on the processing of deciduous leaves or conifer needles (Webster & Benfield 1986).
- A specialized group of fungi, the aquatic hyphomycetes, dominates the decomposition of leaves and other CPOM. Typically, fungal biomass and production on leaves exceed bacterial values by a factor of at least 10 (Gessner et al. 2003).
- Total annual productions of bacteria, fungi and invertebrates in pristine streams are similar – within an order of magnitude of each other (Suberkropp 1977, Suberkropp: unpubl. results). In these streams, the majority of invertebrates consume decaying leaves enriched with fungal biomass (which can account for up to 18 % of total detrital mass; Gessner et al. 2003).
- As streams widen (increasing stream order) or flow through open country (agricultural areas, deserts), the contribution of terrestrial CPOM becomes less important, and periphyton will play a bigger role, BUT only a few studies have attempted to quantify this.
- Streams outside woodland/forests may still receive substantial amounts of leaves from upstream, from trees growing on stream banks.
- In the absence of tree leaves, emergent and submerged macrophytes probably also provide a resource for stream fungi and bacteria, but there are few hard data on this.
- Any effect on fungal production would be expected to have a knock-on effect on detritivorous invertebrate production because fungi can improve the palatability and nutritional value of leaves.
- Conversely, if invertebrate production is reduced for some reason, then fungal production would be expected to increase. This is because detritivorous invertebrates compete for leaves with fungi, and also consume the fungi.
- Bacterial production would also be expected to increase if invertebrates are reduced, although relatively few invertebrates (e.g. some daphnids and chironomids) actively feed on bacteria.
- It should also be noted that bacteria are preyed on from ‘below’ by certain viruses, and ‘above’ by some protozoa.

Lakes

- Leaves are a much less important food source in the open waters of lakes, the main origin of photosynthetic production being phytoplankton.
- Furthermore, most living phytoplankton is eaten directly by zooplankton, and is not utilized by fungi. An exception to this rule are Chytrids (fungi which attack phytoplankton) (Kendrick, 2003). In contract, bacterial degradation of algae does occur and at the end of algal blooms (Lignell et al, 1993) and may be sufficient to deoxygenate the water and place other organisms (e.g. fish) under severe oxygen stress.
- However, in shallow lake reed beds, lake margins and wetlands, the fungi play an important role as degraders of plant matter, at least in some systems (e.g. Kuehn et al, 2004, Kominkova et al. 2000).
- Consequently, if fungicides impact on microbial decomposers, there is more potential for secondary effects in streams than in lakes.

Ponds/slow-flowing ditches

- Even where there are no trees, these systems will have an important degrader community if there are abundant submerged, floating or emergent macrophytes.
- However, although degradation of these carbon sources is more poorly understood, it is possible that bacteria are more important than fungi for the degradation of the less fibrous submerged species (De Boer et al . 2005).

2. How important is the microbial loop in determining energy flow in freshwater ecosystems and what are the consequences of its perturbation?

Importance for energy flow

- In streams, both aquatic hyphomycetes and bacteria are important for energy flow (Parkyn et al 2005, Fuller et al, 2004).
- In lakes, fungal decomposition may be less important but the microbial loop is estremely important. Bacteria, nanoflagellates (including mixotrophs and autotrophs), ciliates, and mesozooplankton reveal spatial and seasonal changes in biomass composition and are importance in the carbon budget. Constraints in growth of microbial communities include resource competition and predation by organisms occurring at several trophic levels. *Daphnia* species play a key role in breaking the microbial loop and establish direct routes from several microbial compartments to higher trophic levels (Riemann and Christoffersen, 1993).
- In lakes results suggest that structural and functional characteristics of the 'microbial loop' may be operating differently in stressed versus unstressed ecosystems. The possibility of using autotrophic picoplankton as an early warning indicator of environmental perturbation is proposed (Munawar and Weisse, 1989).
- Bacteria are a key component of the microbial loop, which may play a crucial role in the food webs of both stream and lakes.
- The importance of microbial degradation for energy flow in ponds and slow-flowing ditches is probably intermediate between streams and lakes, but there are few data on this.
- In streams, there is no doubt that fungi are more important than bacteria for promoting energy flow through the breakdown of coarse particulate organic matter (Gessner et al, 2003)

Consequences of perturbation

- Due to unidirectional water flow in streams, some disturbances (e.g., contamination by toxins) will spread more quickly and show a more immediate effect in stream than in lake communities (Hynes 1971). However, unless the disturbance persists, stream communities may also recover more quickly by recolonization from upstream sections.
- If the carbon cycle is interrupted by fungicidal activity, leaf material can accumulate in forest and woodland streams, and macrophyte biomass can accumulate in shallow still waters (Kaushik & Hynes 1971; Bärlocher 1992b).
- This in turn reduces the food supply for detritivorous invertebrates.
- It is also theoretically possible that fish which feed on fungally-based periphyton could suffer a reduction in their food supply, and the same applies to fish which feed on invertebrates. However, we know of no data to support this, and because there are more food choices available at higher levels in the food chain, it seems inherently unlikely that these processes would be important.
- Speed of recovery will partly depend on the extent of the water area affected – because most recolonisation occurs via the water rather than through the atmosphere. Although an unlikely event, the destruction of all fungi in a stream would therefore result in slower recovery than if only a short stretch was impacted.
- Fungal reproduction is typically more sensitive to perturbation than fungal growth (Bärlocher 1992c), and the germination of aquatic fungal spores is more severely inhibited by contaminants than metabolism and growth of mycelia (Kempt et al. 2002), which further limits the speed of recolonisation.

- Some re-colonisation by fungi can occur from within the leaf structure or from other micro-habitats protected from the fungicide (e.g. sediment).
- Providing there are some remaining sources of re-colonisation, full recovery from fungicide poisoning of an entire freshwater fungal community will take at least a week, and in some cases several months (Bärlocher 1992c).
- This expectation for speed of recovery is supported by observations of the time taken for transplanted leaf matter to be colonised by a functioning fungal community, and the time taken for fungi to re-colonise ephemeral streams (Maamri et al. 2001).
- Speed of re-colonisation is also a function of temperature.
- Indirect effects of fungicides on detritivorous invertebrates may be expected due to the reduced food supply, although species that compete with fungi for food may have an increased food supply.

3. How specific are microbe-consumer interactions?

- In the laboratory, these interactions have been shown to be quite specific - a particular fungal species may provide a food source for one invertebrate organism but not another.
- These experiments have studied growth rate, survival and food preference, and specificity has been demonstrated for all of these measures (for review, see Bärlocher 1987, 1992b, c).
- On the other hand, we do not know if similar specificity occurs in the field.
- However, there is a greater range of alternative food sources in the field compared with experimental conditions, so it is likely that food specificity is less of an issue under natural conditions.
- Because of these specificities, fungal diversity will to some extent drive invertebrate diversity, although the degree of coupling is poorly understood.
- Bacteria are also known to exhibit some specific interactions with invertebrates. The few studies available from freshwater systems suggest that the consumption of bacteria covers between 5 and 11% of the energy demands of deposit-feeding macroinvertebrates (Johnson et al. 1989 and cited references). In black water streams, that are extremely heterotrophic, up to 50% of deposit-feeders carbon demands can be obtained by bacteria (Edwards and Meyer, 1990). The ingested bacteria could be more or less easy to digest. It may therefore be possible that the invertebrates can distinguish the more palatable ones and feed selectively on these.

Recommendations

- Due to the many uncertainties and data-gaps described above, it seems premature to propose the development of regulatory tests for fungicides which seek to investigate the trophic interactions of detritivorous freshwater micro-organisms and invertebrates.
- Nevertheless, there are grounds for believing that such interactions may be damaged by some fungicides, so further research is required to strengthen the knowledge-base. Research needs to include the following, subdivided into fungicide-related and general questions. The questions should be tiered; if we cannot find fungicide-mediated impacts of the type hypothesised there would be no need to consider differences in potential impacts driven by such factors as habitat-type and rates of recovery etc.

Fungicide-related questions

- Is there evidence from old mesocosm/microcosm experiments with fungicides of indirect impacts on invertebrates due to damaged fungal degrading capacity? If not, mesocosm/microcosm experiments should be specifically designed with this objective in mind.
- More information is needed on the rates of recovery of impacted fungal communities, and more especially on the consequent rates of recovery in secondarily-affected detritivorous invertebrates.

- Do fungicides have less of an impact in such systems due to the relative importance of bacteria?

General questions
- How does degrading capacity vary between stream types?
- How important are aquatic macrophytes and associated fungi as a source of organic matter for detritivores in slow-flowing ditches and ponds, and in lake margins?
- Is it possible to observe microbe-consumer specificity under field conditions? This applies both to bacteria and fungi.
- To what extent does fungal biodiversity drive detritivorous invertebrate biodiversity?

4 Outcome of the workshop and recommendations

4.1 Related to aims

4.1.1 Differences in risk assessment approaches between pesticides and biocides

Exposure profiles for pesticides and biocides are expected to be different. It is expected that the exposure to pesticides is relatively high and is characterised by a pulsed exposure while the concentrations of biocides are lower, but long-lasting. Interpretation of effects data, however, should be similar when the same compound is of concern. The biocide assessment, however, seems more precautionary because sometimes larger assessment factors are used on the same data. It is therefore recommended to harmonise the guidance documents for pesticides and biocides. For the risk assessment of fungicidal pesticides and biocides, read across from soil microbial studies and water-sediment fate packages (C N mineralization) may be possible for effects on the microbial community. This is, however, not an option for all biocides since such data are only required for some biocidal product types. Additional information for aquatic systems should be gathered by performing litter bag studies when using microcosms or mesocosms. From the limited data currently available the standard tier 1 studies appear to be protective of microbial processes in terms of function (decomposition).

4.1.2 Use of microbial endpoints in risk assessment

Sensitivity

Microorganisms is very diverse group of organisms. It is therefore likely that some species will be affected by fungicides, but it is envisaged that the bacterial community have a high capacity for recovery. It should be investigated whether one could extrapolate effects on terrestrial microbial processes (C and N mineralization) to aquatic system. This because the group felt that it can provide some information but that further research is needed before any extrapolation can be made. In terrestrial tests, for instance, only aerobic degradation is studied, while also exposure may be difficult to extrapolate (e.g. (from mg/kg to mg/L). Furthermore, terrestrial microbial tests are mainly driven by bacteria and not by fungii which may be more sensitive (and possibly more important in aquatic ecosystems. It was also questioned what the implications are of the expected rapid recovery, adaptation and functional bottlenecks are for risk assessment

Functional redundancy

Community structure and function are related, but measuring many functions and defining bacterial diversity is difficult. One needs to define and focus on the system of interest and its most relevant processes. We also need to distinguish fungal from bacterial activity and may need to monitor fungal and/or bacterial diversity.

Trophic interactions

There are major differences in the importance of heterotrophic microbes in decomposing organic matter in different aquatic systems. In streams the fungal decomposers and fungal-consumer interactions are most important, while in lakes bacterial decomposers and bacterial-consumer interactions most important in open water, and fungi are more important at the margins. In ponds and ditches bacteria may be most important. Recovery of affected fungal communities depends on extent of impact and can take weeks/months. Fungal-consumer interaction is specific in laboratory, but specificity is not known for field populations. There is a potential link between fungal and invertebrate diversity, so a potential for indirect effects .

4.2 Research recommendations

- Compare additional single species data and microbial endpoints (structure and function) to evaluate protective value of standard species data sets.
- Evaluate use of ACR from Tier 1 studies to extrapolate the acute SSD to chronic SSD. This because it is expected that pesticide use results in a pulsed exposure while biocide use results in a more chronic exposure.
- Quantify use patterns for biocides to be applied in higher tier exposure assessment.
- Develop higher tier exposure assessment tools for biocides
- Determine which taxonomic groups are responsible for 'key processes' in different aquatic systems.
- Assess the sensitivity and recovery rates of 'key processes' and associated taxa, exposed to fungicides.
- Can impact on microbial function be assessed at process level and ignore diversity (functional redundancy)
    - Target N-cycle due to sensitive bottlenecks?
- Investigate the effects of adaptation of microbes to fungicides on community functioning (and structure?) in aquatic systems.

4.3 Policy recommendations

- Focus biocide and pesticide studies on constant chronic exposure, where relevant.
- Use information on MoA and species sensitivities to focus additional studies (i.e. whole ecosystem or sensitive components).
- Harmonise guidance documents of 91/414 and 98/8, where appropriate. Include detailed guidance on higher tier exposure and effects assessment for biocides.
- Consider setting different standards (e.g. Maximum acceptable concentration: MAC, annual average concentration: AA of the Water Framework Directive) to account for different uses and hence exposure profiles.
- From the current knowledge it appears sufficient to apply standard assessment factor to tier 1 data to protect functioning of aquatic microbial communities.
- Consider microbial processes and detrital food chain effects in higher tier studies when there is an indication that microbial systems may be at risk.
- Premature to recommend a new regulatory test on trophic interactions between microbes and their consumers.

5 Final programme

Workshop rapporteur: Lorraine Maltby

5.1 Sunday 6 November 2005

Opening address and social

12:30 – 13:30	Steering Committee lunch meeting
17:00 – 18:00	Meet in bar and registration

18:30 – 18.45 Welcome (Paul Van den Brink)
19:00 – 20:30 Workshop dinner

5.2 Monday 7 November 2005

Regulatory background and risk assessment approaches

Session 1: Background to fungicidal pesticides and biocides (Paul Van den Brink)
08:30 – 08:45 Introduction to the workshop (Paul Van den Brink)
08.45 – 09:15 Overview of fungicidal chemicals (Steve Maund)
09:15 – 09:45 Regulation of pesticides and biocides (Lina Wendt-Rasch / Floor Peeters)
09:45 – 10:15 Presentation of the European Science Foundation (ESF) (Rudy Rabbinge and Milena Horvat)
10:15 – 10:45 Coffee break
10:45 – 11:30 Emissions, fate and exposure in the aquatic environment (Colin Brown)
11:30 – 12:00 Plenary discussion
12:00 – 13:00 Lunch

Session 2: Effects and risk assessment of fungicidal pesticides and biocides (Peter Matthiessen)
13:00 – 13:45 Introduction to effect assessment approaches (Paul Van den Brink)
13:45 – 14:30 Species identity to be included into the SSD (Lorraine Maltby)
14:30 – 15:15 Comparing lower and higher tier assessments (Theo Brock)
15:15 – 15:30 Explanation of case studies (Floor Peeters / Lina Wendt-Rasch)
15:30 – 15:45 Coffee break
15:45 – 17:15 Case studies
17:15 – 18:00 Report back of breakout groups and plenary discussion

Evening Steering Committee meeting with rapporteurs

5.3 Tuesday 8 November 2005

Microbial Systems: Functional and Structural Endpoints

Session 3: Structural and functional endpoints (Ralf Schulz)
08:30 – 09:15 Role of fungi in aquatic ecosystems (Felix Bärlocher)
09:15 – 10:00 Measuring microbial structure and functioning in aquatic ecosystems: approaches and limitations (Roger Pickup)
10:00 – 10:45 Lessons learned from studying soil microbial systems (Chris Leake)
10:45 – 11:15 Coffee break
11:15 – 11:45 The use of functional endpoints in semi-field experiments (Helene Roussel)
11:45 – 12:15 Plenary discussion
12:15 – 13:15 Lunch

Session 4: Break out groups (Fred Heimbach)
13:15 – 13:30 Introduction to breakout groups (Paul Van den Brink / Lorraine Maltby)
13:30 – 15:30 Breakout groups on functional redundancy, sensitivity and trophic interaction
15:30 – 16:00 Coffee break
16:00 – 18:00 Reporting back and plenary discussion

Evening Steering Committee meeting with rapporteurs

5.4 Wednesday 9 November 2005

Scientific state of the art and Outlook (Theo Brock)

08:30 – 11:00	Update of report of rapporteurs of Monday and Tuesday breakout groups
11:00 – 11:30	Coffee
11:30 – 12:30	Reporting back of workshop rapporteur and final discussion
12:30 – 12:45	Closing remarks
12:45	Lunch and good bye
13:30 – 15:00	Steering Committee meeting
Afternoon	Departure

6 Final list of participants

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7 Statistical information on participants

Figure 1 provides an overview on some statistics describing the background of the workshop participants. Most people were between 30 and 50 years old and wre born and living in the UK, germany or The Netherlands. The participants were evenly distributed over the stakeholder groups academia, business and regulators.

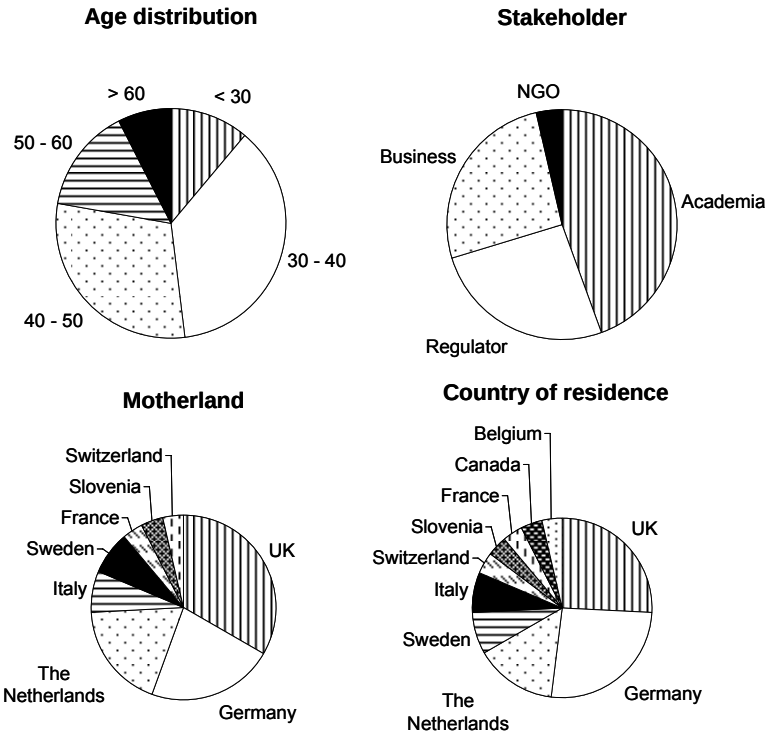

Figure 1: Statistical distribution of the participants over age, stakeholder groups, mothercountry and country of residence.

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Appendix 1

Breakout group Monday: Fungicidal Biocide A

Mode of action

The active substance of Fungicide A is a so called multi site inhibitor interfering at many locations in the metabolism of fungi. The compound is not systemic.

First tier data

Application and most relevant physico-chemical data

The application concerns an antifouling application on ships, PT 21 of the Biocidal Product Directive

solubility in water:		0.9 mg/L(metabolite: soluble in water)
hydrolysis		($t_{1/2}$) at 22°C
	pH 4	12 days (metabolite: stable)
	pH 7	29 hours
	pH 9	<< 10 min
water-sediment metabolism study		
	DT50 water:	4 hours (metabolite: 59 days)
	logPow	3.9 (metabolite: 2.0)

PEC (surface water) using MAMPEC

Main routes of entry	Direct emission from leaching into water and sediment (salt water), chronic exposure.

Scenario	Medium	PEC water (µg / L)
Marina	Fresh + salt	1.4
Commercial harbour	Salt	0.9
Estuarine harbour	Salt	0.9
Shipping lane	Salt	0.02
Open sea	Salt	0.03

Toxicity data for aquatic species (most sensitive species of each group)

Test species	Study Type / Duration	Test Substance	Ecotoxicological Endpoint
	Fish	acute	
Rainbow trout	flow-through system, 96 h	formulation	LC_{50}
Rainbow trout *)	static system, 96 h	tech.	LC_{50}
Golden Orfe *)	static system, 96 h	tech.	LC_{50}
Bluegill *)	static system, 96 h	formulation	LC_{50}
Guppy *)	static system, 96 h	formulation	LC_{50}
Zebra fish *)	static system, 96 h	formulation	LC_{50}
Japanese ricefish *)	static system, 96 h	formulation	LC_{50}
Russian sturgeon *)	static system, 96 h	formulation	LC_{50}
Fathead minnow *)	static system, 96 h	formulation	LC_{50}
Goldfish *)	static system, 96 h	formulation	LC_{50}
Rudd *)	static system, 96 h	formulation	LC_{50}
Common Carp *)	static system, 96 h	formulation	LC_{50}
Common Carp	static system, 72 h	formulation	LC_{50}
Zebra fish	static system, 96 h (pH buffered)	formulation	LC_{50} (pH 6.0) LC_{50} (pH 7.0) LC_{50} (pH 8.0)
Golden Orfe	static system, 96 h	metabolite	LC_{50}
Rainbow trout	static system, 96 h	metabolite	LC_{50}

Multi Species: HC_5, species with *)	static system, 96 h	tech., formulation	HC_5
	Invertebrates	acute	
Daphnia magna	flow-through, 48 h	tech.	EC_{50}
Daphnia magna	static system, 48 h	tech.	EC_{50}
Daphnia magna	static system, 48 h	metabolite	EC_{50}
	Green	algae	
Scenedesmus subspicatus	static system, 72 h	tech.	E_rC_{50}
Pseudokirchneriella subcapitata	static system, 72 h	formulation	E_rC_{50}
Pseudokirchneriella subcapitata	static system, 72 h	metabolite	E_rC_{50}

Test species	Study Type / Duration	Test Substance	Ecotoxicological Endpoint
	Fish	chronic	
Rainbow trout	flow-through system, 21 d	tech.	NOEC
Rainbow trout	flow-through system, 21 d	formulation	NOEC
Rainbow trout	semi-static system, 21 d	metabolite	NOEC
Fathead minnow	early life stage, flow-through system, 32 d	metabolite	NOEC
	Invertebrates	chronic	
Daphnia magna	semi-static system, 21 d	tech.	NOEC
Daphnia magna	semi-static system, 21 d	formulation	NOEC
Daphnia magna	semi static system, 21 d	metabolite	NOEC
Chironomus riparius	sediment-water system, static 28 d	metabolite	NOEC
Chironomus riparius	sediment-water system, static 28 d	metabolite	EC_{15}

PNEC (surface water – see presentation regulation biocides)

Assessment factors for fresh water

Available data	Assessment factor
At least one short-term L(E)C50 from each of three trophic levels of the baseset (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

Assessment factors for salt water

Data set	Asessment factor
Lowest short-term $L(E)C_{50}$ from freshwater or saltwater representatives of three taxonomic groups (algae, crustaceans and fish) of three trophic levels	10.000
Lowest short-term $L(E)C_{50}$ from freshwater or saltwater representatives of three taxonomic groups (algae, crustaceans and fish) of three trophic levels, + two additional marine taxonomic groups (e.g. echinoderms, molluscs)	1.000
One long-term NOEC (from freshwater or saltwater crustacean reproduction or fish growth studies)	1.000
Two long-term NOECs from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish)	500
Lowest long-term NOECs from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing three trophic levels	100
Two long-term NOECs from freshwater or saltwater species representing two trophic levels (algae and/or crustaceans and/or fish) + one long-term NOEC from an additional marine taxonomic group (e.g. echinoderms, molluscs)	50
Lowest long-term NOECs from three freshwater or saltwater species (normally algae and/or crustaceans and/or fish) representing three trophic levels + two long-term NOECs from additional marine taxonomic groups (e.g. echinoderms, molluscs)	10

Risk assessment using first tier data

PEC/PNEC < 1 no refined risk assessment necessary
PEC/PNEC > 1 refined risk assessment necessary

Further tier 1 data included in the annex II or III standard data package (PPP)

Effects on earthworms

Acute toxicity	LC_{50}	> 1000 mg ai/kg d. wt. soil (techn. ai)
Acute toxicity	LC_{50}	> 961 mg ai/kg d. wt. soil (Formulation A)
Reproduction	NOEC	12 kg ai/ha (Formulation A)

Effects on soil micro-organisms

Nitrogen mineralization	no influence up to 14.4 kg ai/ha
Carbon mineralization	no influence up to 14.4 kg ai/ha

Effects on biological methods of sewage treatment

Activated sludge (mixed populations of different microorganisms, from an aeration tank of a waste water plant treating predominantly domestic sewage) was exposed for 3 hours to nominal concentrations ranging from 32 to 320 mg/L.
Results: The EC_{50} was determined as 230 mg/L

Higher tier data

Test species	Study Type / Duration	Test Substance	Ecotoxicological Endpoint
	Indoor	microcosm	
Rainbow trout	water-sediment, multiple application, 28 d	formulation	NOEC
Daphnia magna	water-sediment, multiple application, 40 d	formulation	NOEAEC
	Outdoor	mesocosm	
Rainbow trout	outdoor enclosures, multiple application, 35 d	formulation	NOEC
Aquatic community	outdoor enclosures, multiple application, 51 d	formulation	NOEAEC

Fish indoor microcosm study

Test item: Fungicide A (formulation, content of active ingredient: 51 %)
Test species: Rainbow trout (*Oncorhynchus mykiss*), body wet weight 1.2 g (0.7 - 1.7 g)

Test design: This indoor microcosm study was designed to simulate spray drift on fish in natural standing water. Rainbow trout, one replicate with 20 fish per test level, were exposed to repeated spray applications in a water/sediment system (total volume: 100 L, water column about 34 cm, sediment layer about 2 cm) for 28 d under static test conditions. Aqueous solutions of the test substance were sprayed four times on the water surface of the aquaria in weekly intervals. The test included a water/sediment control and a water control.

Test conditions: Reconstituted water (according to ISO), artificial sediment (according to OECD-Draft 219, 2/2000), temperature 12.9 to 15.3 °C, test aquaria were aerated in order to avoid oxygen depletion below 60% of the saturation value, pH ranged from 7.0 to 8.1, fish were fed on workdays with 24h old larvae of *Artemia salina*.

Results: The NOEC in this indoor microcosm study in a water-sediment system after 4 applications of fungicide A in 7 day intervals onto the water surface was 44 µg ai/L (based on initial test concentrations per application). The highest test concentration (4 times 53 µg ai/L) yielded 10 % mortality.

Fish outdoor microcosm study

Material and methods: The effects of repeated applications of the test substance (same formulation with fungicide A as above) on growth and survival of juvenile rainbow trout (approx. 7 cm in length) was studied under outdoor field conditions. The study was carried out using 6 enclosures. The height of the enclosures above water surface was approx. 0.15 m, water depth was 0.50 m. Each enclosure contained approx. 433 L of water. All enclosures contained some macrophytes and had a bottom layer of sediment.

The treatment consisted of 4 applications of the test substance at one week intervals, simulating spray drift. Nominal treatment levels were 46 µg a.i./L and 60 µg a.i./L. Treatments were duplicated, using 2 enclosures per treatment level and 2 controls. The test lasted for 35 days after the first application. The concentrations of the active ingredient and its main metabolite in the water phase were followed over time. The weight and length of the fish were determined 4 days prior to the first application of the test substance, when they were

transferred to the enclosures, and at the end of the experiment. Dynamics in chlorophyll-a content of phytoplankton, macrophyte species composition and cover and community metabolism were followed over time. The following datasets were analyzed through one-way analysis of variance (one-way ANOVA): alkalinity, chlorophyll-a content, fish weight and length, community metabolism parameters (temperature, dissolved oxygen and pH).

Results: Chlorophyll-a content of all treatment levels, including controls, increased after day 13. The highest levels were found in controls. Overall, no clear dose-response relationship was discernible for effects of the test substance on chlorophyll-a content of phytoplankton.

Enclosure Nr.	Nominal initial treatment level (µg/L)	Mortality on day 28 (%)
3	Control	0
5	Control	0
2	46	0
6	46	10
1	60	0
4	60	20

Conclusions: According to the study reporter the NOEC is > 60 µg/L.

Daphnia - microcosm study

Material and methods: Fungicide A formulation (same as above) was used in this study. Populations of different age-classes of *Daphnia magna* in a static test system were exposed for 40 days to 6 concentrations (with 3 replicates) between 32 and 560 µg ai/L (nominal initial, four applications on days 0, 7, 14 and 21) in a water-sediment system. Test containers (20 L glass aquaria) were prepared two weeks before application. Artificial sediment (5 % sphagnum peat) was used for this study. The sediment layer had a depth of approximately 2 cm, the overlying water column approximately 20 cm (15 L). The water in all aquaria was aerated slightly throughout the study period. The pH, dissolved oxygen and the temperature of the test water were determined several times during the study. The pH of the water varied between 7.9 and 9.0 during the test.

Results: No effect on the *Daphnia magna* abundance was observed at the concentration of 32 µg ai/L. In all higher treatments populations decreased after each application (the second application seemed to have the severest effects on the population level), but none of the populations became extinct. Full recovery until day 40 (3 weeks after the last application) was observed at concentrations up to 180 µg ai/L. At 320 and 560 µg ai/L recovery started from day 33 onwards (except in one replicate of 560 µg ai/L), but populations did not reach control levels until day 40 in all replicates.

Conclusions: Based on nominal initial concentrations following results were found: NOEC 32 µg ai/L, LOEC: 56 µg ai/L. The population recovered without an artificial insertion of new organisms up to 180 µg ai/L within 40 days. Even higher concentrations up to 560 µg ai/L demonstrated a recovery of the population although full recovery could not be observed until the end of the study three weeks after the last application.

Aquatic community –outdoor microcosm study

Material and methods: The effects of repeated applications of fungicide A (same formulation as above) on the population and community dynamics of aquatic invertebrates (zooplankton, macro-invertebrates, emergent insects) and planktonic algae was studied under outdoor field conditions. The study was carried out using 13 enclosures in an experimental ditch. The height of the enclosures above water surface was approx. 0.15 m, water depth was 0.50 m. Each enclosure contained approx. 433 L of water. All enclosures contained some macrophytes and had a bottom layer of sediment.

The treatment consisted of 4 applications of the formulation at one week intervals, simulating spray drift. Nominal treatment levels were 10, 21.5, 46, 99 and 214 µg a.i./L. Treatments were duplicated, using 2 enclosures per treatment level and 3 controls. The test lasted for 51

days after the first application. The concentrations of the active ingredient and its metabolite in the water phase were followed over time, as were effects on phytoplankton, zooplankton, macroinvertebrates and community metabolism.

The following datasets were analyzed through univariate analysis: abundance phytoplankton taxa, abundance zooplankton taxa, abundance macro-invertebrates on artificial substrata, abundance of macro-invertebrates in emergent traps, decomposition rate of Populus leaves in litter bags and community metabolism parameters (temperature, dissolved oxygen and pH). NOEC calculations at taxon or parameter level (p = 0.05) were carried out using the Williams test (ANOVA; Williams, 1972). Multivariate statistical analysis (the Principal Response Curves (PRC) method) was used to assess changes in community structure. The statistical significance of treatment effects at a community level were also tested, using Monte Carlo permutation.

Results: The concentration of the test substance in the water column decreased very fast after application to the enclosures. At all treatment levels and after each of the applications the concentration of the test substance had decreased more than 45 % within 4 h of the application. The concentration of the metabolite always rapidly increased within several hours after each application of the test substance. The concentration of the metabolite decreased only very slightly between applications of the test substance.

Mean pH values of the water ranged between applications from 7.65 to 8.77.

The most sensitive treatment-related responses were observed for phytoplankton. According to univariate statistical analysis only 2 (*Dictyospaerium* spec., *Gonium pectorale*) of the 32 phytoplankton taxa observed in the enclosures showed consistent treatment-related decreases in densities, while one taxon (*Kirchneriella* spec.) showed an increase in abundance. The most sensitive phytoplankton species (*Gonium pectorale*) had a NOEC of 10 µg/L on several consecutive sampling dates, but recovered at all treatment levels within 16 days after the last application. For the other two affected phytoplankton taxa a NOEC of 99 µg/L was calculated. According to multivariate techniques the NOECphytoplankton was 21.5 µg/L for the total community. Both effects at species and community level were not persistent.

For 5 taxa of the 37 zooplankton taxa observed significant differences were observed on consecutive sampling days with a consistent decrease of abundance. These taxa and their NOECs are given in the following Table. Data for *Anuraeopsis fissa* should be interpreted with caution since their densities were very low when significant differences were observed. *Anuraeopsis fissa* had already recovered 6 days after the last application of the test substance. All affected taxa had recovered within 30 days of the last application of the test substance. The NOEC for the most sensitive zooplankton species and the zooplankton community resulting both from univariate and multivariate statistical analysis, was 46 µg/L. These effects, however, were not persistent.

NOEC values (µg/L) for zooplankton taxa whose abundance was consistently affected and results of permutation tests and the Williams test.

Sampling date	*Anuraeopsis Fissa*	*Keratella cochlearis*	*Keratella quadrata*	Calanoid Copepods	Nauplii	P-value	$NOEC_{community}$
- 13						> 0.05	> 214
- 5						> 0.05	> 214
2			99		21.5	> 0.05	99
6	21.5			99	46	> 0.05	> 214
9					99	> 0.05	> 214
13			99	10	99	> 0.05	> 214
16		46	99	99	99	> 0.05	> 214
20	21.5	46	46			< 0.005	46
23	46	46	46			0.015	46
27		99	46			0.035	> 214
30		46	99			0.025	99
37		46	99			> 0.05	> 214

44	99	99	> 0.05	> 214
51			> 0.05	> 214

In the enclosures 76 macro-invertebrate taxa on artificial substrates and 15 taxa in emergent traps were identified. The only taxon for which a significant change in density was observed on 2 or more consecutive sampling dates was *Erpobdella octoculata*. The number of organisms per artificial substrate of this taxon was, however, quite low, varying from 0-7 for each of the treatment levels. Effects on the abundance of this taxon were non-consistent over time. In view of the lack of a clear dose-relationship it seems unlikely that the observed fluctuations were the result of treatment with the test substance. No treatment-related effects on the macro-invertebrates were observed. The NOECmacro-invertebrates was > 214 µg/L.

No treatment-related effects on the macrophyte species composition and cover were observed as well as on the overall decomposition rate of Populus leaves in litter bags and on community metabolism endpoints (temperature, pH and dissolved oxygen content).

Conclusions: Based on the most sensitive endpoint observed in the enclosure experiment the lowest NOEC can be set at a treatment level of 10 µg ai/L (response of *Gonium pectorale*). At the community level (multivariate analysis) phytoplankton showed the most sensitive response with a NOECphytoplankton of 21.5 µg ai/L. Both effects at species and community level were not persistent. A conservative NOEAEC for aquatic primary producers and invertebrate populations can be set at 46 µg ai/L. At this treatment level consistent effects on phytoplankton were transient and confined to one species, while treatment-related responses of other ecological endpoints (zooplankton, macro-invertebrates, macrophytes, community metabolism, and decomposition of Populus in litter bags) that lasted longer than a single sampling date could not be demonstrated. An NOEAEC can also be set at 99 µg ai/L, since at this treatment level consistent but transient effects were observed for phytoplankton and zooplankton endpoints only. All affected endpoints recovered within 3 weeks after the last application.

Breakout group Monday: Fungicidal Biocide B

Mode of action

Fungicide B inhibits fungal mitotic microtubule formation.

First tier data

Application

The application concerns a slimicide, product type 12 of the Biocidal Product Directive. For example for the use in paper industry.

PEC (surface water) using (E)USES

Method of calculation	PEC calculated with the rate constants from the respective systems DT50 = 10.8 d
Main routes of entry	Chronic exposure to surface water and sediment (river) via STP (Seawage treatment plant).

Results for chronic PEC (surface water – river): = 0.40 µg/L

Toxicity data for aquatic species (most sensitive species of each group)

Laboratory tests

Group	Time-scale	Endpoint	Toxicity (mg/l)
Cyprinus carpio	96h (st)	LC_{50}	0.44
Oncorhynchus mykiss	96 h (st)	LC_{50}	0.83
Oncorhynchus mykiss	79 d (fl) ELS	NOEC mortality embryo	0.011
Daphnia magna	48 h (st)	EC_{50}	0.15
Daphnia magna	21 d (ss)	NOEC reproduction	0.0015
Chironomus riparius	28 d (st)	NOEC emergence	0.0133*
Chlorella pyrenoides	96 h (st)	E_rC_{50}	0.34**
Scenedesmus subspicatus	72 h (st)	EC_{50}	> 8.0

*) calculated a.i.value, because test substance was 500SC-formulation
**) no analytical determination of the test substance in the test medium

PNEC (surface water – see presentation regulation biocides)

Assessment factors for fresh water

Available data	**Assessment factor**
At least one short-term L(E)C50 from each of three trophic levels of the baseset (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

Risk assessment using first tier data

PEC/PNEC < 1 no refined risk assessment necessary
PEC/PNEC > 1 refined risk assessment necessary

Further tier 1 data included in the annex II or III standard data package (PPP)

Effects on earthworms

Acute toxicity	LC_{50}
	5.4 mg as/kg (14 d)
	3.9 mg as/kg (28 d)
Reproductive toxicity	NOEC
	1.0 mg as/kg

Effects on soil micro-organisms

Nitrogen mineralization	No effects up to 1.8 kg as/ha
Carbon mineralization	No effects up to 1.5 kg as/ha

Effects on biological methods of sewage treatment

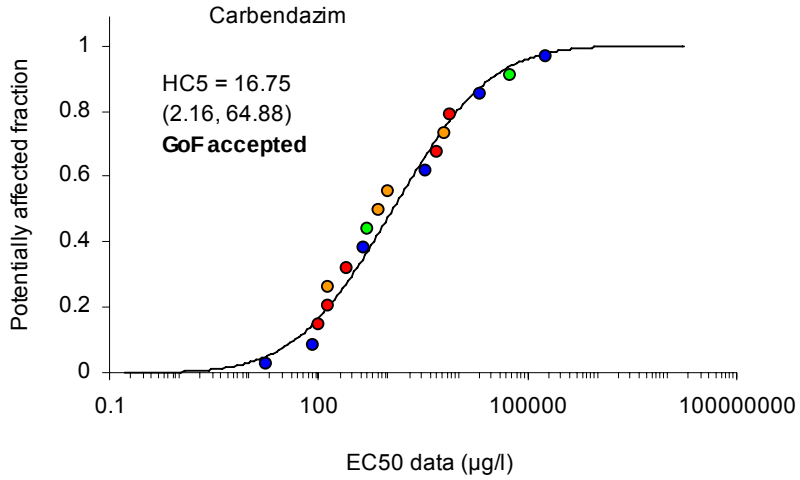
Fungicide B had no significant effects on dehydrogenase activity on soil micro-organisms in a laboratory test with 4.5 kg/ha of a formulation containing 500 g as/L. There was a significant increase in the nitrate concentration in fungicide B treated soils, without a concomitant increase in the ammonia concentration. The increase in the nitrate concentration was probably due to enhanced nitrogen mineralization from an endogenous source.
Due to low bacteriostatic potential suggested from studies concerning soil micro-organisms and soil algae, the effect to water bacteria is expected to be similar and minimal.

Higher tier assessment

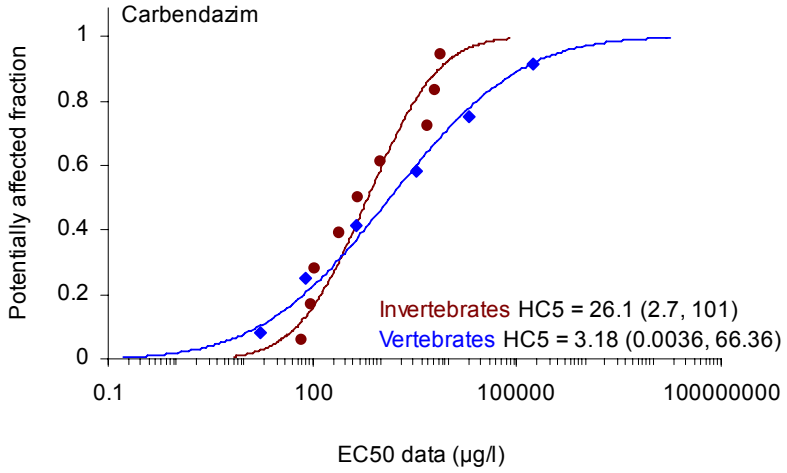
Data from studies on further species

Species Latin Name	Group		Species geo. mean EC_{50} (µg/L)
Ictalurus punctatus	VERT	Fish	16.9
Cyprinus carpio communis	VERT	Fish	80.0
Americamysis bahia	ARTH	Mysid shrimp	98.0
Gammarus pulex	ARTH	Amphipod	132.8
Dugesia lugubris	NONARTH	Planarian worm	137.2
Daphnia magna	ARTH	Crustacean zooplankton	241.3
Oncorhynchus mykiss	VERT	Fish	431.1
Chlorella pyrenoidosa	ALGAE	Green algae	484.3
Stylaria lacustris	NONARTH	Oligochaete	729.1
Dero digitata	NONARTH	Oligochaete	980.0
Poecilia reticulata	VERT	Fish	3400.0
Simocephalus vetulus	ARTH	Crustacean zooplankton	4948.0
Tetrahymena pyriformis	NONARTH	Ciliated freshwater protozoan	6400.0
Cancer magister	ARTH	Crustacean	7600.0
Rana hexadactyla	VERT	Amphibian	19862.5
Scenedesmus subspicatus	ALGAE	Green algae	54000.0
Rana limnocharis	VERT	Amphibian	173786.0
Cyprinodon	VERT	Fish	>1160

variegatus			
Ictalurus punctatus	VERT	Fish	>560
Lepomis macrochirus	VERT	Fish	>1400
Crassostrea virginica	NONARTH	Oyster	>1160
Simocephalus vetulus	ARTH	Crustacean zooplankton	>solubility
Elodea nuttallii	MACR	Macrophyte	> 10000
Myriophyllum spicatum	MACR	Macrophyte	> 10000
Potamogeton crispus	MACR	Macrophyte	> 10000
Elodea canadensis	MACR	Macrophyte	> 10000



SSD for Fungicide B based on the data available for all species.



SSD for Fungicide B based on the data available for invertebrates and vertebrates.

Breakout group Monday: Fungicidal Pesticide A

Mode of action, physico-chemical data, toxicity data for aquatic species, further tier 1 data and higher tier data are the same as fungicidal biocide A sheet.

Tier 1 data

Application

Spray application: PEC (surface water) using FOCUS

PEC water:	main route of entry: spray drift (fungicide A hydrolyses completely between applications and thus the maximum values were obtained by calculations for single applications)
PEC sediment:	residues of fungicide A in sediment (in water/sediment tests) did not reach 10 % of the applied radioactivity at any time

Crop	Width of buffer strip (m)	PECsw of fungicide A (μg/L) Northern Europe	Southern Europe
1	0	109.55	78.65
	5	74.62	42.05
	10	44.31	18.00
	15	20.82	9.05
	20	10.39	5.45
2	0	48.12	53.47
	5	21.72	24.13
	10	7.38	8.20
	15	3.90	4.33
	20	2.52	2.80
3	0	23.08	11.54
	5	4.75	2.38
	10	2.42	1.21
	15	1.67	0.83
	20	1.25	0.63

Risk assessment using first tier data

Toxicity/exposure ratios for the most sensitive aquatic organisms:

Appl. rate (kg ai/ha)	Crops	Organism	Time-scale	TER[1)]	TER Annex VI trigger[2)]	refined risk assessment
1.1 - 2.5	1, 2, 3	fish	acute flow-through	0.21 - 64	100	refined risk assessment necessary
			acute static	0.58 - 173		
			laboratory microcosm	0.59 - 176	5	
		daphnia	acute flow-through	2.5 – 760	100	refined risk assessment necessary
			acute static	4.8 - 1440		
		Algae	chronic static	67 - 20040	10	not necessary

[1)] Calculated for different PEC depending on crop
[2)] A refined risk assessment is performed in case the TER-tier 1 is lower than the Annex VI-trigger.

Risk assessment based on higher tier data

Considering all experimental data, fish are clearly the most sensitive aquatic organisms. Therefore, the final risk assessment for fish can be considered to cover all aquatic organisms. Since the multiple application outdoor mesocosm study with rainbow trout (NOEC ≥ 60 µg a.i./L) considers multiple applications under practical use conditions together with an assessment of effects on a species of fish which is amongst the most sensitive species, this outdoor mesocosm study can be considered as the final study being relevant for the risk assessment for aquatic organisms.

It seems justified to use the NOEC of ≥ 60 µg a.i./L for the evaluation of the risk, since the outdoor mesocosm study with rainbow trout considers aspects as species sensitivity, exposure under natural conditions, multiple applications, and the range of pH in natural water bodies. However, due to the pH-dependent hydrolytic instability of fungicide A, an assessment factor of 1.4 should be implemented into the risk assessment (Zebrafish: LC50 pH8 / LC50 pH6 = 1.4) in order to extrapolate the findings to a pH of 6 which is the lower end of the pH-range considered as a relevant for aquatic ecosystems in agricultural areas in Europe (Aquatic Guidance Document 2002; Heneghan 2000: Internet-database PondFX of February 14, 2000; and other authors).Overall the risk is considered acceptable, if a TER-value of 3 is used for the final risk assessment.

Final TER-values for toxicity of fungicide A to fish based on the result of the multiple application outdoor microcosm study and the maximum PECsw.

Toxicological Endpoint: Rainbow trout, chronic, 35 d, NOEC multiple application outdoor

	TER based on maximum PECsw of fungicide A[1]					
Width of Buffer Strip [m]	1	5	10	15	20	30
Crop 1 (Northern Europe): 1.1 kg ai/ha; 7 applications / 7 d interval	---	0.80	1.35	2.88	5.77	15.4
Crop 1 (Southern Europe): 1.5 kg ai/ha; 3 applications / 7 d interval	---	1.43	3.31	6.59	10.9	22.2
Crop 2 (Northern Europe): 1.8 kg ai/ha; 8 applications / 10 d interval	---	2.78	8.11	15.4	24.0	46.2
Crop 2 (Southern Europe): 2.0 kg ai/ha; 3 applications / 8 d interval	---	2.49	7.32	14.0	21.4	40.0
Crop 3 (Northern Europe): 2.5 kg ai/ha; 3 applications / 8 d interval	2.61	12.5	25.0	35.3	46.2	72.3
Crop 3 (Southern Europe): 1.25 kg ai/ha; 3 applications / 7 d interval	5	25.0	50.0	72.3	96.8	143

[1] after the last application; in case of apples in Northern Europe maximum value during the application period

TER > 3 is met with corresponding buffer zone (risk is considered acceptable)

Conclusion: The final ecotoxicological endpoint of ≥ 60 µg ai/L (fish outdoor mesocosm with multiple applications) was compared to environmental concentrations calculated according to actual drift rates. The results demonstrate that fungicide A can be used in Southern Europe in crop 3 without any buffer zone. In Northern Europe a buffer zone of 5 m is needed necessary to exclude unacceptable risks for aquatic organisms. Depending on the use rate of the application in crop 1 and 2, a buffer zone of 10 or 20 m is sufficient to exclude unacceptable risks for aquatic organisms.

Breakout group Monday: Fungicidal Pesticide B

Mode of action, first tier toxicity data, further tier 1 data and higher tier data are the same as fungicidal biocide B sheet.
First tier data

Tier 1 data

Application

Spray application: PEC (surface water) (Annex IIIA, point 9.2.3)

Method of calculation	PEC calculated with the rate constants from the respective systems DT50 = 10.8 d Water depth: 30 cm; PEC calculated for 1 m, 5 m and 10 m distance
Application rate	2 x 500 g as/ha (oilseed rape); Interval: 21 d
Main routes of entry	Spray-drift; 82nd percentile Run-off; 0.5 % of soil residues enter the water body

Results for spray drift, 1 m distance

$PEC_{(sw)}$ [µg/L]	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	-	-	5.07	-
Short term 24h				
2d 4d	-	-	4.69	4.88
	-	-	4.36	4.70
	-	-	3.79	4.39
Long term 7d				
14d 21d 28d	-	-	3.14	3.99
42d	-	-	2.16	3.30
	-	-	1.58	2.81
	-	-	1.19	2.45
		-	0.69	1.94

Results for spray drift, 5 m distance

$PEC_{(sw)}$ [µg/L]	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	-	-	1.0	-

Results for spray drift, 10 m distance

$PEC_{(sw)}$ [µg/L]	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
Initial	-	-	0.51	-

PEC (sediment)

Method of calculation	PEC calculated with the rate constants from the respective systems A and B Water depth: 30 cm; Sediment depth: 0.05 m; Bulk density of sediment: 0.8 g/cm^3 PEC calculated for 1 m, 5 m and 10 m distance
Application rate	2 x 500 g as/ha (oilseed rape); Interval: 21 d

Main routes of entry	Spray-drift; 82nd percentil

Results for spray drift, 1 m distance ($PEC_{(sed)}$ [µg/kg])

Days after max. conc. (after 1st appl.)	Single application Actual	Single application Time weighted average	Multiple application Actual	Multiple application Time weighted average
0 (37)	–	–	37.13	
1 (38)	–	–	37.09	37.11
2 (39)	–	–	37.02	37.08
4 (41)	–	–	36.78	37.00
7 (44)	–	–	36.29	36.80
14 (51)	–	–	34.82	36.19

Risk assessment using first tier data

Long term TER-values for aquatic invertebrates *Daphnia magna* ($NOEC_{21d}$: 1.5 µg/L)

Scenario: Oilseed rape, 82nd percentile drift factor
Application rate: 2 x 0.500 kg as/ha

Distance	Drift rate [%]	$PEC_{ini;sw,}$ [µg as/L]	TER	Annex VI Trigger
1 m	2.38	3.97	0.4	10
5 m	0.47	0.78	1.9	10
10 m	0.24	0.40	3.8	10
15 m	0.16	0.27	5.6	10
20 m	0.12	0.20	7.5	10

Taking into account all toxicity data available, adequate risk mitigation measures (i.e. buffer zones) are considered necessary in order to avoid unacceptable effects to aquatic organisms.

Composition of the Monday breakout groups

	Chairmen	Rapporteurs
Group 1 (biocides)	Theo Brock	Peter Dohmen
Group 2 (biocides)	Ralf Schulz	Udo Hommen
Group 3 (pesticides)	Felix Bärlocher	Jane Howcroft
Group 4 (pesticides)	Martina Ross-Nickoll	Steve Maund

Assignment:

Group 1	Group 2	Group 3	Group 4
Theo Brock	Ralf Schulz	Felix Bärlocher	Chris Leake
Peter Dohmen	Udo Hommen	Jane Howcroft	Steve Maund
Helene Roussel	Roger Pickup	Fred Heimbach	Anneli Widenfalk
Peter Lawlor	Eric Bruns	Martina Ross-Nickoll	Naomi Blake
Colin Brown	Milena Horvat	Elena Redolfi	Peter Matthiessen
Domenica Auteri	Paul Van den Brink	Mechteld van Dijk	Ivo Roessink
Lorraine Maltby			

Floaters

Floor Peeters
Lina Wendt-Rasch

Appendix 2

Fungicidal pesticide A

1. No data on aquatic fungal species but it is unclear how important these are to the aquatic ecosystem. There are several fundamental questions related to natural variability, recovery and impact from their loss which need to be answered, One also needs to differentiate between lakes and streams. There is a lot of information available for lakes and importance of fungi in reed areas; species, processes, nutritional requirements, growth rates and recovery to natural pressures. The fungi present above the water on the reeds are important for the breakdown of the foliage when the plant dies at the end of the season. However, this pesticide is not persistent and the level from spray drift will be much reduced on the reeds. Therefore the impact on non-target fungi cannot be defined as the effective concentration and of recovery of these are unknown. One needs a suitable test system.
2. Carbon mineralization from effects on soil microorganisms can provide some information, but this is limited.
3. The degradation test using Populus leaves in field mesocosm studies. Litter degradation is critical measure to decide whether the functionality of the ecosystem, is effected. This is an accepted approach for the terrestrial environment. The question is if a functional endpoint is sufficient or do we also need a structural endpoint, which are possibly more sensitive (but are these feasible ?). Also tests which report the effects on pH and/or Oxygen levels post application of the pesticides to natural aquatic systems could provide indicators.
4. The lack of specificity of the MOA of Pesticide A means that the effects on aquatic organisms cannot be predicted. The data suggests that fish are the most sensitive, so mitigation could be triggered (prevent spray drift). However, we have no data on the effect against non-target fungi, which could be exposed on aquatic macrophytes. However, as spray drift is a reduced amount of effective concentration in field this may have no impact. With this being a non-systemic, aquatic fungi might not be affected at all if its fate is also considered. Are the safety factors used with the data sufficiently protective of the non-target fungi, i.e. 100 ? If so then there should be no trigger for higher tier testing based on MOA alone.
5. If there was an effect on fungal population in mesocosm, which had an impact on fish then these studies will take this into account. The aquatic community study addresses this.
6. What is the role of aquatic fungi in the ecosystem? We need to identify all of the roles to then consider the wider impacts. However, the study states that all of the affected endpoints recovered within 3 weeks after the last application.
7. This was a multi-site fungicide therefore the approach taken with a full range of species was correct. However, if we had a fungicide with a specific MOA then only/or at least those species which are thought to be affected must be tested in the mesocosm studies.

Fungicidal biocide A

1. The following information is lacking : PEC data for the metabolite (however, low toxicity); PEC sediment for a.s., BCF for the a.s., sediment species for the a.s., fish eating birds and mammals (log Pow > 3). While the main risk to fish has been identified, no specific major risks are identified due to the fungicidal mode of action.
2. Yes, at least get some useful indication of potential risk or about likelihood of risk (e.g. no indication for major effects on microbial activity, see nitrogen and carbon mineralization, STP activity; low toxicity to oligochaets). But information on the stability of the compound in soil would be useful to make sure, that sufficient exposure was given in the terrestrial tests.
3. Yes, e.g. fish bioaccumulation and additional marine species (additional NOECs to lower A F)
4. Not the MoA alone, but in combination with the problems not solved in 2. and 3. would be a trigger for higher tier testing
5. Yes since the acute HC5 (based on mean) is more than 20 times higher than the maximum PECs (chronic) and thus significantly above a suggested trigger of 1-5 (however, this was based on fish data only and thus not really according to the TGD). In addition the acute to chronic ratio is small (2-5).
6. No

7. The freshwater mesocosm studies simulated the rapid degradation of the a.s. and may therefore not sufficiently cover chronic risk. However, it provides information on which groups of organisms might be particularly sensitive and thus whether the data available are adequate to address the risk. There is no indication in this case that another taxonomic invertebrate group was substantially more sensitive than aquatic the standard test species (e.g. daphnia). However, algae were affected in the mesocosm at significantly lower levels than expected from standard tests.

Fungicidal pesticide B

1. We need to know if there were any bioaccumulation potential. We need to know the log P, and any subsequent BCF values. This would give an indication of bioaccumulation potential. These data are available under 91/414. Soil micro data and lab/field soil and water-sediment fate studies (degradation occurring), sewage studies gives some reassurance about potential effects on function. Data from the efficacy dossier might give an indication of which fungal taxa might be expected to be sensitive. Although we have reassurance about function, structural endpoints are not covered which may lead to a more resilient community. The question is, is this a problem? There are also concerns that there are potential differences in sensitivity from the standard test species. Will there be more sensitive species? Are all modes of action captured by the standard tests ? Data we've seen earlier looks encouraging but considering the broad range of modes of action of fungicides, we would be happier to see more data of this sort. When doing so, one need to consider the mode of action, and the indications from other parts of the dossier. We expect chronic issues because of its mode of action – borne out by Daphnia ACR. Would want to know when the effect was observed in the Daphnia chronic study. Algae might be more sensitive because of the mode of action, but might expect low levels of phytotoxicity because these synthetic organic agricultural fungicide do not affect this. Mode of action is not everything, you also have to consider the ADMEK issues too.
2. Yes
3. Does structure matter? There is a lack of tests to measure effects on fungi community structure. Our concern for fungi is really focused on function, since they are important for aquatic fungi for breakdown of leaf litter. We do not know the correlation between structure and function. However the evidence to data is that function is OK, so we could probably feel reassured. Do we need to know how robust the function is? Our biggest uncertainty is what is going on in freshwater? Do we know how well the soil tests read across to the water-sediment test. How relevant are the degradation data for telling us what is going on with fungi. Is it bacterially mediated? Do we need to equivalent of a aquatic litter bag test? But we need to think about how we would use the data in decision making.
4. No
5. Daphnia do seem to be pretty sensitive, since there are no organisms that are hugely more sensitive. The higher-tier data do not address the concerns about fungal communities. Catfish data, however, look odd The acute data used for the higher-tier assessment and the compound clearly implicate chronic concerns. Are these adequately addressed for the acute data? Interesting point is that the 'all organisms' SSD would meet the criteria for using all the data together. However, the fish SSD is significantly more sensitive than invertebrates. However the data set is smaller, so the HC5-95 may be being affected by this. We debated the merits of separating the taxa. Pros are that the analysis is more robust and there is no evidence to suggest fish and invertebrates are significantly different. Breaking out the fish has an enormous impact on the confidence intervals.
6. In this case, we don't think the higher-tier data take us any further forward.
7. Maybe a mesocosm study would have been a good idea and perhaps we could have included decomposition bags. Pay particular attention to the detritivores in the study and make sure that there are no secondary effects on invertebrate populations. The risk assessment should consider the mode of action carefully, which in this case is clearly a chronic concern to fish and invertebrates. Maybe an alternative would be to set up a Gammarus population study to look at leaf litter bags.

Fungicidal Biocide B

1. No information is provided about effects on aquatic organisms like bacteria and especially fungi which might be sensitive due to the mode of action of the test item. Also no information is available about sensitivity of non-arthropod invertebrates. Due

to the unspecific MoA of fungicides arthropods can not be assumed to be the most sensitive invertebrate group. A NOEC for algae is missing to get a feeling for the slope of the dose-reponse curve for this group. Also no information is available about sensitivity of macrophytes.

2. No, because the additional data do no refine the risk for invertebrates, fish and algae. However, the earthworm test provides information about an additional invertebrate, but concentration in mg as / ha are difficult to compare with water concentrations. The other tests provide some data about possible effects on microbial communities, but the tests might be driven by bacteria and not fungi which might be more sensitive. In addition, the tests analyse function and not structure. It was assumed, that if exposure can be extrapolated (from mg as/kg soil to mg as/kg sediment or mg/L), also the effects can be extrapolated. Aquatic microbial communities are considered to show similar sensitivity than soil communities. It was also noted, that dehydrogenase activity would not always be the best endpoint to analyse effects on fungi. Test on ready biodegradability and inherent biodegradability test were considered to be more related to fate than to effects. If a substance is not biodegraded it might be due to a toxic effect on the microbes but it could also be a results of the stability of the substance.
3. One option could be to test additional invertebrates (molluscs, worms) and fish to reduce uncertainty in species to species extrapolation. Due to the focus on long-term exposure and chronic effects, chronic tests with NOECs are desirable. Alternatively additional acute test could be used to identify sensitive species which can than be tested for chronic effects. Acute chronic ratios (here between 80 for trout and 100 for Daphnia) could be used to extrapolate from an SSD of acute data to an HC5 for chronic effects (NOECs). Because in the standard test Daphnia was the most sensitive species, micro- and mesocosm studies would provide data for invertebrate communities under more realistic exposure conditions.
4. No, because it might be possible to refine the PEC estimation before doing higher tier testing.
5. No, only acute data are provided. Applying an acute-chronic ratio of 100 to the HC5 of 16.75 µg/L for the whole data set or 26.1 µg/L for the invertebrates only, the PEC/PNEC ratio is still higher than 1. There is also still uncertainty about the sensitivity of non-arthropod invertebrates (only one mollusc species was tested). Macrophytes seem to be not at risk (4 species with EC50 > 10000 µg/L).
6. There is still uncertainty about the sensitivity of fungi and bacteria, while also data on relevant non-arthropods are still missing (snails, rotifers).
7. The following options were discussed:
    - Covering broad range of non-arthropods
    - SSD should be based on NOECs (or LC50s and ACR based not only on one species)
    - Consider analysis of effects on fungi (more details Tuesday?)
    - Consider more detailed analysis of destruents in micro-/mesocosms
        - Bacterial / fungal biomasses
        - Detritivorous (abundance, function)
        - Microbial bioassays with sediment taken from the mesocosms