

Biodegradation and elimination of industrial wastewater in the context of whole effluent assessment

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*Das Wasser ist ein freundliches Element für den, der damit bekannt ist
und es zu behandeln weiß.*

Johann Wolfgang von Goethe (1749 - 1832)

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LIST OF ABBREVIATIONS

AbwAG	Abwasserabgabengesetz (German Wastewater Charges Act)
AbwV	Abwasserverordnung (German Wastewater Ordinance)
ANOVA	Analysis of Variance
AOX	Absorbable Organic Halogens
ASTM	American Society for Testing and Materials
BAT	Best Available Techniques
BCF	Bioconcentration Factor
BOD	Biological Oxygen Demand
BREF	Best Available Techniques Reference Document
CAS	Chemical Abstracts Service
CMR substances	Carcinogenic, Mutagenic or Reprotoxic Substances
COHIBA	Control of hazardous substances in the Baltic Sea region
COMMPS	Combined Monitoring-based and Modelling-based Priority Setting scheme of the WFD for selecting priority substances
DIN	Deutsches Institut für Normung
COD	Chemical Oxygen Demand
DMDTC	Dimethyldithiocarbamate
DMN	2,3-dimethylnaphthalene
DOC	Dissolved Organic Carbon
DTA	Direct Toxicity Assessment
DYNAMEC	OSPAR's Dynamic Selection and Prioritisation Mechanism for Hazardous Substances
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
EC ₅₀	50% Effect Concentration
EN	European Norm
EPA	US Environmental Protection Agency
EU	European Union
HELCOM	Helsinki Commission for the protection of the marine environment of the Baltic Sea
IPPC Directive	Integrated Pollution Prevention and Control Directive
ISO	International Organization for Standardization
LID	Lowest Ineffective Dilution
Log K _{ow}	Octanol Water Partition Coefficient
NOEC	No Effect Concentration
OECD	Organisation for Economic Co-operation and Development
OSPAR	Oslo/Paris Convention for the protection of the marine environment of the North East Atlantic
PBS	Potentially Bio-accumulative Substances

PBT	Persistent, Bioaccumulative and Toxic
PEC	Predicted Environmental Concentration
PNEC	Predicted no Effect Concentration
REACH	Regulation on Registration, Evaluation and Authorisation of Chemicals
SETAC	Society of Environmental Toxicology and Chemistry
SPME	Solid Phase Micro-Extraction
TGD	Technical Guidance Document (European Commission 2003)
TIE	Toxicity Identification Evaluation
TOC	Total Organic Carbon
UBA	Umweltbundesamt (Federal Environment Agency of Germany)
US EPA	U.S. Environmental Protection Agency
WEA	Whole Effluent Assessment
WEER	Whole Effluent Environmental Risk (Dutch WEA concept) ⁷
WET	Whole Effluent Toxicity
WFD	Water Framework Directive
WHG	Wasserhaushaltsgesetz (German Federal Water Act)
WWTP	Wastewater Treatment Plant

1 GENERAL INTRODUCTION

1.1 Wastewater evaluation with bioassays

Substance specific versus whole mixture toxicity approach

Most industrial effluents can be considered as complex mixtures of substances where only a fragmented knowledge of the components is available. Substance specific analytical methods do not comprise all compounds or exceed the available resources in terms of finances and time. The chemical specific approach is also limited since even if all substances could be analysed there is often a lack of ecotoxicity data. Additional degradation-products and combined effects of substances present in the discharges are not being taken into account with chemical analysis. Thus wastewater analysis mainly consists in the application of sum parameters like TOC, COD, or AOX whose ecotoxicological relevance remains unclear. Therefore researchers and authorities have developed different approaches for direct ecotoxicological assessments of complex effluents. With bioassays the effects of all compounds present in a complex sample are accounted for. Any synergistic or antagonistic interactions between the compounds are inherently captured in the observed responses of the exposed organisms. Toxic effects of bioavailable substances are measured directly and therefore all kinds of hazardous substances including their degradation products are considered.

Effluent testing has often been referred to as an important example of the whole mixture toxicity approach (Kortenkamp et al. 2009).

Already in the 1940s and 1950s numerous investigations on the effects of wastewater contaminants have been published. Liebmann et al. (1958) gives a first overview about the German-speaking publications. Here, the focus was on effects of well known contaminants such as chlorine, ammonium, cyanide, heavy metals and selected organics to several target organisms (mainly fish but also invertebrates). Both the contaminants themselves and the wastewater containing them have been analysed, often under different environmental conditions (temperature, pH, oxygen supply...). The test design of these experiments followed the ecological question to be answered such as the impact of discharges on fish survival.

Development of test guidelines

In the early 1970s the first testing guidelines were developed for wastewater evaluation with bioassays, which were mainly adapted from those used for chemical analysis. In 1978 the toxicity test with luminescent bacteria became commercially available. In 1980 the US-EPA began developing short-term toxicity tests for estimating chronic toxicity. Environment Canada also developed several test guidelines. In the 1990s the Direct Toxicity Assessment approach was initiated by regulators in the United Kingdom and a series of guidelines have been produced for specific test methodologies (Whitehouse et al. 2004). The OSPAR background document on Whole Effluent Assessment (WEA) gives an overview on existing international and national test guidelines applied for effluent assessments (OSPAR 2000). Updates have been presented by ECETOC (2004) and OSPAR (2007). Since the 1980s the number of ecotoxicological tests and the experience in performing tests have grown rapidly and acute and chronic toxicity testing have increasingly been used to identify and control discharges to surface water. Several authorities provide very useful information about available testing guidelines and strategies for effluent testing with bioassays (US EPA 2010, Environment Canada 2010, NIWA 2010).

Testing strategy

In parallel to the guidelines also different concepts for testing strategies have been developed. A first review about Environmental Hazard Assessment of Effluents was published by Bergmann et al. (1986). In 1995 a workshop on whole effluent toxicity at the University of Michigan provided a detailed overview (Grothe et al. 1996). The Society of Environmental Toxicology and Chemistry (SETAC) held a conference at Luton University in July 1996 and a major symposium and workshop was hosted by Zeneca (Brixham Environmental Laboratory), in Torquay in October 1996. In 1997, an OSPAR workshop on the "ecotoxicological evaluation of wastewater" was organised by the Federal Environment Agency in Berlin (German Federal Environment Agency 1997). In March 1999 another workshop on "Effluent Ecotoxicology: A European Perspective" was held in Edinburgh and experience with numerous test methods from different European countries was presented. The proceedings of this workshop, including the reviews of Chapman (2000) and La Point et al. (2000), have been published in a special issue of the journal *Environmental Toxicology and Chemistry* in January 2000.

For effect-based effluent testing a wide range of terms is used. In the United States (US) and some European countries the term ‘Whole Effluent Toxicity’ (WET) is used, in Canada the term ‘effluent toxicity test’ is generally accepted. In the UK and Australia, the term ‘Direct Toxicity Assessment’ (DTA) is used, covering both effluent and receiving environmental sample testing. In the Netherlands the term ‘Whole Effluent Environmental Risk’ (WEER) refers to both effluent and receiving water toxicity. Also scientific organisations have dealt with whole effluent toxicity concepts. The Society of Environmental Toxicology and Chemistry published a technical paper on whole effluent testing and dedicated numerous publications on this item (Grothe et al. 1996, Chapman 2000, SETAC 2004). The European Centre for Ecotoxicology and Toxicology of Chemicals as scientific organisation of the European chemical industry published a technical report on Whole Effluent Assessment (ECETOC 2004).

In principle, two major testing approaches can be distinguished: the emission-based approach and the immission-based approach. The first approach has traditionally been adopted in continental Europe and is also called the “Fixed Emission Limit” approach. Here, a uniform limit of pollutant load and concentration per unit of production is applied to all effluents (at least within an industrial sector) regardless of dilution. This approach clearly is hazard-based and follows the precautionary principle. The second approach, also called the “Environmental Quality Objective” approach, takes into account the volume, nature and use of the receiving waters. This approach assesses the concentration of pollutants and their effect on the use of the receiving water after dilution and can be considered as “risk-based”. In practice both approaches have their limitations: There is the risk that site-specific discharge limits privilege some dischargers (which would not be consistent with the IPPC objectives of implementing BAT in a sector) while fixed emission limits may lead to unnecessary investment without significant benefits to the environment or, where dilution is limited, may fail to prevent significant pollution (Whitehouse et al. 2004). Thus often a combination of both is applied.

While the different approaches differ in their strategy some common principles apply for all: It is generally recognised that no single bioassay can be used to assess the toxic effects from different modes of action. Therefore a battery of test species representing different trophic levels, typically algae (primary producers), invertebrates (primary consumers) and fishes (secondary consumers) is required. Often screening tests, adapted from test methods used for ecotoxicity assessment of chemicals (e.g. ISO, EN or OECD test guidelines), are used for effluent control, but high throughput tests, e.g. with bacteria for screening

purposes have also been developed. Acute and (short and long term) chronic tests are being applied next to tests for detecting specific endpoints such as genotoxicity. The need for quality assurance by determining the precision and accuracy of the methods applied and by implementing general principles of laboratory quality measures such as standard operating procedures and internal and external quality control is accepted, especially when the outcome is used as a basis for regulatory decisions.

Most of these efforts mainly focus on the toxicity of the effluents and/or on their impact on the environment with the notable exception of the Dutch WEER concept, which combines effect assays with degradability tests, and the ECETOC report, which addresses both the WEA approach including ecotoxicity, bioaccumulation and persistency and the biological monitoring of receiving waters.

Regulative wastewater surveillance in Germany

In Germany, the assessment of wastewater with bioassays has been put into routine regulatory practice since 1976 by introducing the acute fish toxicity test with *Leuciscus idus*. Later on other ecotoxicity tests with bacteria (*Vibrio fischeri*), daphnids (*Daphnia magna*) and algae (*Desmodesmus subspicatus*) have been considered in the Wastewater Ordinance. Further milestones were the inclusion of the umu-assay with *Salmonella typhimurium* TA1535/pSK1002 for determining genotoxic effects in 2002 and the replacement of the acute fish toxicity test though the fish egg assay with *Danio rerio* in 2004 for animal protection reasons. According to § 57 of the German Federal Water Act (WHG), discharge permits shall be granted only if the waste load is kept at least on the current BAT level (Best Available Technology). The requirements based on BAT are established by the federal government in the appendices of the Wastewater Ordinance (AbwV) for the different industrial branches and processes. They are updated according to the further development of BAT. For wastewater evaluation there are two legal regulations where whole effluent toxicity is tested:

- the AbwV (Ordinance on Requirements for the Discharge of Wastewater into Waters, Wastewater Ordinance - AbwV) based on WHG. Here, for several industrial sectors limit values for selected bioassays have been established based on the Lowest Ineffective Dilution (LID) concept, which is defined as the reciprocal volume fraction of the wastewater sample at which only effects not exceeding the test-specific variability are observed (ISO 5667-16: 1998, Annex A)

- the Wastewater Charges Act (Act pertaining to Charges levied for Discharging Wastewater into Waters, Abwasserabgabengesetz – AbwAG). In the AbwAG the fish egg toxicity test is implemented for industrial and municipal direct discharges to a receiving water body. For a limit LID of 2 no charge based on fish toxicity is imposed.

Discharge limits to different wastewater sectors are set in 57 annexes of the Wastewater Ordinance. In 25 wastewater sectors the fish egg test is part of the licensing of wastewater permits.

1.2 Whole Effluent Assessment in the context of OSPAR

OSPAR WEA Expert group

In 1999 a whole effluent assessment (WEA) expert group was established within the OSPAR convention for the protection of the North-East Atlantic (Oslo-Paris-Commission). Here, representatives from authorities and industry were asked to examine the value of effect-based wastewater analyses in helping to achieve the OSPAR objectives for protection of the marine environment. The WEA concept intends to support the objectives of the OSPAR Hazardous Substances Strategy by controlling point emissions to the aquatic environment. The author of this thesis participated in this expert group on behalf of the German Federal Environment Agency in order to support their activities in the development of effect-based wastewater evaluation. Several background reports about suitable methods for determining "genotoxicity", "endocrine disrupters", and "biodegradation and persistence" were prepared and two practical programmes with real wastewater samples were organised. In 2007 the work was concluded with a guidance document on how to apply WEA testing in practice (OSPAR 2007b).

Relationship between WEA and the control of priority substances

The OSPAR strategy on hazardous substances has the objective to achieve (very) low levels of hazardous substances in the marine environment by continuously reducing discharges, emissions and losses of hazardous substances. Hereby the hazard characteristics of substances are assessed with respect to their persistence, bioaccumulation potential and toxicity (PBT criteria) according to the DYNAMEC system (Dynamic Selection and Prioritisation Mechanism for Hazardous Substances, OSPAR Commission 2006). In principle, the selection of priority substances consists of a set of criteria on selected intrinsic properties of the substances and a safety net where other criteria, which

give rise to an equivalent level of concern, are considered. This approach is similar to the selection of candidate substances in the context of the Water Framework Directive 2000/60/EC (WFD) according to the COMMPS approach (Combined Monitoring-based and Modelling-based Priority Setting scheme) (Denzer et al. 1999). Table 1.1 compares some basic principles of both approaches.

Table 1.1: Selection of priority substances according to OSPAR and the WFD

Cut of values	OSPAR DYNAMIC	WFD COMMPS
Persistency (P)	Half-life ($T_{1/2}$) of 50 days *)	Attribution of degradation factors based on aquatic biodegradation: Ready biodegradable 0.1 Inherent biodegradable 0.5 Persistent 1.0
Liability to Bioaccumulate (B)	$\log K_{ow} \geq 4$ or $BCF \geq 500$	Score Log K_{ow} BCF 0 < 3 < 100 1 3 - < 4 100 - < 1000 2 4 - < 5 1000 - < 10000 3 > 5 > 10000
Toxicity (T)	LC50 or EC50 acute ≤ 1 mg/l, long-term NOEC ≤ 0.1 mg/l or chronic mammalian toxicity or carcinogenic, mutagenic or reprotoxic substances (CMR)	Scoring of Predicted No Effect Concentrations (PNECs) extrapolated from chronic or acute data according to the TGD (European Commission 2003). CMR properties as well as chronic effects on human (oral uptake) (e.g. score 2 for R45, R46, R47, R60 or R61)

*) According to the TGD a half-life of 50 days in surface water has been attributed to substances, which are readily biodegradable, but failing 10-d window (European Commission 2003)

The OSPAR list of chemicals for priority action currently includes 42 substances or groups of substances while Annex X of the WFD refers to 33 priority substances and Annex VIII of the WFD refers to 12 other main pollutants such as organohalogens, organophosphorus or organotin compounds. Since 2007 OSPAR's work on the selection and prioritisation of substances has stopped in the light of other activities in the chemicals sector in the European Community, namely developments under the Water Framework Directive and the regulation on registration, evaluation and authorisation of chemicals (REACH). Instead, OSPAR collaborates with the EC on these issues (<http://www.ospar.org/>).

The process for including further priority substances into Annex X of the WFD is going on. Annex III of Directive 2008/105/EC indicates further substances that are subject to review for possible identification as priority substances.

The Commission commissioned a study on monitoring-based prioritisation of further potential priority substances candidates (James et al. 2009). For 316 substances selected as candidates for prioritisation, monitoring data were analysed and predicted no-effect concentrations (PNEC) in water, sediment and/or biota were derived. Priority was assigned according to risk ratios, i.e. PEC/PNEC. In total 44 organic substances have been selected for further evaluation. Alongside this research project the European Chemicals Bureau coordinates an advisory group to the European Commission which has elaborated a new concept for an optimised prioritisation strategy for future ranking. For substances for which monitoring data are not available at the required quality level, a modelling-based approach to assess potential exposure needs to be implemented. Information such as overall tonnage used, fractions of this tonnage going to particular uses and emissions from these uses may be used as input to a simple partitioning model (Lepper et al. 2008). It is expected that as a result of all these activities about 10-20 priority substances will be selected for inclusion in Annex X of the WFD by January 2011. However, according to Brack et al. (2007, 2009) numerous studies did not demonstrate a clear cause-effect relationship between environmental concentrations of priority pollutants and ecotoxicological effects or the ecological status at many sites under investigation. Thus, the limited number of chemicals on the priority pollutant list may not be the sole or major driving force for poor ecological status at many sites. As chemical analysis of pre-selected sets of toxicants often does not explain ecotoxic effects of complex environmental samples the authors propose a combined biological and chemical-analytical approach for identification of newly emerging toxicants (Brack et al., 2007, 2009).

This discourse on priority substances clarifies that although great efforts are undertaken for selecting and prioritizing hazardous substances in water policy the chemical-based approach is rather limited when applied to complex effluents. There still remain many substances to be assessed and only a few compounds will be included in routine monitoring programmes. It is generally recognised that in complex samples, only a small fraction of the substances present can be analytically identified. Often only a part of adverse effects measured in effluents can be related to the PBT properties of identified substances (OSPAR 2005). According to Whitehouse et al (2004) it is important to bear in mind that while much of the objectives environmental legislation is concerned with the

control of individual chemicals, the ultimate goal is concerned with biological outcomes e.g. protection of human health, the natural environment, or fisheries. The WFD for the first time referred to biological objectives for the protection of water quality by introducing the objective of the “good ecological status” of all groundwaters and surface waters by 2015.

OSPAR WEA strategy

The difference of the OSPAR WEA strategy compared to previous concepts on wastewater evaluation with bioassays is that it does not only focus on the toxicity (T) of the mixture but is extended to include also persistence (P) and bioaccumulation (B). That means that the same PBT-criteria that are used within OSPAR’s Hazardous Substances Strategy for identifying priority substances are applied to the entire effluent sample instead of to the individual substances (Kortenkamp et al. 2009).

The basic WEA flowchart as described in the OSPAR WEA guidance document (OSPAR 2007b) shows the sequence in testing of the parameters persistency, bioaccumulation and toxicity. Persistency is not be assessed as a separate parameter, but combined with other parameters. The flowchart starts with a persistency test (degradation test) to remove the majority of non-persistent substances. After this ‘pre-treatment step’, the treated sample is used for testing toxicity and bioaccumulation. This combination of tests reveals the persistent levels of acute and chronic toxicity and bioaccumulation (Figure 1.1).

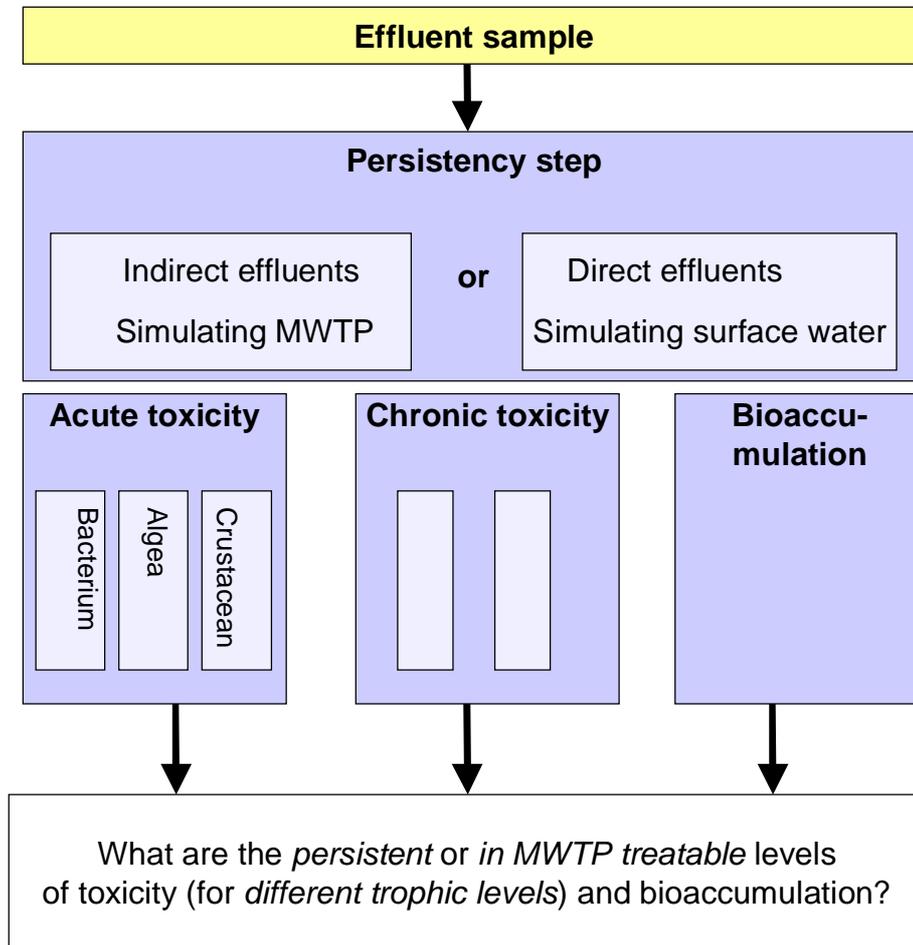


Figure 1.1: OSPAR basic flowchart for WEA

For persistency there is a basic difference between the tests to be applied for direct effluents and for indirect effluents. Effluents directly discharging into surface water should be analysed by methods using low inoculum density (ready biodegradability type tests) of the OECD 301 series. The DOC die-away method according to OECD 301A, which uses up to 10 vol. % surface water as inoculum is one example of this test category. For indirectly discharged effluents the degradability (as a more appropriate term than persistence) should simulate the elimination behaviour in WWTP by using a high inoculum concentration (inherent biodegradability type tests). The Zahn-Wellens test (OECD 302B) is the most prominent test of this test category. In this context, an indirect discharger means a nondomestic discharger introducing “pollutants” to a publicly owned WWTP. The different tests should attempt to identify the extent to which toxicity or bio-accumulative potential will be removed in the receiving waters on the one hand or in a WWTP on the other hand. In principle the (relatively effortful) degradation step can also be omitted, if no outstanding toxicity of the effluents is expected.

The application of the Zahn-Wellens test for indirect dischargers can also be justified with the Water Framework Directive 2000/60/EC and the IPPC Directive 2008/1/EC concerning integrated pollution prevention and control, where the following definition has been included.: *"The emission limit values for substances shall normally apply at the point where the emissions leave the installation, dilution being disregarded when determining them. With regard to indirect releases into water, the effect of a waste-water treatment plant may be taken into account when determining the emission limit values of the installations involved, provided that an equivalent level is guaranteed for protection of the environment as a whole and provided that this does not lead to higher levels of pollution in the environment."* (Article 2 (40), 2000/60/EC and Article 2 (6), 2008/1/EC).

Acute and chronic toxicity should preferably be tested at more than one trophic level (usually bacteria, algae, crustacean, or fish) in order to obtain a broad insight of the effects on the different levels within the ecosystem. This is in line with the assessment of chemicals. Tests that measure acute toxicity in effluents are more developed than those that address chronic toxicity. Thus OSPAR (2007) did not recommend specific chronic tests. Next to aquatic toxicity tests such as the *Daphnia magna* reproduction toxicity test also testing of genotoxicity, mutagenicity, and endocrine effects has been viewed as involving "chronic effects". Several tests on genotoxicity/mutagenicity of water samples have been standardised and one (the umu test according to ISO 13829) is routinely applied in Germany. Compared to genotoxicity evaluation, tests for determining endocrine disruption in effluents are less far developed and standardised. The OSPAR survey on test methods for endocrine effects revealed that standardised and validated test methods designed specifically to identify endocrine effects in aquatic organisms are not available. However, in the scientific literature many in vitro and in vivo methods have been described, which have the potential of eventually becoming a tool to be used in whole effluent assessment (OSPAR 2003).

Considering bioaccumulation, two tests are available, both based on extraction and giving an indication of the presence of potential bioaccumulating substances (PBS). The liquid-liquid extraction method (LLE) is applied in Sweden on a routine basis for many years and reflects the total extraction of potentially bio-accumulative substances, including the fraction bound to particulate matter. The Solid Phase Micro-Extraction (SPME) method is a more recent method, reflecting more closely the possible bioaccumulation in the ecosystem, and only measures bio-available substances. Briefly, after absorption of the PBS to the SPME fibre these are inserted into a gas chromatograph where they are

thermodesorbed. The whole chromatogram is integrated and normalised to a reference compound with the log Kow of interest. In the OSPAR WEA Guidance document preference is given to the SPME method because of its technical advantages (simpler, shorter and cheaper) and because it better reflects the potential to bioaccumulate in an ecosystem (OSPAR 2007b).

The bioassays in the WEA approach can be considered as a kind of sum parameter which complements chemical sum parameters (AOX, TOC, COD, N_{total}, PBS). The advantage is that only a small number of parameters is needed and the constituents of effluent are described in a more comprehensive way than by single chemical analysis. Nevertheless chemical characterisation of effluents is still necessary and information on hazardous substances in effluents should be considered. Thus, WEA must be seen as a safety net for the substance-by-substance approach and does not replace existing approaches with regard to the reduction of releases of hazardous substances. WEA can be used as a complementary tool to the substance-based approach in order to reach the objectives regarding hazardous substances (OSPAR 2005).

Relationship between WEA and the IPPC Directive

A major outcome of the IPPC Directive (2008) is the development of BAT reference documents (BREF). The performance of BAT can also be expressed as the absence or reduction of negative effects in effluents measured by means of WEA parameters. So far, WEA testing has been incorporated in 5 (out of 33) BREF documents (table 1.2).

Table 1.2: Reference to bioassays and WEA in selected BREFs

BREF	Year	References to bioassays
Industrial Cooling Systems	2001	The luminescent bacteria toxicity test is applied for determining the content of biocides and the time for which the circuit should be kept closed before discharging the cooling water.
Common Waste Water and Waste Gas Treatment/Management Systems in the Chemical Sector	2003	Chapter 2.2.1.2 addresses the evaluation of the composition and quantity of waste water and waste gas streams in a stream inventory or register, including data on biodegradability (results from modified Zahn-Wellens-Test, refractory COD/TOC loads). The WEA concept is described in detail. It is stated that WEA is a useful tool for integrated pollution prevention and control, but will complement traditional chemical-based controls, rather than replace them. Experience shows that when measures of P-B-T within a well-designed WEA programme are implemented, they result in reductions of releases of hazardous substances into waste water.
General Principles of Monitoring	2003	Chapter 5 describes “surrogate parameters” that are closely related to conventional direct measurements of pollutants, and which may therefore be monitored and used instead of the direct pollutant values for some practical purposes. Toxicity parameters are referred as a special group of surrogate parameters. Fish/fish egg test, daphnia test, algae test and luminescent bacteria test are all common test methods for the toxicity assessment of complex waste water streams. They are often used to obtain additional information to the information that can be gained from sum parameter measurements (COD, BOD, AOX, EOX...). Toxicity tests, when used in combination with direct measurements of specific substances and with the measurements of sum parameters, are increasingly becoming a set part of any Whole Effluent Assessment strategy (WEA).
Large Volume Organic Chemicals (LVOC)	2003	Chapter 5.4.3 on monitoring of water emissions refers to bioassays as an important tool for the evaluation of LVOC waste waters both before and after treatment. Several national approaches to include bioassays in wastewater permits are described. Chapter 14.2.3 states, that WEA may have greater value for LVOC waste waters. For details reference is given to the horizontal BREF on Common Waste Water and Waste Gas.
Manufacture of Organic Fine Chemicals	2006	Chapter 4.3.8.19 describes WEA as a management tool for treatment of waste water streams. The effectiveness of the treatment of waste water streams can be evaluated with ecotoxicological tests comprising toxicity, persistency and bioaccumulation.
Economics and Cross Media issues	2006	Chapter 2.5.3 addresses aquatic toxicity of discharges to aquatic environment and ranking methodologies based on the level of environmental harm. It is stated that WEA may offer a useful means to address aquatic toxicity of mixtures of substances although care needs to be taken when using data from specific effluent streams in drawing sector-relevant conclusions.

*) The BREFs can be downloaded from the European IPPC Bureau, <http://eippcb.jrc.es/reference/>

In other BREFs such as the BREF on the Pulp and Paper Industry (2001) and the BREF on the Textiles Industry (2003) bio-elimination rates of input chemicals such as sizing agents, dispersants, defoamers, dyes or other additives in the Zahn-Wellens test are considered. The aim is to minimise the contribution of these additives to the total COD load after

biological treatment. In summary, the WEA approach is increasingly recognised in the IPPC activities.

1.3 Sewage Treatment Plants as point emission sources

The main focus of industrial wastewater evaluation is on direct dischargers. The German Wastewater Ordinance distinguishes 57 different industrial sectors, which discharge around 1.3 billion m³ per year of industrial wastewater directly into surface water. In addition around 22 billion m³ per year of cooling water are discharged directly, mainly originating from once-through cooling systems without chemical treatment (Statistisches Bundesamt 2009, see figure 1.2). However, the importance of industrial wastewater discharged into public sewers is often underestimated. The biological treatment of industrial wastewater in municipal treatment plants is very common. In Germany about 1.3 billion m³ industrial wastewater is discharged indirectly per year after passing a municipal treatment plant, including 0.8 billion m³ cooling water. (This cooling water mainly originates from open circuit cooling systems which usually have undergone a chemical treatment). This means that the volume of indirectly discharged industrial wastewater is of the same order of magnitude as that of directly discharged wastewater. When rainwater and infiltration water are ignored, roughly a quarter of the total municipal sewage flow treated in around 9933 activated sludge plants in Germany is to be attributed to industrial wastewater (Statistisches Bundesamt 2009).

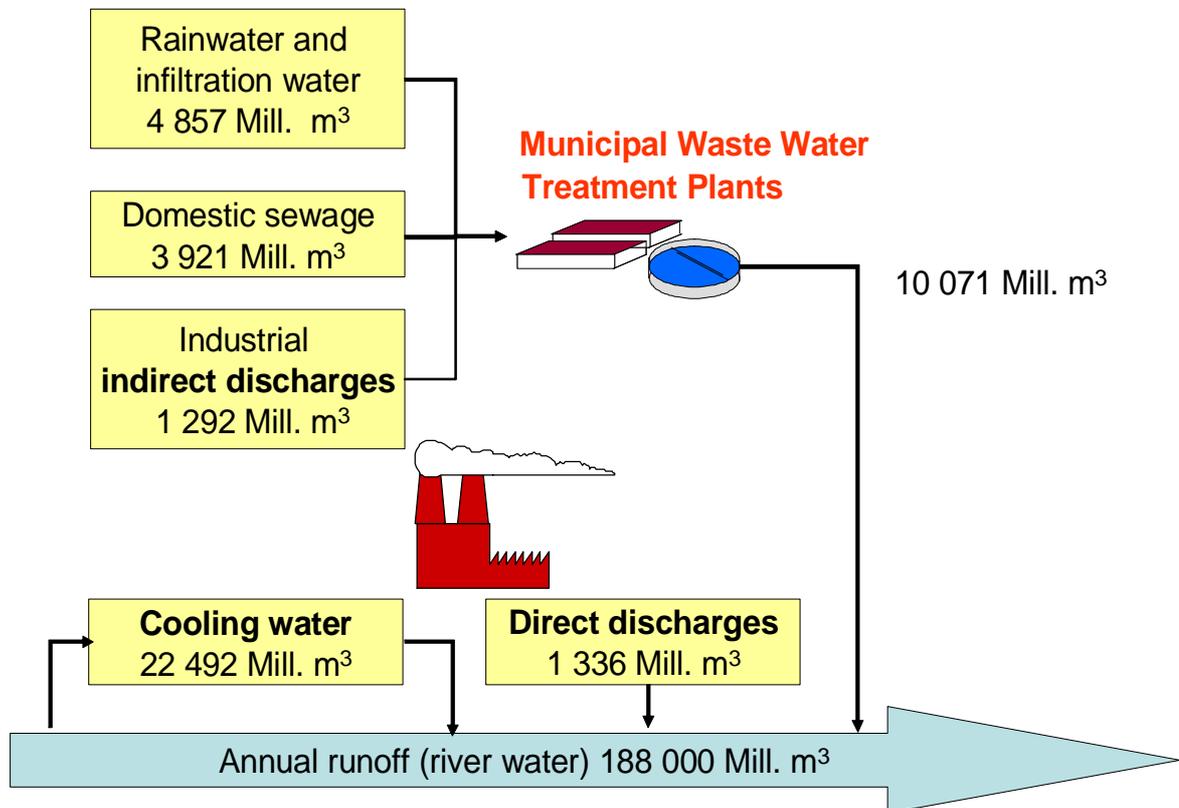


Figure 1.2: Direct and indirect wastewater discharges in Germany
(wastewater database from 2007, mean annual runoff in 1969-1990, source: Statistisches Bundesamt 2009a, 2009b and the website of the German Umweltbundesamt)

According to the water balance of Germany in total 188 billion m³ river water per year runs off across the border of Germany (mean value from 1969 – 1990). Therefrom 71 billion m³ per year originate from upstream riparian flow and 117 billion m³ per year from rain runoff.¹ When the contribution of the upstream riparian flow is disregarded the mean dilution factor of municipal wastewater in German surface water is about 1:10, which corresponds to the default average dilution factor recommended in the Technical Guidance Document (Gartiser 1999, European Commission 2003).

Not surprisingly, municipal treatment plants are important point sources of contaminants. While pharmaceuticals or ingredients of consumer products can be attributed to domestic wastewater (Daughton et al. 1999, Thompson et al. 2005, Yu et al. 2006), there remain contaminants whose origin remains unclear. WWTPs have been identified as an important emission source for pesticides to surface water. Swiss studies suggest that about 20% of the total load of pesticides in surface water is emitted from WWTPs (Hanke et al. 2007). The origin of emissions from WWTPs often cannot be

<http://www.umweltbundesamt-daten-zur-umwelt.de/umweltdaten/public/find.do> „Wasserbilanz“

attributed to specific sources. Often consumer products are considered as the most important source of pollutants in WWTPs next to diffuse emissions from traffic or construction. However, also indirectly discharged industrial effluents have been detected as a relevant source for emissions of hazardous chemicals from WWTPs. For example emissions of perfluorinated alkylated substances (PFAS) from effluents of WWTP have partly been attributed to wastewater from the metal industry and paper industry (Clara et al 2008). Roswell et al. (2010) analysed the sources of priority substances in wastewater and found that the mean level of micropollutants was higher for those WWTP which received both domestic and trade influents than for those only treating domestic wastewater. With 58% industrial chemicals were the dominant input of priority substances to WWTPs, followed by heavy metals (24%) and surfactants (13%). The determination of the main sources of micropollutants is the bases for decision making concerning source management and/or end-of-pipe techniques.

A literature survey on the occurrence of micropollutants such as pesticides in municipal wastewater and rough estimates of removal efficiency from physical sorption and volatilization parameters have been documented by van Beelen (2007). The sorption of organic substances on activated sludge plays an important role for removal efficiency. Substances with an octanol-water partition coefficient ($\log K_{OW}$) below 2.5 are predicted to have low sorption potential with the consequence that they will be released into surface water if they are not biodegradable.

In a literature study for the European Commission Thornton et al (2001) analysed the origin of sewage sludge contaminants. The Urban Waste Water Treatment Directive (91/271/EEC) encourages the use of sludge as manure on agricultural land whenever appropriate. Potentially toxic elements and hydrophobic organic contaminants largely transfer to the sewage sludge during waste water treatment. Thus contaminants might be transposed from water to soil. It is stated that there is considerable uncertainty in quantifying the relevant sources of contaminants. Depending on the chemical class, emissions of potentially toxic elements from industrial point sources were among the major sources of pollution to urban wastewater. However, stringent and more widespread limits applied to industrial users have reduced the levels of potentially toxic elements emitted by industry into urban wastewater considerably. It is recommended to perform a hazard (toxicity), biodegradability and fate assessment for all chemicals which might enter WWTPs.

1.4 Degradation and elimination of wastewater

The focus of this thesis is on the degradability of indirectly discharged wastewater in municipal treatment plants and on assessing indirectly discharged effluents by coupling the Zahn-Wellens test with effect-based tests.

Zahn-Wellens test

The Zahn-Wellens test is the most commonly used test for determining inherent biodegradability of chemicals. International (ISO, EN, OECD) as well as national standard guidelines (EPA, ASTM, DIN) are available. The principle consists of an activated sludge static test with a high inoculum concentration (200 – 1 000 mg/l suspended solids). The test concentration is relatively high compared with the ready-type biodegradation tests (50-400 mg/l DOC). DOC/COD-elimination is determined for the filtered samples over a period of up to 28 days. In parallel with the test vessels containing the test compound, blank vessels are assayed and an abiotic degradation check (abiotic control) is carried out.

In Germany this test has been included in the Wastewater Ordinance. Here the inoculum concentration has been fixed at 1 000 mg/l suspended solids and the test duration varies between 3 and 7 days according to the respective requirements in the different wastewater sectors. A DOC/COD-elimination of 80% (less the part eliminated in the abiotic control) is considered to indicate treatability in municipal treatment plants. The test is also used to determine elimination of other group parameters, such as AOX. Since strictly speaking the amount eliminated by biodegradation and that eliminated by adsorption cannot be distinguished, especially in the case of complex mixtures, results are given as elimination (= bioelimination).

The Zahn-Wellens test has been used to calculate the contribution of single process waters to determine the recalcitrant portion of COD or DOC (resistant to degradation, also described as "refractory" COD/DOC) in the effluent of real treatment plants. Stuhlfauth (1995) found that the recalcitrant DOC of 63 process waters from a chemical industry company, as calculated from the Zahn-Wellens test results, was nearly identical with the corresponding value determined in the real biological treatment plant of the company. Similarly Killer et al. (1993) determined the bioelimination rates of textile and domestic wastewater, as the principal dischargers of a municipal treatment plant, in the Zahn-Wellens test and confirmed the additivity of recalcitrant DOC loads by comparison with the real municipal treatment plant. Thus 84% of the recalcitrant DOC load could be attributed to four textile mills. The DOC elimination of several single chemicals also

showed comparable results in the Zahn-Wellens test and real biological treatment plants (Pagga 1995). The Zahn-Wellens test has been successfully applied to predict the recalcitrant or refractory COD/TOC-loads of different wastewater streams in biological treatment plants (industrial or municipal) especially in the chemical industry (European Commission 2002) and therefore is an effective management tool in stream inventories. It can be concluded that the Zahn-Wellens test is a suitable test to assess the treatability of industrial effluents discharged to municipal wastewater treatment plants.

All methods used to determine DOC-elimination in wastewater elimination tests in principle do not distinguish between biodegradation and elimination by adsorption. Thus, the transfer of hazardous substances to sewage sludge and, subsequently, to farmland cannot be excluded. On the other hand, the methods commonly used to determine ultimate biodegradability use low inoculum concentrations and therefore underestimate adsorption processes and elimination in sewage treatment plants. For that reason, new methods were developed combining both endpoints CO_2 and DOC (Strotmann et al., 1995, Baumann et al., 1996, Meinecke et al. 2000, Gartiser et al. 2007).

Treatment plant simulation model

A laboratory sewage flow-through treatment plant is used to determine degradability of organic compounds. This test is also known as the Coupled units test or OECD Confirmatory test (OECD 303 A). The test item is dissolved in a synthetic sewage matrix and continuously dosed into the activated sludge vessel (3-litre capacity). A control unit is fed only with the synthetic sewage. Both units might be coupled by interchanging a defined volume of activated sludge once a day. DOC is measured in the effluent, and the daily DOC-elimination is calculated after correcting for the material transfer due to the transinoculation procedure. ISO, OECD and EPA methods are available. The test design allows certain modifications. For example the concentration of synthetic sewage might be halved in order to guarantee stable nitrification conditions. Schöberl and Scholz (1991) coupled the OECD Confirmatory test with continuous ecotoxicity tests for assessing the degradability of surfactants and the ecotoxicity of their metabolites. This test has occasionally been used to assess elimination of effluents in sewage treatment plants (Zander-Hauk 1993, Gartiser et al. 1996), but the considerable effort involved prevents its broader application. Further extensions of the test method with an additional anoxic vessel for denitrification processes have been developed (DIN EN ISO 11733: 2004 Determina-

tion of the elimination and biodegradability of organic compounds in an aqueous medium - Activated sludge simulation test).

DOC die away test

The ‘DOC Die away assay’ according OECD 301 A has been proposed by OSPAR as an example of the ready biodegradation test category to be performed for direct discharges (OSPAR 2007b). OECD 301 A allows using the outflow of the final clarifier of a municipal treatment plant as inoculum at a ratio of up to 10%, which corresponds to the upper limit allowed by OECD 301 A and is equal to the mean dilution factor of municipal wastewater in surface water of Germany. OSPAR considers this test as a suitable tool for assessing directly discharged wastewater which did not receive a biological treatment before. While for some contracting parties of OSPAR this kind of effluents has certain importance (especially for direct discharges to the marine environment) in Germany this is of minor importance. Additionally it could be put in question whether direct discharges of effluents containing higher loads of biodegradable organics comply with BAT. In the Netherlands Tonkes et al. (1997) combined a DOC die away test with effect tests and also determined the degradability of potentially bioaccumulating substances (PBS).

1.5 Coupling of degradation tests with ecotoxicity tests

Degradability of wastewater constituents may be of special interest if effluent samples indicate ecotoxic or genotoxic effects. Then the question arises whether these effects are persistent or not. In the United States of America a guideline for "Assessing microbial detoxification of chemically contaminated water" exists using a degradation test not specified and the *Vibrio fischeri* assay. The percentage difference between the EC₂₀ of the treated and the untreated sample is used to assess the progress of detoxification (ASTM D 5660-96). De Groot (1999) proposed to combine a 28 day biodegradation test with the chronic Daphnia reproduction test and the early life stage test with fish. Whale et al. (1999) used a respirometer biodegradation test to assess the recalcitrant ("hard") or readily biodegradable ("soft") toxicity of three effluents (OSPAR 2000). The OSPAR WEA concept included ready biodegradability tests for directly discharged effluents and inherent type tests for indirectly discharged effluents, the Zahn-Wellens test being the most prominent in this category (see chapter 1.2).

The rationale of coupling the Zahn-Wellens test with bioassays is that persistent toxicity of an indirect discharged effluent can be detected and attributed to the respective

emission source. When testing the outflow of WWTPs the presence of persistent hazardous compounds might not be detected because the respective partial wastewater stream has been diluted in the treatment plant with wastewater from other origins. Because acute toxicity tests usually applied for wastewater evaluation as a rule are less sensitive than chronic tests, the absence of acute toxicity in the outflow of a WWTPs is no guarantee that the wastewater discharged is safe. Additionally, even if toxicity is observed in the outflow of WWTP by means of bioassays these effects often cannot be attributed to a specific discharger. Thus a suitable strategy consists in moving the sampling site from the outflow of the WWTP to the sources of pollution - that means, to the indirectly discharged partial effluents from conspicuous industrial sectors. When testing these effluents before the degradation step (Zahn-Wellens test) their toxicity might be overestimated by compounds which are effectively removed during wastewater treatment, such as ammonium or readily biodegradable organics. Additional testing of raw effluents might be disturbed by suspended solids influencing photometrical tests. For municipal wastewater Diehl et al (1998) reported moderate toxicities in the inflow and outflow of WWTPs (table 1.3). The highest values were observed with the *Vibrio fischeri* assay, which is relatively insensitive to ammonium toxicity (Zander-Hauk (1993) reported an EC20 of 670 mg L⁻¹ NH₄-N). After treatment the luminescent bacteria toxicity was reduced to the background level, indicating that luminescence measurement is disturbed due to the turbidity of the raw effluent.

Table 1.3: Ecotoxicity in the inflow and outflow of municipal WWTPs in Germany

	Fish <i>Leuciscus idus</i> [LID]	Crustacean <i>Daphnia magna</i> [LID]	Algae <i>Desmodesmus</i> <i>subsp.</i> [LID]	Bacteria <i>Vibrio fischeri</i> [LID]
Number of data sets	33	42	29	178
Inflow (mean)	7.4	2.8	2.0	49.7
Inflow (median)	4	2	2	16
Number of data sets	308	157	29	510
Outflow (mean)	2.2	2.1	2.2	8.6
Outflow (median)	2	1	1	2

The combination of the Zahn-Wellens test with bioassays allows the classification of the effects as "inherent degradable" or "hard". This approach may also be considered as a part of a TIE-procedure (toxicity identification evaluation, see chapter 5). Practical experience in coupling the Zahn-Wellens test with ecotoxicity and genotoxicity tests has already been achieved in research projects on hospital and textile effluents (Gartiser et al.,

1996, 1997). As these studies confirmed the practicability of the Zahn-Wellens test for this purposes this approach was included in the OSPAR WEA strategy.

Higher tier tests such as the OECD Confirmatory Test (OECD 303A) have occasionally been coupled with continuous ecotoxicity tests (here Daphnids) for assessing the ecotoxicity of breakdown products of detergents (Schöberl 1991, Scholz 1991). Zander-Hauk et al. (1993) adapted this approach for wastewater and analysed leachates from landfill sites. A high correlation between the Daphnia toxicity and ammonium concentration ($500 - 2000 \text{ mg L}^{-1} \text{ NH}_4\text{-N}$) was observed. After treatment of these effluents in laboratory flow through activated sludge treatment plants under nitrifying conditions Daphnia toxicity was reduced to values around $\text{LID} = 4$. This combination of a treatment plant simulation model with ecotoxicity tests has been integrated in the German Wastewater Ordinance for the sector "landfill leachate" and "waste treatment". Here the limits regarding effluent toxicity (fish egg test $\text{LID} \leq 2$, daphnia and luminescent bacteria test $\text{LID} \leq 4$) may be achieved after the biological treatment.

1.6 Challenges in determining persistency of wastewater

Persistence can be considered as the inverse of degradability. Apart from biodegradation, hydrolysis and photolysis may also contribute to the observed degradation. Further, adsorption and evaporation can erroneously contribute to the apparent degradation. These effects may be considered in abiotic controls in the test design. Making a definition of persistence or degradability in the context of whole effluents is complex. In chemical risk assessment degradation is considered as half-life or period required for 50 percent dissipation (see chapter 1.2). The ready biodegradability tests used for measuring the biodegradability of a substance do not give a quantitative estimate of the removal percentage in a wastewater treatment plant or in surface water. Therefore, in order to make use of the biodegradation test results it is necessary to assign rate constants to the results of the standard tests for use in WWTP-models or for half life estimates in surface water. Since direct measurements of degradation rates at environmentally relevant concentrations are often not available, this has been a pragmatic solution to this problem. Thus it is very common to use results from ready or inherent screening tests for estimates of the elimination in sewage treatment plants and/or surface water (European Commission 2003).

For WEA persistence is taken as the persistence of toxicity and/or liability to bioaccumulate after a period of degradation. For some contracting parties of the OSPAR WEA group, among them Germany, persistence alone is seen as a criterion of its own.

However, few data on persistence of toxicity and bioaccumulation of wastewater were available. Thus, the OSPAR WEA group realised two practical studies to assess the suitability of the approach (OSPAR 2005, 2007a). Later on, the German Environmental Agency sponsored a more detailed project for two sectors, the metal surface treatment industry and the paper manufacturing industry. The results of these data provide the basis for this thesis.

The OECD tests for ready biodegradation of substances require a pass level of 60% (BOD or CO₂ production) or 70% (DOC). The background for these relatively low pass levels is that it is assumed that a substantial part of the organic degraded is used for biomass growth. The measurement problem of using an unspecific sum parameter like DOC is recognised as a key problem in persistency assessment of complex water samples (OSPAR 2005). Beek et al. (2001) stated that tests based on sum parameters like BOD, COD, DOC, CO₂ production are only applicable to single substances, because a decrease of the amount of the different compounds cannot be differentiated and metabolites formed cannot be quantified. Thus it cannot be distinguished whether the observed partial degradation results from the complete degradation of one constituent or from several substances undergoing only partial degradation. From industry it is stated that it is incorrect to discuss the "persistence of effluents". Only compounds in the effluent or a property that is an indirect effect of these compounds (such as toxicity) can be persistent (ECETOC 2004). One problem with determining the persistence of mixtures is the presence of rapidly degradable (non-persistent) compounds together with persistent ones. A low concentration of a highly persistent chemical, in the presence of much higher concentrations of more easily degradable substances (such as an organic solvent) is likely to give a result that suggests that the effluent contents are not persistent, even though the effluent may contain highly persistent material. This dilemma led Beek and co-authors (2001) to the conclusion that tests using such measurements that do not differentiate between compounds that are easily degraded and compounds that are not are unsuitable for the testing of complex samples (OSPAR 2005).

1.7 Objectives of the work

The emission-based strategy for wastewater surveillance favoured in Germany has been successfully applied in the past and follows the precautionary principle. In routine wastewater evaluation the emission-based strategy relies on sum parameters, determination of compounds (e.g. volatile organohalogenes) or elements (e.g. heavy metals) and toxicity

tests. Degradation is only considered for determining the treatability of wastewater in biological WWTPs by means of the removal of organic carbon.

One objective of the studies was to support the activities of the German Federal Environment Agency in the development of a "Whole Effluent Assessment" (WEA) concept within the framework of OSPAR (Oslo - Paris Convention) and the integration of bioassays for determining Best Available Techniques. For this the author participated in the OSPAR expert group on Whole Effluent Assessment where several emission- and immission-based approaches were discussed.

Another objective of the studies presented in the thesis was to elaborate a new proposal for integrating persistency and bioaccumulation in the present methodology with the main focus on indirect discharged effluents.

Further on, the studies was aimed to identify the primary toxicants present in the wastewater (if any) and to link the effects measured in the effluents to the respective processes. For this the OSPAR practical studies were accompanied by chemical-specific analysis while within the follow-up study the main input chemicals and processes in the paper making and metal processing industries have been analysed.

The main objective of the studies presented in the thesis was to put theory into practice and to gain experience from the investigation of real wastewater samples. For this, two practical studies were organised within the OSPAR WEA group and several industrial wastewater samples were analysed in order to demonstrate the added value of WEA compared to the chemical analyses. In a follow up study the WEA approach has been further adapted on a broader scale for paper and metal working effluents with the objective to evaluate the usefulness of WEA for defining and surveillance of BAT in these sectors according to the IPPC Directive.

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1.9 Basis for the cumulative dissertation

This dissertation is based on the following reviewed publications published in international journals:

- Gartiser, S., Hafner, C., Oeking, S., Paschke, A. 2009: Results of a "Whole Effluent Assessment" study from different industrial sectors in Germany according to OSPAR's WEA strategy. *J. Environ. Monit.* 11, p. 359–369.
- Gartiser, S., Hafner, C., Hercher, C., Kronenberger-Schäfer, K., Paschke, A. 2010: Whole Effluent Assessment of Industrial Wastewater for Determination of BAT Compliance Part 1: Paper Manufacturing Industry. *Environ. Sci. Pollut. Res.* 11, p. 359–369
- Gartiser, S., Hafner, C., Hercher, C., Kronenberger-Schäfer, K., Paschke, A. 2010: Whole Effluent Assessment of Industrial Wastewater for Determination of BAT Compliance Part 2: Metal Surface Treatment Industry. *Environ. Sci. Pollut. Res.* 17, p. 856-865

In German professional journals the following publications cover the same topic:

- Gartiser, S., Hafner, C., Oeking, S., Paschke, A., Maue, G.: Einsatz wirkungsbezogener Methoden zur Bewertung und Kontrolle von Industrieabwässern im Rahmen von OSPAR-Aktivitäten. *KA Abwasser Abfall* 54 (2007), H. 11, S. 1146-1155

- Gartiser, S., Hafner, C., Hercher, C., Kronenberger-Schäfer, K.: Bewertung von Direkteinleitern mit Biotests. Teil 1: Abwasser in der Papierindustrie. Korrespondenz Abwasser, Abfall 56, 2009, S. 915-920
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- Gartiser, S.: Bestimmung der Mineralisierung und DOC-Elimination von Abwasserproben im Zahn-Wellens-Test. gwf-Wasser-Abwasser 150, 9, 2009, S. 700-710.

Additionally the following posters have been presented during international Conferences:

- Gartiser, S., Hafner, C., Paschke, A.: Einsatz wirkungsbezogener Methoden zur Bewertung und Kontrolle von Industrieabwässern im Rahmen von OSPAR Aktivitäten. SETAC German Language Branch - 10. Jahrestagung in Basel, Schweiz, 28. - 30. September (2005)
- Gartiser, S., Hafner, C., Oeking, S., Paschke, A.: Results of a Practical Whole Effluent Assessment Study in Germany. SETAC Europe 16th Annual Meeting in The Hague, The Netherlands, 7 - 11 May 2006
- Gartiser, S., Hafner, C., Hercher, C., Kronenberger-Schäfer, K., Paschke, A.: Effect-related Evaluation of Paper Manufacturing and Metal Surface Treatment Wastewater. SETAC Europe, 18th Annual Meeting in Göteborg, Schweden, 31. Mai - 4. Juni 2009

The posters can be downloaded from the website of the Hydrottox GmbH www.hydrottox.de.

1.10 Own contribution to the research results

The author Stefan Gartiser was involved in the preparation, realisation and evaluation of the research projects with regard to the following points:

- Evaluation of wastewater samples with bioassays: Project manager of the research projects „*Contaminating substances and the waste water situation in hospitals*”(FKZ 102 06 514, project duration 1992 - 1995), „*Environmentally sound disinfectants in hospital wastewater*“ (FKZ 29727526, project duration 1997-1999) and „*Use of environmentally compatible cooling water treatment chemicals*“ FKZ 2002433, project duration 2000 - 2001). All these projects have been funded by the Federal Environment Agency of Germany (Umweltbundesamt, UBA).

- Preparation of the draft of the background document "*Ecotoxicological Evaluation of Wastewater within Whole Effluent Assessment*" as German contribution to OSPAR (UBA FKZ 360 07 018, in 1999)
- Member of the OSPAR Intersessional Expert Group „*Whole Effluent Assessment*“ from 2001 - 2007 (within the UBA projects FKZ 20119304 and FKZ 205 44 324/01)
- Project manager of the UBA projects “*Suitability of bioassays for the controlling of wastewater within the frame of OSPAR’s strategy on dangerous substances*“ (UBA FKZ 20119304 and UBA FKZ 205 44 324/01, project duration from 2001 - 2007) and „*Effect-related test data of specific wastewater sectors for the new conception of the Wastewater Ordinance*“ (UBA FKZ 206 26 302, project duration from 2006 – 2008). The waste water samples analysed in the last mentioned projects have been used for the publication based dissertation.

The contribution of the author on the projects referred to above relates to the following subjects:

- Overall concept of the investigation strategy
- Selection of the companies, wastewater sampling and evaluation of the production characteristics of the companies.
- Pointwise contribution to the laboratory work
- Evaluation of all results and of the literature researches
- Presentation of the projects to the sponsor, the Federal Government and Federal States Working Groups (BLAK) on paper and metal working industries, the OSPAR WEA expert group, and industry.
- Independent preparation of the final reports and of all the publications (posters and papers in German and English technical and scientific journals).

2 CHAPTER 2

Results of a “Whole Effluent Assessment” study from different industrial sectors in Germany according to OSPAR’s WEA strategy

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Abstract

The results of a Whole Effluent Assessment (WEA) of 8 wastewater samples from different industrial sectors as the German contribution to the OSPAR-WEA expert group are presented. The testing strategy followed the WEA principles described in the OSPAR WEA-Guidance document considering persistency (P), potentially bio-accumulative substances (B) and toxicity (T). All wastewater samples have been tested before and after a biodegradation test. The Zahn-Wellens test has been applied with wastewater indirectly discharged to a municipal treatment plant, the DOC Die away assay for wastewater directly discharged to surface water. The DIN standardized bioassays referred to in the German wastewater ordinance which partly are related to screening versions of the respective OECD guidelines have been applied. The potentially bio-accumulative substances (PBS) were determined by solid phase microextraction (SPME) and referred to the reference compound 2,3-dimethylnaphthalene. Generally low to moderate ecotoxic effects of wastewater samples have been determined with maximum values of $LID_A = 8$ in the algae test, $LID_{lb} = 24$ in the luminescent bacteria test and $LID_{Egg} = 6$ in the fish egg test. Low levels of PBS were determined in the effluents after biological treatment.

The Zahn-Wellens test proved to be a suitable screening tool for the biological treatment of wastewater samples. The mutagenicity of one wastewater sample from the chemical industry was investigated by additional chemical analysis and backtracking. A nitro-aromatic compound (2-methoxy-4-nitroaniline) used for batchwise azo dye synthesis and its transformation products are the probable cause for the mutagenic effects analysed.

2.1 Introduction

Effect-based test methods are increasingly used for the evaluation and monitoring of complex wastewater samples in many parts of the world. These tests detect combined toxic effects of all substances present in wastewater samples on aquatic organisms and are complementary to the “single substances approach” where environmental hazards of identified contaminants are assessed on a case by case basis. Because only a limited number of substances are routinely analysed and identified in environmental samples and degradation products or combined effects are not covered with single substances analysis, the Whole Effluent Assessment (WEA) concept has been developed. Here, the wastewater samples are assessed with regard to persistency (P), presence of potentially bio-accumulative substances (B) and toxicity (T). Within the Integrated Pollution Prevention

and Control Directive (IPPC, 96/61/EC) the WEA concept has been included as a suitable monitoring strategy on effluent in several Best Available Techniques Reference Documents (BREFs), notable the BREFs on “Organic Fine Chemicals”, “Large Volume Organic Chemicals”, and on “Common waste water and waste gas treatment and management systems in the chemical sector” (European Commission 2003a, 2003b, 2006). Some European countries such as Germany, Ireland, Spain or Sweden include bioassays in their regulatory routine measurements. However, in most countries the application of WEA has (until now) been restricted to the parameter acute toxicity, thus only covering the “T” part of the PBT criteria. An expert group on the “Whole Effluent Assessment” approach has been established under the Hazardous Substances Committee of the OSPAR Commission (Oslo-Paris-Convention on the protection of the marine environment of the North-East Atlantic) in order to explore the applicability of the WEA concept and to describe robust WEA tools. The working group finished its work in 2007 and the results have been published at the OSPAR’s website (www.orspar.org). The testing strategy and WEA principles have been described in a Guidance document OSPAR 2007a). While the general WEA approach is described in different flowcharts several test methods have been suggested in a common toolbox and an optional toolbox. Here the methods generally applied by the Contracting Parties have been included. In 2003 and 2005, the OSPAR-WEA expert group organised two practical studies with the aim to evaluate and optimise the applicability of the WEA-concept in practice (OSPAR 2005, 2007b). In total 43 wastewater samples from different industrial sectors have been analysed, among them, as a German contribution, 8 wastewater samples. The results of the practical WEA analysis with the German wastewater samples are described in this article.

2.2 Materials and methods

2.2.1 Wastewater samples

The OSPAR WEA expert group organised two practical studies for which in a total of 8 wastewater samples have been tested as a German contribution. The origin and characterisation of the wastewater samples are given in table 2.1. The samples A and C–E originated from the first testing series in 2003, samples B and F–H from the second series in 2005. The treated and untreated samples were stored at -20°C in 500 mL PE-bottles before testing. The degradation tests performed with samples A, B and F–H have been

started at the day of sampling. Samples C–E have been taken, frozen and sent by local authorities. Here the biodegradation test started one week after sampling. All wastewater samples consisted of 24-h mixed samples.

Table 2.1: Origin and characterisation of wastewater samples

	Description	Wastewater treatment	Characterisation (selection)	Elimination in degradation test
Indirect dischargers				
A	Cotton, Polyester and Polyamide- dyeing factory (textile industry)	Only equalisation basin with pH adjustment as on-site treatment	TOC 279 mg L ⁻¹ AOX 0,65 mg L ⁻¹ NH ₄ -N 7,25 mg L ⁻¹	30% after 3 h 71% after 7 d
B	Automobile factory (processes: carriage, the pressing of sheet metal, finishing and assembling of 400.000 motorcars per year)	700 m ³ ·d ⁻¹ Water cycle within production is almost closed (1 million m ³ ·d ⁻¹ water used per day); chemical, physical treatment (neutralisation, flocculation, precipitation, gravel filter)	TOC 61,5 mg L ⁻¹ DOC 56,3 mg L ⁻¹ AOX 0,3 mg L ⁻¹	29 % after 3 h 96% after 7 d
Direct dischargers				
C	Pharmaceutical industry (e.g. cytotoxic and cytostatic drugs)	500 m ³ ·d ⁻¹ precipitation with FeClSO ₄ , Activated sludge treatment (nitrification, de-nitrification) and activated carbon filter Non-biodegradable mother liquors (criteria >90% elimination in the Zahn-Wellens test) are collected and disposed of via waste incineration.	TOC 5,4 mg L ⁻¹ AOX 0.056 mg L ⁻¹ NH ₄ -N <0,05 mg L ⁻¹ PO ₄ -P (total) 0,6 mg L ⁻¹ Chloride 2695 mg L ⁻¹	26% after 3 h -1% after 14 d
D	Chemical and Pharmaceutical industry (e.g. intermediate products for pharmaceuticals and food additives such as caffeine).	4000 m ³ ·d ⁻¹ de-nitrification, activated sludge (8-10 g d.s./L) treatment with pure oxygen supply	TOC 24,7 mg L ⁻¹ AOX 0,081 mg L ⁻¹ NH ₄ -N <0,08 mg L ⁻¹ PO ₄ -P (total) 0,7 mg L ⁻¹	-4% after 3 h 12% after 14 d
E	Textile finishing industry (finishing of laminates or prints textile fabrics or knitted fabrics made of natural fibres)	1000 m ³ ·d ⁻¹ Activated sludge treatment with activated carbon and a bio filter (Katox-technology) followed by flocculation with ferrous sulphate, calcium hydroxide and organic flocculation aids	TOC 33,1 mg L ⁻¹ AOX 0,084 mg L ⁻¹	39% after 3 h 10% after 14 d
F	Speciality chemical industry (e.g. dyes, pigments, optical brighteners, biocides; in total batch-wise synthesis of around 350 different chemicals; only few continuous processes)	8000 m ³ ·d ⁻¹ Wastewater from batch processes with known recalcitrant COD (results from Zahn-Wellens test) are nanofiltrated or extracted and then passed to the central treatment plant, the concentrates being burnt. Central wastewater treatment (neutralisation, flocculation, sedimentation, activated sludge treatment) together with municipal wastewater; 50% of the hydraulic load, but 90% of the TOC load are of industrial origin.	TOC 51,8 mg L ⁻¹ DOC 48,7 mg L ⁻¹ AOX 0,1 mg L ⁻¹ Sulfate 1740 mg L ⁻¹ Chloride 2000 mg L ⁻¹	2% after 3 h 31% after 14 d
G	Chemical industry (e.g. inorganic special chemicals)	1900 m ³ ·d ⁻¹ chemical/physical or biological treatment depending from the origin Phosphoric acid, ammonium/urea, acetic acid and alcohols added from external sources for the biological treatment process (inlet COD about 300 mg L ⁻¹ , outlet COD 15 mg L ⁻¹).	TOC 3,0 mg L ⁻¹ DOC 1,8 mg L ⁻¹ AOX 0,05 mg L ⁻¹	Not suitable due to low concentration
H	Paper mill (higher quality coated papers, raw material consist of ground wood pulp and ready-to-use cellulose pulp, but no recycling paper; bleaching with sodium hydroxide and hydrogen peroxide)	12000 m ³ ·d ⁻¹ storage tank, flocculation with iron chloride, activated sludge treatment and percolating filter. Co-treatment of municipal wastewater. 75% of the hydraulic load and 90% of COD load to be attributed to industrial wastewater. Addition of urea and phosphoric acid as nutrients.	TOC 46,1 mg L ⁻¹ DOC 39,0 mg L ⁻¹ AOX 0,03 mg L ⁻¹ Sulfate 284 mg L ⁻¹	25% after 3 h 44 % after 14 d

2.2.2 Testing strategy and degradation tests

According to the WEA concept, the PBT-criteria (persistence, bioaccumulation and toxicity) should be assessed. The toxicity should be tested by acute as well as chronic tests, because OSPAR focuses on long term effects in the marine environment. From the optional toolbox mutagenic and genotoxic effects can be determined. The overall test concept consisted in coupling the effect-based tests with biodegradation tests (see figure 2.1).

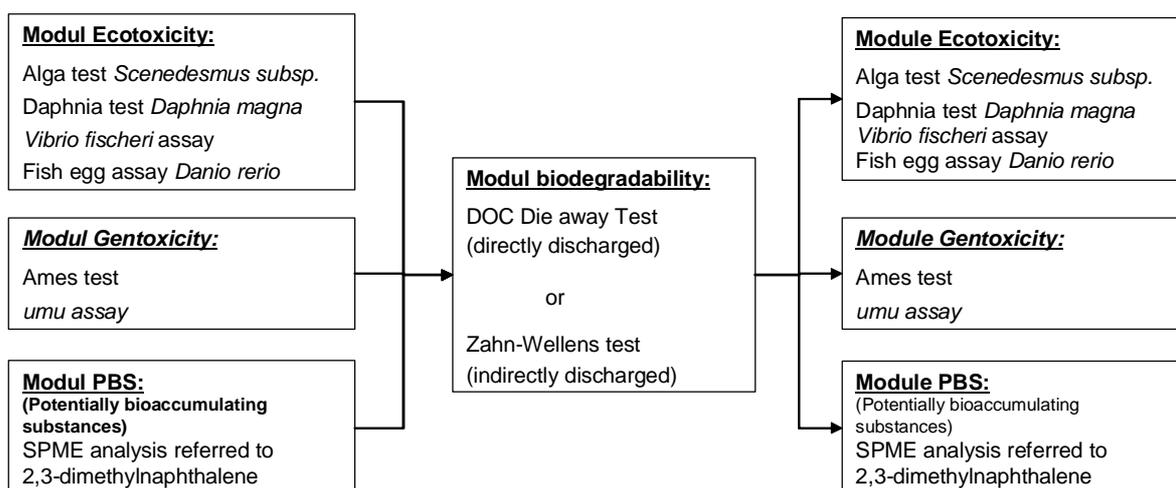


Figure 2.1: Practical WEA study test concept

In both practical studies all German wastewater samples have been tested before and after the performance of a biodegradation test, in order to assess the elimination of ecotoxic and genotoxic effects through biological treatment in municipal treatment plants (indirect dischargers) or the persistence of effects in surface water (direct dischargers). According to the OSPAR WEA testing strategy an inherent type degradation test with high inoculum concentration (Zahn-Wellens test) has been applied for wastewater samples which are treated in biological treatment works while a ready type degradation test with low inoculum concentration (DOC Die away test) is used for wastewater directly discharged to surface water.

The Zahn-Wellens test (adopted from OECD 302 B) was performed with activated sludge (1 g d.s. L⁻¹) from the municipal treatment plant Breisgauer Bucht (600,000 inhabitant equivalents). The wastewater samples were supplemented with an inorganic nutrient solution and continuously stirred and aerated with an aquarium pump. The pH was adjusted to pH 7–8 each working day. COD determination was done using ready to use

cuvette tests from Hach-Lange, Germany. The bio-elimination extents were referred to the expected initial start concentration calculated from the COD of the original sample and the dilution by adding mineral medium and activated sludge (less than 20% of total volume). After treatment for 7 days the activated sludge was allowed to settle for about 1 h and the supernatant was decanted.

The ‘‘DOC Die away assay’’ (adopted from OECD 301 A) was performed using the outflow of the final clarifier of the same municipal treatment plant as inoculum. Inoculum density was 10% of total volume, which corresponds to the upper limit allowed by OECD 301 A and is equal to the mean dilution factor of municipal wastewater in surface water of Germany. All vessels were continuously stirred and aerated with an aquarium pump. The pH was measured and adjusted at least two times per week with NaOH (1 mol L⁻¹) or H₂SO₄ (0.5 mol L⁻¹) if the range was out of pH 7–8. DOC analysis was performed with a total carbon analyser TOC-5000A, Shimadzu, Germany. DOC-elimination was referred to the expected start concentration calculated from the TOC of the sample and the dilution with inoculum (10%). Test duration was 14 days. After treatment the vessels were allowed to settle for about 1 h and the supernatant was decanted.

2.2.3 Ecotoxicity and genotoxicity test methods

The test methods applied corresponded to those which have been considered in the German wastewater ordinance for routine measurements (see table 2.2). The test methods referred to in the German wastewater ordinance are screening versions of the corresponding OECD guidelines with fewer replicates (e.g. Daphnia test), reduced points of evaluation time (Algae test), fewer tester strains (Ames test) or shorter test duration (Zahn-Wellens test) (Anonymous, 2004). Generally a dilution series of the wastewater samples is tested using dilution steps of 1.5 and 2 (thus testing wastewater at dilution factors of 1, 2, 3, 4, 6, 8, 12 ...). As far as possible the original wastewater samples have been tested after pH-adjustment with hydrochloric acid or sodium hydroxide solution to 7.0 ± 0.2 without any further pre-treatment. Where suspended particles might have an influence on the test results by mechanically interfering with the test organisms (Daphnia) or by light absorbance (Algae, Luminescent bacteria test) the sediments were allowed to settle for 1–2 h immediately before starting the incubation period. In parallel to the wastewater samples one concentration of suitable reference compounds (ecotoxicity: 3,4-dichloroaniline or potassium dichromate; genotoxicity: 2-aminoanthracene, nitrofurantoin, 4-nitro-1,2-phenylenediamine, 4-nitroquinolineoxide) has been tested accor-

ding to the Analytical Quality Assurance bulletin (AQS) of the German Working Group of the Federal States on water issues (LAWA).

Table 2.2: Test methods used from the wastewater ordinance

Waste-water Ordinance	Method	Standard	Test-organism	Test-duration	Criteria
No. 401	Zebrafish embryo assay LID _{Egg} -value	DIN 38415-6: 2003 ISO 15088: 2007	<i>Danio rerio</i>	48	Development of embryos (coagulated eggs, heart beat, somites and tail differentiation) < 10%
No. 402	Daphnia acute toxicity LID _D -value	DIN 38412-30: 1989	<i>Daphnia magna</i>	24 h and 48 h	90% of Daphnia maintain ability to swim
No. 403	Alga growth inhibition test LID _A -value	DIN 38412-33: 1991	<i>Scenedesmus subspicatus</i>	72 h	Inhibition of biomass production < 20%
No. 404	Fluorescent bacteria test LID _{ib} -value	DIN 38412-34: 1997 EN ISO 11348-2: 1998	<i>Vibrio fischeri</i>	30 min	Inhibition of light emission < 20%
No. 407 / 408	Zahn-Wellens test	DIN EN ISO 9888: 1999	Activated sludge	2-7 d	COD/DOC-elimination
No. 410	Genotoxic potential <i>umu</i> test, LID _{EU} -value	DIN 38415-3: 1996 ISO 13829: 2000	<i>Salmonella typhimurium</i> TA1535/pSK1002	2 h	Induction rate < 1.5
-	Mutagenic potential Ames-test	DIN 38415-4:1999 ISO 16240: 2005	<i>Salmonella typhimurium</i> TA98, TA100	48 h	Induction difference <80 (TA100) and < 20 (TA 98) revertant colonies

In Germany, the short term fish egg assay (also called fish embryo assay) with fish eggs of *Danio rerio* has replaced the acute fish toxicity test with *Leuciscus idus* in wastewater evaluation for animal protection considerations. The test is classified as a suborganism test because the central nervous system of fish embryos is not fully developed (Oberemm 2000). The fish were cultivated at 26 °C and 16: 8 h light: dark cycle and were daily fed with TetraMIN® flakes and two times per week with newly hatched brine shrimps (*Artemia* sp.) The fertilised eggs were collected in a rectangular glass spawning box, covered by a stainless steel mesh and artificial plants, and were separated manually from unfertilised eggs using an inverted microscope. The eggs were incubated over 48 h, which covers the time from the blastula to the stage with fully developed blood circulation. The test performance consists in exposing 10 fertilised eggs for each concentration in 24-well cell culture plates (2 ml each).

The Daphnia toxicity test was performed using the clone 5 of *Daphnia magna* STRAUS of the German Federal Health Agency. *Daphnia* were held in Elendt M4 medium and were fed daily with living algae cells (*Desmodesmus subspicatus* CHODAT, formerly called *Scenedesmus subspicatus*). Each concentration (dilution) was tested in two

replicates with 5 daphnia and incubated at 20°C in the dark. The test was evaluated after 24 h (DIN standard) and was prolonged to 48 h in order to comply with OECD 202.

For the Algae growth inhibition test *Desmodesmus subspicatus*, a planktonic freshwater alga, was used. Considering the higher variability of this test, three instead of two parallel vessels as suggested in the DIN standard have been tested for each concentration. After adding an algal nutrient solution the vessels were inoculated with 10^4 algae per ml and incubated under defined light conditions (6000–10 000 lux) at $23\text{ °C} \pm 1\text{ °C}$. After 72 h, the number of cells was determined microscopically as a measure for the biomass increase.

The Luminescent bacteria toxicity test with the marine bacteria *Vibrio fischeri* was performed using the LUMIS-tox system of the company Dr. Lange, Düsseldorf with liquid dried bacteria of the strain *Vibrio fischeri* NRRL-B-11177. The wastewater samples were tested after salinizing with sufficient sodium chloride to obtain a 2% solution with two replicates at an incubation temperature of $15\text{ °C} \pm 1\text{ °C}$ after 30 minutes contact time.

The Ames test is a bacterial mutagenicity test using different tester stains of *Salmonella typhimurium*. For wastewater evaluation usually only the two tester strains TA98 and TA100 are applied. The strain TA98 detects frameshift mutagens; strain TA100 is susceptible for base pair substitution mutagens (point mutations). The number of back-reverted mutated bacteria (revertants) compared to the spontaneous back-reversion rate provides a measure of the mutagenic potential of a substance or a sample. In higher organisms certain mutagens are first activated by metabolic processes (promutagens) or become inactivated. Therefore, the enzymes required are added to the bacterial system as a rat liver extract S9 (Moltox Co, USA). The water samples were sterilized by membrane filtering (0.2 µm, Whatman S&S FP 30/02, Germany). Up to 1 ml of wastewater per Petri dish could be added. Each concentration has been tested with 5 petri dishes. A sample is classified as mutagenic according to DIN 38415-4 if in one of the strains with or without S9 an induction difference compared to the control (solvent alone) of 80 (for TA100) or 20 revertants (for TA98) is induced and a dose-effect relationship is found. The lowest ineffective dilution (LID)-value corresponds to the last dilution step at which the induction difference established for that strain is not exceeded. Since the wastewater sample in the test is diluted by a factor of 3 with medium/inoculum, the lowest possible LID_{EA} -value = 3 (non-mutagenic).

The umu test is a genotoxicity test with the bio-technologically modified bacterial strain *Salmonella typhimurium* TA1535/pSK1002. Gene toxins induce the umuC-gene, which belongs to the SOS-repair system of the cell. By coupling of the umuC-gene promoter with the lacZ-gene for β -galactosidase, the activation of the umuC-gene can be indirectly measured spectrophotometrically at 420 nm through the formation of a coloured product from the β -galactosidase substrate o-nitrophenyl-galactopyranoside (ONPG). The bacteria are exposed for 4 hours to the wastewater with and without metabolic activation using microplates and the genotoxin-dependent induction of the umuC-gene is compared to the spontaneous activation of the control culture. Each concentration has been tested threefold in 96-well microtiter plates (Greiner Bio-One, Frickenhausen, Germany). The induction rate (IR) corresponds to the increase of the extinction at 420 nm relative to the negative control. Bacterial growth and inhibition are determined turbidimetrically from the optical density at 600 nm. For growth factors below 0.5 (50% growth inhibition) the results are not evaluated. The result given is the smallest dilution step at which an induction rate < 1.5 is measured.

All results of ecotoxicity and genotoxicity testing are given as the Lowest Ineffective Dilution (LID), which is defined as the reciprocal volume fraction of the wastewater sample at which only effects not exceeding the test-specific variability are observed (EN ISO 5667-16: 1998, Annex A). This corresponds to the lowest dilution factor where non-significant effects have been observed. The following were considered as non-significant effects: a mortality of $\leq 10\%$ (Fish egg test, Daphnia test), an inhibitory effect $\leq 20\%$ (Luminescent bacteria test, Algae test), an induction rate ≤ 1.5 (umu test) or a tester strain specific induction difference of revertant bacteria compared to negative controls (Ames test). The reciprocal value of the LID corresponds to the volume fraction of the wastewater sample at which no significant effects are observed. The indication of the LID as test result might be interpreted as a No Effect Concentrations (NOEC) although these values are directly taken from the test results and not statistically derived. Therefore, additionally the NOEC of the algae tests were calculated by ANOVA analysis. However, ANOVA analysis requires at least three replicates per concentration while the screening tests applied in Germany only require two replicates each. Therefore only the algae test where three replicates have been tested could be evaluated by ANOVA analysis. Additionally the EC50 values were calculated where possible because in other European states mostly EC50 of wastewater samples are reported. The statistical programme ToxRAT (ToxRAT Solutions GmbH, Alsdorf, Germany) has been used for statistical analysis.

2.2.4 Potentially bioaccumulative substances

The potentially bioaccumulative substances (PBS) were determined by solid phase microextraction (SPME) (Verbruggen et al. 2000, Leslie et al. 2002) according to a protocol adapted by Leslie and Leonards (2005) for the OSPAR WEA group. Briefly, a 1 cm long quartz glass fibre coated with 100 nm polydimethylsiloxane (PDMS) from Supelco (Bellafonte, CA, USA) was exposed at room temperature to 250 mL wastewater which was continuously stirred at 500 rpm over 24 h. The Erlenmeyer flask used was headspace-freely filled with the sample and wrapped with aluminium foil during SPME. Gas chromatographic analysis was performed after thermodesorption of SPME fibre in splitless mode using a CP 9001 (Chrompack, Frankfurt a. M.) with flame-ionisation detector and a 10 m long capillary column OPTIMA-1 with 0.25 mm I.D. and 0.1 mm film thickness (Macherey-Nagel, Düren, Germany). The whole chromatogram was integrated between the retention times of nonane (n-C9) and octatriacontane (n-C38). All data were normalised to the reference compound 2,3-dimethylnaphthalene (DMN; log K_{ow} = 4.4) and expressed as mmol L^{-1} DMN equivalents. Additionally two blank values from two PE bottles filled with distilled water (one new, one used before) were determined according to the same procedure.

2.2.5 Accompanying chemical analysis

All samples from the first series in 2003 have been analysed by extended chemical analysis (GC/MS screening) by working group members from the Dutch Ministry of Transport, Public Works and Water Management in order to determine the added value of effect based testing. The toxicity could not be attributed to single substances, thus proving that effect based tests provide a complementary tool to the single substance approach for wastewater evaluation. Aliquots of the samples of the second series from 2005 were analysed after liquid–liquid extraction or stir-bar sorptive extraction with Twister bars (Gerstel, Mülheim a.d.R., Germany) followed by thermal desorption, using gas chromatographic separation and mass selective detection in SCAN mode. (A more detailed description of the analytical methods is given in the electronic supplementary information available from the web-site of the journal www.rsc.org/jem). These analyses were used within a backtracking study concerning the mutagenic effect in wastewater sample F from the chemical industry by literature research on specific substances.

2.3 Results

2.3.1 Biodegradability

The results of the biodegradation tests are shown in figures 2.2–2.4. The COD-elimination of indirect discharged wastewater sample in the Zahn-Wellens test showed a typical course, while the variability of the DOC-elimination of direct discharged samples, which had already passed a biological treatment, was considerable. This can be explained with the low initial TOC concentration and the complex interaction of adsorption and desorption processes.

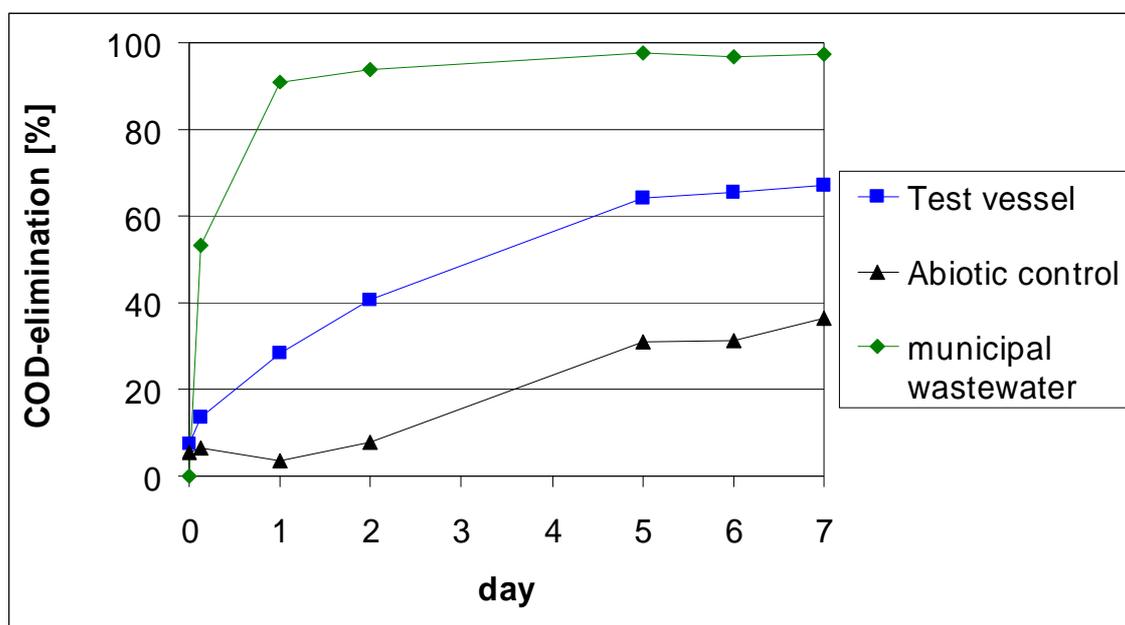


Figure 2.2: COD-elimination of textile wastewater A in the Zahn-Wellens test

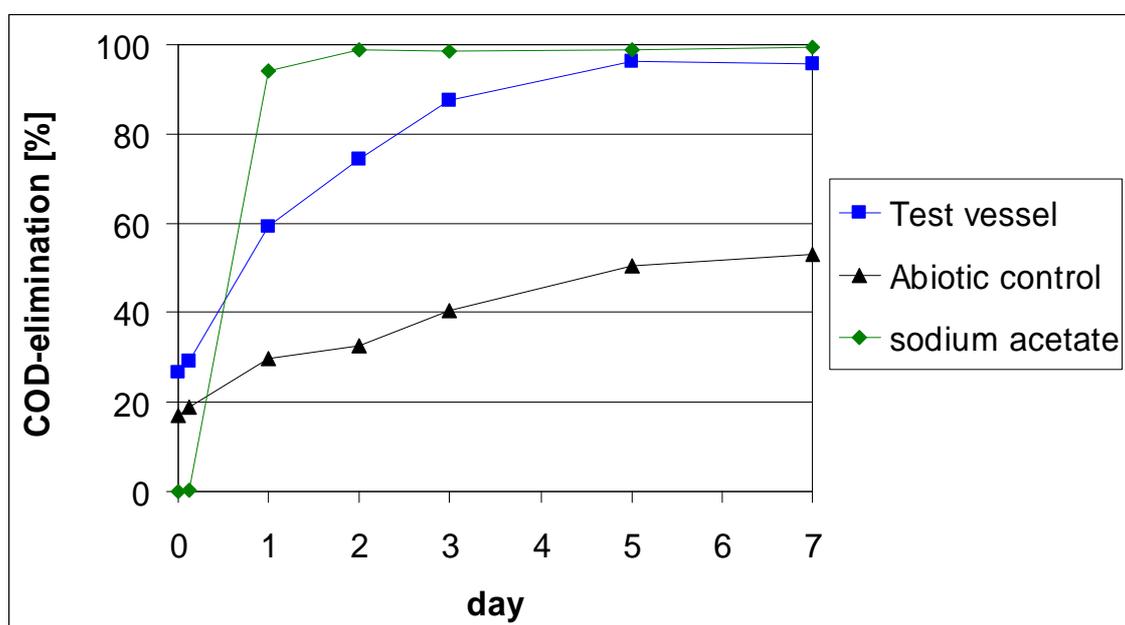


Figure 2.3: COD-elimination of metal working wastewater B in the Zahn-Wellens test

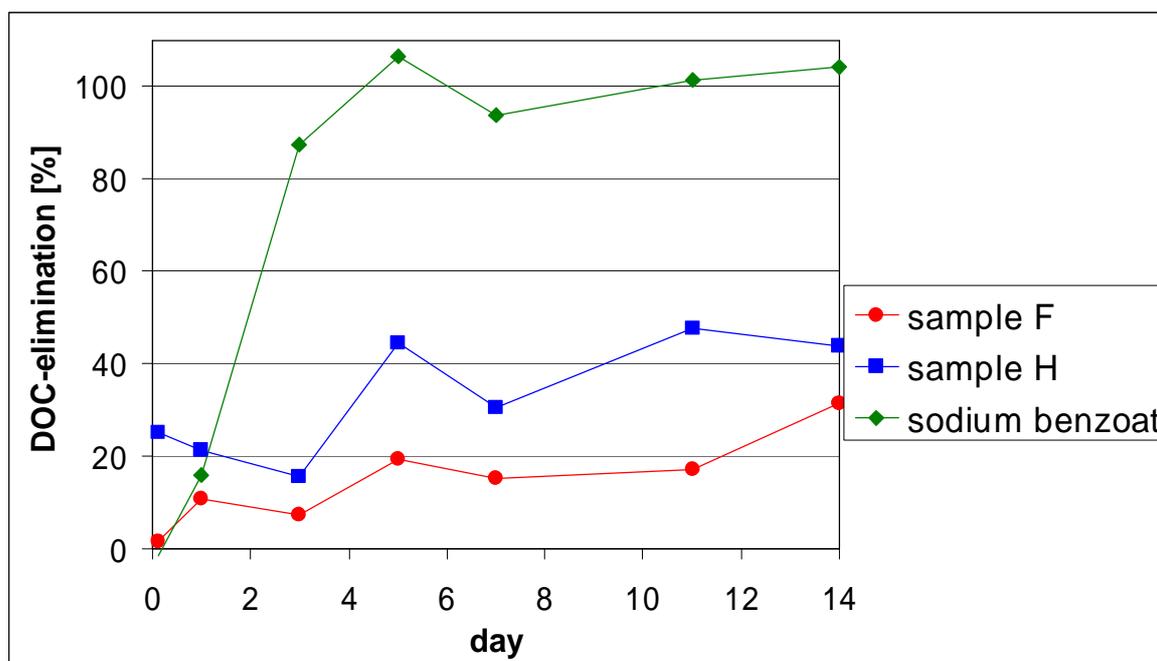


Figure 2.4: DOC-elimination of wastewater from chemical industry (F) and paper mill (H) in the DOC-Die-Away test

2.3.2 Ecotoxicity

The summary of results is given in figure 2.5 (a–h). Generally low to moderate ecotoxic effects of wastewater samples have been determined. Referring to native samples from direct dischargers or to samples from indirect dischargers, which have been biologically pretreated in the Zahn-Wellens test maximum values of $LID_A = 8$ in the algae test (pharmaceutical industry C), $LID_{lb} = 24$ in the luminescent bacteria test (textile plant A, influence of colouration on test results possible) and $LID_{Egg} = 6$ in the fish test (chemical industry F) have been determined. No sample was toxic against *Daphnia magna*. Some samples (D, H) showed slightly higher algae toxicity after biological treatment, but at an overall low level.

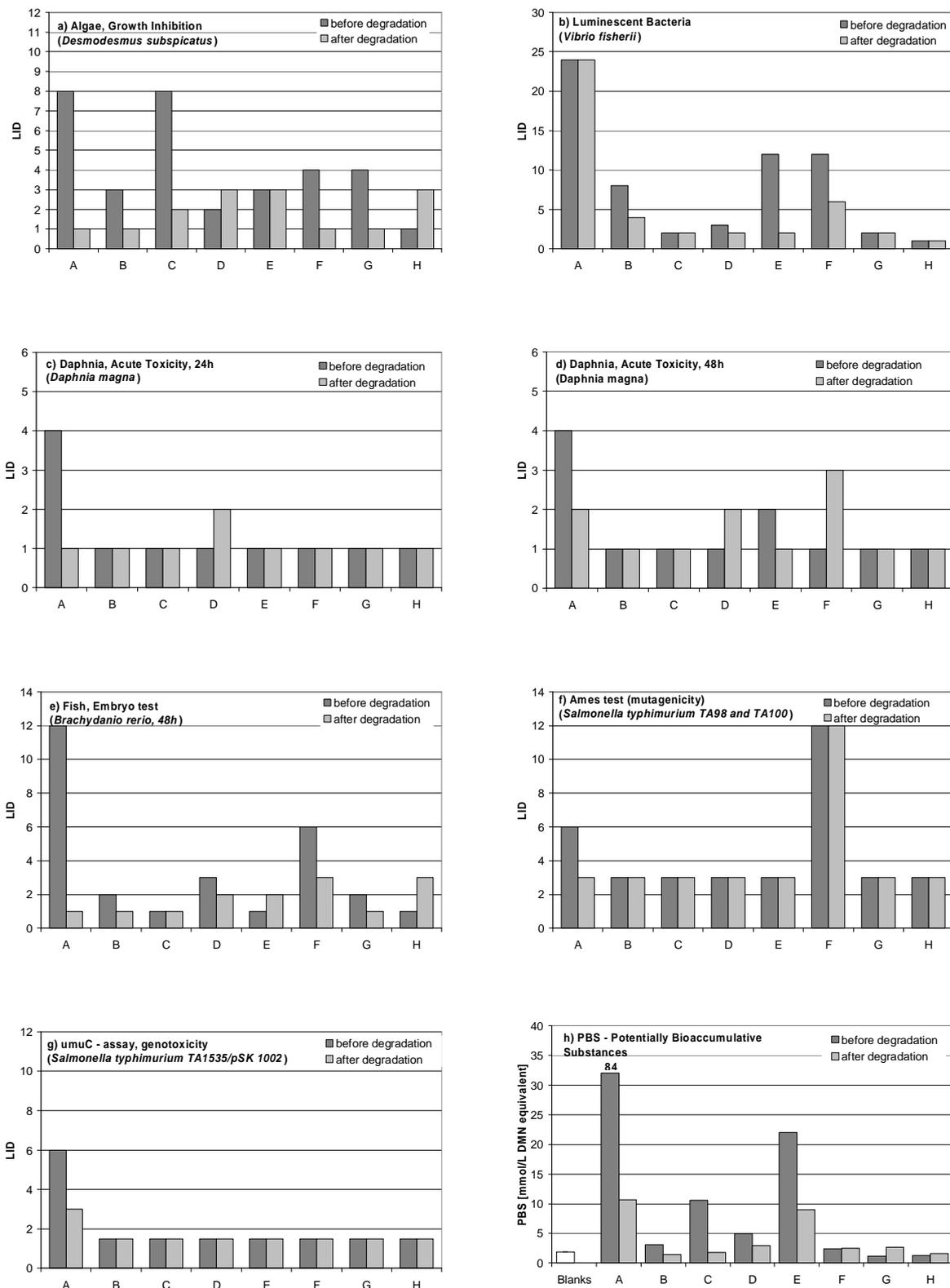


Figure 2.5 (a-h): Summary of test results with wastewater samples A to H
LID: Lowest Ineffective Dilution (ISO 5667-16, Annex A)

Table 2.3 shows the summary of the statistically analysis of ecotoxicity data. Because of the moderate toxicity for most wastewater samples no 50% effect concentration (EC50) could be calculated as the number of concentrations to be evaluated was not sufficient (at least one concentration should have effects above 50%). For the algae test the NOEC derived from the LID ($\text{NOEC}_{\text{LID}} = 100/\text{LID}$) corresponded quite well with the statistically significant $\text{NOEC}_{\text{ANOVA}}$ calculated from ANOVA-analysis.

Table 2.3: Statistical analysis of ecotoxicity results

Sample		A		B		C		D		E		F		G		H	
		before	after	before	after	before	after	before	after	before	after	before	after	before	after	before	after
Luminescent bacteria	NOEC _{LID} % ww	4	4	12,5	25	50	50	33	50	8,3	50	8,3	16,6	50	50	50	50
<i>Vibrio fischeri</i>	EC50 % ww	19,2 (15,2-25,2)	47,8 (44,4-51,7)	45 (40,4-50,5)	344 (215-685)	133 (103-183)	129 (100-175)	129 (71-305)	134 (108-173)	38,0 (30,9-49,5)	144,6 (133-158)	68,2 (61,5-76,8)	130 (116-147)				
Daphnia test 24h	NOEC _{LID} % ww	25	100	100	100	100	100	100	50	100	100	100	100	100	100	100	100
	EC50 % ww	43,6 (36,9-51,6)	>100	>100	>100	>100	>100	>100		>100	>100	>100	>100	>100	>100	>100	>100
Daphnia test 48 h	NOEC _{LID} % ww	25	50	100	100	100	100	100	50	50	100	100	>33	100	100	100	100
<i>Daphnia magna</i>	EC50 % ww	29,7 (25,7-34,4)	>100	>100	>100	>100	>100	>100		>100	>100	>100		>100	>100	>100	>100
Fish egg test	NOEC _{LID} % ww	8,3	100	50	100	100	100	33	50	100	50	16,6	33,3	50	100	100	33,3
<i>Danio rerio</i>	EC50 % ww	10			>100			67		>100	>100	72,2		>100	>100	>100	1832
Algae test	NOEC _{LID} % ww	12,5	100	33	100	<12,5	50	50	<33	33	33	25	100	25	100	100	33
	NOEC _{ANOVA} % ww	25,0		33		<12,5			33	50	33	25		25			<25
<i>Desmodemus subspicatus</i>	EC50 % ww	29,7 (18,0-n.d.)	>80	74,8 (n.d.-n.d.)	>80	8,2 (0,9-13,5)	>80	78,1	33-50	108,8 (81,9-208,4)	45,3 (n.d.)	34,8 (n.d.-n.d.)	>100	>100	>100	>100	

ww: wastewater

NOEC_{LID} % ww = 100/LID [% wastewater]

LID: Lowest ineffective dilution

2.3.3 Genotoxicity

The textile wastewater A was mutagenic in the Ames test (TA98 + S9, maximum induction factor 2.2) and genotoxic in the umu test (maximum induction factor 3.2). After treatment in the Zahn-Wellens test the mutagenicity in the Ames test was eliminated completely while in the umu test genotoxicity could still be observed (maximum induction factor 1.9). The wastewater sample F from chemical industry was mutagenic before and after the performance of the DOC Die-Away test (TA98 + S9, see figure 2.6).

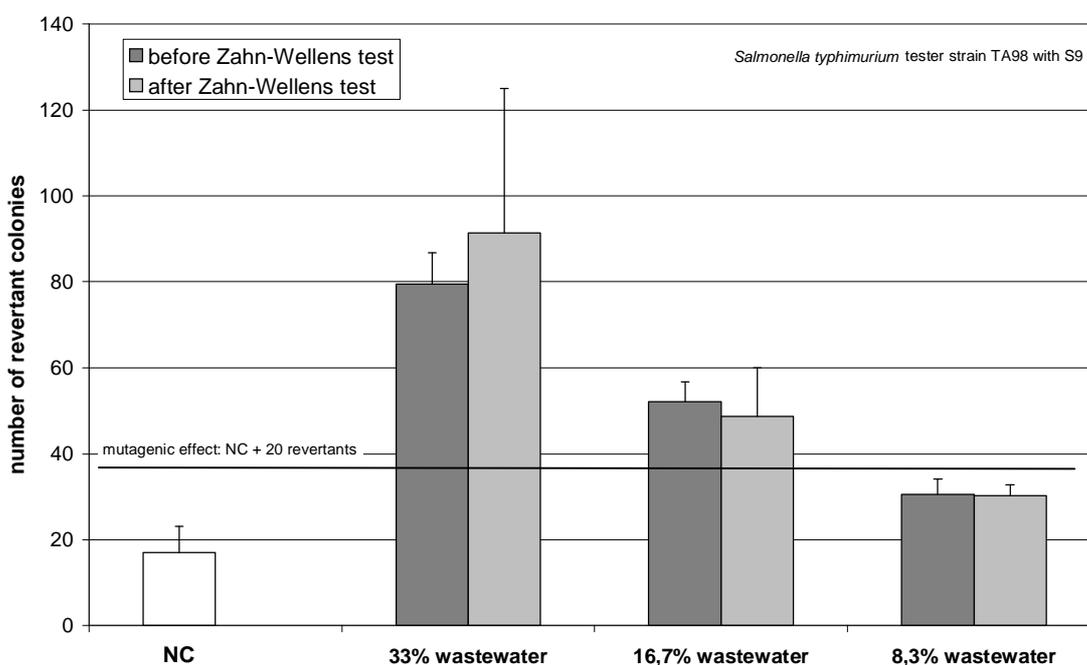


Figure 2.6: Ames test sample F from chemical industry

2.3.4 Potential potentially bio-accumulative substances (PBS)

Considering the PBS values, in particular the textile wastewater samples A and E stood out. The untreated samples A and E showed 84 and 22 mmol L⁻¹ respectively, the DMN equivalents exceeding the blank values by factors of 46 and 12. After biological treatment low levels of PBS (1.6–10.7 mmol L⁻¹) were determined. It should be noted that PBS concentrations refer to the volume of the fibre and not to the water phase (not exhaustive extraction). The relative standard deviations of the mean of duplicate PBS determinations for the samples B, F, G, and H are between 3.5% and 30%, which is in accordance with the outcome of an interlaboratory study on this method, performed as part of the OSPAR

Demonstration Project 2005–2006 by five laboratories with five different samples (Leslie 2006).

2.3.5 Backtracking studies on sample site F

The source of the mutagenicity of wastewater sample F from chemical industry was investigated by additional chemical analysis and backtracking. According to historical data the algae and fish egg toxicity at this site also did not always comply with wastewater permits according to Annex 22 of the German Wastewater Ordinance (limit values for fish egg and algae toxicity are LID 2 and 16 respectively, see table 2.2). Therefore both tests have also been considered in follow-up investigations. Chemical analysis of wastewater sample F revealed that a nitro-aromatic compound (2-methoxy-4-nitroaniline, CAS 97-52-9) used for batch-wise azo dye synthesis and its transformation products are the probable causes of mutagenic effects in the wastewater. 2-methoxy-4-nitroaniline has a low water solubility, is non-easily degradable and mutagenic in TA98 after metabolic activation with S9 (but not without S9) (Berufsgenossenschaft Chemie 1995). Testing the mother liquor from dye production containing 100 mg L^{-1} 2-methoxy-4-nitroaniline, confirmed that this partial wastewater stream was mutagenic in the Ames test (table 2.4).

Table 2.4: Ames test mother liquor azo dye synthesis

	Dilution	Induction factor	
		-S9	+S9
TA98	1:3	11.8	9.1
	1:6	8.0	4.0
	1:12	5.1	3.2
	1:24	2.8	2.5
TA100	1:3	6.8	4.3
	1:6	2.9	2.4
	1:12	1.8	1.7
	1:24	1.3	1.4
Characterisation of sample	pH 8.1, conductivity 109.1 mS/cm, 100 mg L^{-1} 2-Methoxy-4-nitroaniline, colour dark-blue, pasty consistency		
Pre-treatment of sample	Centrifugation 40.000 rpm, 10 minutes, followed by filtration 0,45 micrometer		

The LID_{EA} of the mother liquor was above 24. However the pattern of mutagenic effects shows that also transformation products of the reference compound 2-methoxy-4-nitroaniline must have been present in the sample. In contrast, two further total wastewater samples (directly discharged) taken at distinct time points where no azo dye synthesis took place showed no mutagenicity or genotoxicity in the Ames and umu tests (data not shown).

Considering elevated algae toxicity occasionally observed in total wastewater samples behind the treatment plant, the first suspicion was that production of the antimicrobial triclosan, a chlorinated biphenyl ether, and its derivatives might cause these effects. Triclosan is known to be highly ecotoxic to aquatic organisms, algae being the most susceptible organisms (*Scenedesmus subspicatus* 96 h study: EC50 = 1.4 mg L⁻¹) (Orvos et al. 2002). However a partial wastewater stream from triclosan production (original COD 35.000 mg L⁻¹) which was treated in the Zahn-Wellens test over 14 days after diluting the sample by factor of 35 (COD start concentration 1000 mg L⁻¹) showed no or only moderate ecotoxicity with the Daphnia test and fish egg tests: LID = 1, algae and luminescent bacteria tests: LID = 4, Ames test negative, see Table 2.5). Around 99% of the COD of that wastewater sample was removed immediately after addition of the activated sludge inoculum by absorption. Triclosan is known to be ultimately biodegraded (Federle et al. 2002). Indeed no triclosan was detected in that partial stream after treatment in the Zahn-Wellens test by chemical analysis.

Table 2.5: Partial wastewater stream from triclosan production

Algae test	LID _A	4
Daphnia test	LID _D	1 (after 24 and 48 h)
Fish egg test	LID _{Egg}	1
Luminescent bacteria test	LID _{lb}	1
Ames test	LID _{Ames}	3 (non mutagenic) TA98/TA100 +/-S9
Characterisation Of sample	COD 35.000 mg L ⁻¹	
Pre-treatment of sample	Zahn-Wellens test with a 1:35 dilution of original sample, calculated COD start concentration 1000 mg L ⁻¹ , COD-elimination 99%-100% immediately after adding activated sludge through adsorption/filtration.	

No clear correlation was found between algae toxicity of total wastewater samples and the triclosan concentration measured by analytical means (data not shown).

2.4 Conclusion

The overall results show moderate toxicity in general with LID values between 1 and 24. Only the fish egg toxicity of sample F from chemical industry did not comply with wastewater permits (table 2.6). The time series on that sampling sites show high variability.

Table 2.6: Effect-based limit values of German wastewater ordinance

Annex	Wastewater source sector	Fish egg test <i>Danio rerio</i> [LID]	<i>Daphnia magna</i> [LID]	<i>Scenedesmus subspicatus</i> [LID]	<i>Vibrio fischeri</i> [LID]	UmuC Genotoxicity [LID]	Elimination Zahn-Wellens test
22	Chemical and pharmaceutical	2 (DD)	8 (DD)	16 (DD)	32 (DD)	1,5 (DD)	80% - 95% TOC (DD, ID)
38	Textile industry	2 (DD)					
40	Metal processing	2-6 (DD depending from sub-sector)					

LID: lowest ineffective dilution

DD: direct discharge to a receiving water

ID: indirect discharge via public sewers to a wastewater treatment plant

Usually toxicity was removed during the biodegradation tests, but a slight increase of toxicity was also observed for samples D and H. Several surveys on results with bioassay in different wastewater sectors have been elaborated, considering distinct time-frames (1993–1996 and 1997–2000). Herein in total around 25,000 test results have been edited and statistically evaluated (see table 2.7, Diehl et al. 1998, 2003).

Table 2.7: Comparative ecotoxicity data of wastewater sectors

Annex	wastewater sector	year	No test (No sites)	maxima				median				90% percentil			
				LID _A	LID _D	LID _F	LID _b	LID _A	LID _D	LID _F	LID _b	LID _A	LID _D	LID _F	LID _b
22	Chemical / pharmaceutical Industry	1993-1996	2695 (105)	1024	768	24	1024	1	2	2	2	16	4	3	12
		1997-2000	4148 (85)	6144	96	48	512	2	2	2	3	16	8	3	24
28	Paper mill	1993-1996	324 (20)	8	8	16	64	2	1	2	2	4	2	2	12
		1997-2000	30 (3)	2	1	2	2	2	1	2	2				
38	Textile industry	1993-1996	84 (10)	16	6	6	16	2	1	2	2		2	4	4
		1997-2000	197 (17)	2	1	16	8	2	1	2	2	2	1	8	3
40	Metal processing	1993-1996	1235 (112)	3072	256	64	256	2	1	2	2	12	6	3	12
		1997-2000	2022 (126)	1024	512	512	2048	2	2	2	2	24	16	6	48

LID_A Algae test *Desmodesmus subspicatus*

LID_b Daphnia test *Daphnia magna*

LID_F Acute fish toxicity test with *Leuciscus idus*

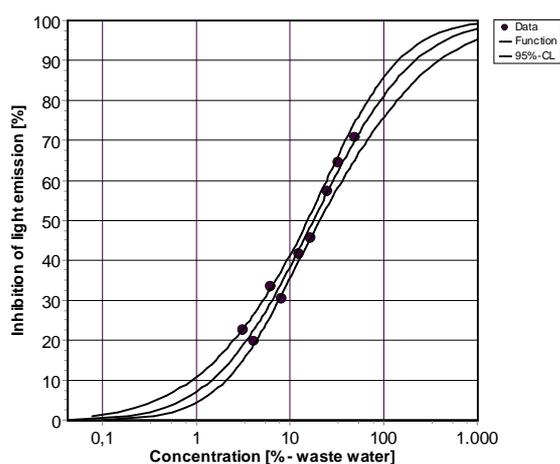
LID_b Luminescent bacteria test *Vibrio fischeri*

From the comparative data of table 2.7 it becomes clear that in general only moderate ecotoxicity is measured in all wastewater sectors (median LID 1-2) while the maximum LID values especially from chemical/pharmaceutical and metal processing wastewater sectors are remarkably high. The own result are in line with this observation. Most PBS values were in the same range as the blanks, thus only minor potentially bio-accumulative substances are present. An interlaboratory study has proposed the following classification of water samples when using SPME as a screening method for PBS (Leslie 2006):

< 5 mmol L ⁻¹ PBS	very low level of PBS (clean) effluent
5-20 mmol L ⁻¹ PBS	low level PBS effluent
>20 mmol L ⁻¹ PBS	high level PBS effluent
<40 mmol L ⁻¹ PBS	narcotic toxicity expected on this level

Thus, with the exception of two indirectly discharged native textile wastewater samples, all other effluents showed low or very low levels of PBS. After treatment in the Zahn-Wellens test the PBS of both textile wastewater samples were considerably reduced to low levels, not indicating a specific risk. Experience from the Netherlands also indicates that textile effluents show high PBS values compared to other wastewater sectors (OSPAR 2005). Slightly higher PBS values in some samples after the degradation test indicate that the inoculum contributed to the PBS. The statistical evaluation of ecotoxicity data revealed that EC50 calculation gives useful information but applicability was limited by the low observed ecotoxicity values. For most samples, EC50 could not be calculated because often no effects above 50% at all have been detected. Here the indication of dilution factor LID as test result is a suitable method. However, also examples have been found where the EC50 supported the interpretation of data (figure 2.7). Considering the LID, sample A showed no decrease of ecotoxicity through biological treatment in the Zahn-Wellens test at all, while the EC50 was considerably higher after the treatment step, thus indicating a reduction of toxicity. The NOEC derived from ANOVA analysis corresponded quite well with the observed LIDs.

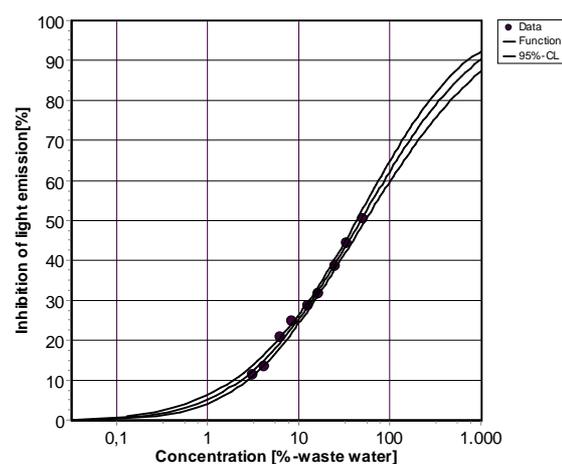
Sample A before Zahn-Wellens test



LID: 24

EC₅₀: 17.9% (95%-CI: 15.6% – 20.9%)

Sample A after Zahn-Wellens test



LID: 24

EC₅₀: 47.8% (95%-CI: 43.1% – 53.8%)

Figure 2.7: Luminescent bacteria test sample A

The origin of mutagenicity of sample F from chemical industry in the Ames test (TA 98 + S9) before and after biological treatment could be explained with partial streams from batch-wise azo dye synthesis with the nitro-aromatic compound 2-methoxy-4-nitroaniline

and its (more water soluble) transformation products. The compound itself has low water solubility, is noneasily degradable and mutagenic in TA98 after metabolic activation with S9 (but not without S9). No mutagenicity of this substance is observed in other in vitro mutagenicity tests (HPRTtest with V79 mammalian cells) (Berufsgenossenschaft Chemie 1995).

The varying algae and fish egg toxicity observed at the same sample site might be explained with partial wastewater streams from the production of triclosan, one of the continual processes of the chemical factory although no unambiguous relation has been detected. The behaviour of triclosan, an antimicrobial widely used in consumer products, in municipal wastewater treatment plants is known from several studies (Singer et al 2002, Thompson et al. 2005). Here, varying removal efficiency of triclosan in wastewater treatment plants (58%–98%) has been observed. Because triclosan is an ionisable molecule, its water solubility and adsorption are affected by pH, the removal efficiency being better at neutral or acid pH than in alkaline conditions. On the other hand literature data suggest that aquatic toxicity of triclosan is determined by the un-ionised form of triclosan being higher at neutral pH (Orvos et al. 2002). These complex and varying conditions might explain that algae toxicity up to now could not unambiguously be attributed to triclosan production. The cause of algae toxicity is currently being investigated in further testing programmes.

The overall results show that most wastewater samples were inconspicuous with the exception of sample F which was mutagenic and toxic to fish eggs. The genotoxicity determined after biological treatment of textile wastewater “A” also gives reason of concern. Generally the WEA testing strategy of combining biodegradation and toxicity tests revealed feasible results for the assessment of indirectly discharged wastewater. The Zahn-Wellens test proved to be a suitable screening tool for the biological treatment of wastewater samples by avoiding the much higher effort needed for laboratory flow through activated sludge simulation tests (OECD 303 A and EN ISO 11733). Also in Germany this concept has still not been considered in regulatory wastewater permits (here bioassay focus on direct discharges, see table 5) but offers new future perspectives for the evaluation of indirect dischargers. Taking into account the processes in the municipal treatment plant for indirect effluents is in line with the IPPC Directive (Article 2 Nr. 6) and the Water Framework Directive (2000/60/ EC, Article 2 Nr. 40), where it is stated that “The emission limit values for substances shall normally apply at the point where the emissions leave the installation, dilution being disregarded when determining them. With regard to

indirect releases into water, the effect of a waste-water treatment plant may be taken into account when determining the emission limit values of the installations involved.’’

The combination of the OECD DOC Die Away Assay with effect based tests resulted in fewer interpretable data probably because all effluents received a biological treatment beforehand at the local sites.

Acknowledgements

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3 CHAPTER 3

**Whole effluent assessment of industrial wastewater for
determination of BAT compliance
Part 1: paper manufacturing industry**

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Abstract*Background, aim and scope*

The applicability of the Whole Effluent Assessment concept for the proof of compliance with the “best available techniques” has been analysed with paper mill wastewater from Germany by considering its persistency (P), potentially bio-accumulative substances (B) and toxicity (T).

Materials and methods Twenty wastewater samples from 13 paper mills using different types of cellulose fibres as raw materials have been tested in DIN or ISO standardised bioassays: the algae, daphnia, luminescent bacteria, duckweed (*Lemna*), fish-egg and umu tests with lowest ineffective dilution (LID) as test result. The potentially bio-accumulative substances (PBS) were determined by solid-phase microextraction and referred to the reference compound 2,3- dimethylnaphthalene. Usually, a primary chemical–physical treatment of the wastewater was followed by a single or multi-stage biological treatment. One indirectly discharged wastewater sample was pretreated biologically in the Zahn–Wellens test before determining its ecotoxicity.

Results

No toxicity or genotoxicity at all was detected in the acute daphnia and fish egg as well as the umu assay. In the luminescent bacteria test, moderate toxicity (up to $LID_{lb} = 6$) was observed. Wastewater of four paper mills demonstrated elevated or high algae toxicity (up to $LID_A = 128$), which was in line with the results of the *Lemna* test, which mostly was less sensitive than the algae test (up to $LID_{DW} = 8$). One indirectly discharged wastewater sample was biodegraded in the Zahn–Wellens test by 96% and was not toxic after this treatment. Low levels of PBS have been detected (median 3.27 mmol L^{-1}). The colouration of the wastewater samples in the visible band did not correlate with algae toxicity and thus is not considered as its primary origin. Further analysis with a partial wastewater stream from thermomechanically produced groundwood pulp (TMP) revealed no algae or luminescent bacteria toxicity after pre-treatment of the sample in the Zahn–Wellens test (chemical oxygen demand elimination 85% in 7 days). Thus, the algae toxicity of the respective paper mill cannot be explained with the TMP partial stream; presumably other raw materials such as biocides might be the source of algae toxicity.

Discussion

Comparative data from wastewater surveillance of authorities confirmed the range of ecotoxicity observed in the study. Wastewater from paper mills generally has no or a moderate ecotoxicity (median LID 1 and 2) while the maximum LID values, especially for the algae and daphnia tests, are considerably elevated (LID_A up to 128, LID_D up to 48).

Conclusions

Wastewater from paper mills generally is low to moderately ecotoxic to aquatic organisms in acute toxicity tests. Some samples show effects in the chronic algae growth inhibition test which cannot be explained exclusively with colouration of the samples. The origin of elevated algae ecotoxicity could not be determined. In the algae test, often flat dose–response relationships and growth promotion at higher dilution factors have been observed, indicating that several effects are overlapping.

Recommendations and perspectives

At least one bioassay should be included in routine wastewater control of paper mills because the paper manufacturing industry is among the most water consuming. Although the algae test was the most sensitive test, it might not be the most appropriate test because of the complex relationship of colouration and inhibition and the smooth dose–effect relationship or even promotion of algae growth often observed. The Lemna test would be a suitable method which also detects inhibitors of photosynthesis and is not disturbed by wastewater colouration.

Keywords Wastewater ordinance, Paper manufacturing industry, Ecotoxicity, Genotoxicity, Algae test, *Vibrio fischeri* assay, Daphnia test, Umu assay, Fish-egg test, Lemna test, Zahn–Wellens test, Potential bio-accumulating substances, Whole effluent assessment, WEA, OSPAR

3.1 Introduction: Background, aim and scope

Effect-based test methods detect combined toxic effects of all substances present in complex wastewater samples and are complementary to the “single substances approach”. The aim of the study was to analyse the applicability of effect-based tests for the proof of compliance with the “best available techniques” using the examples of wastewater from the paper manufacturing and the metal surface treatment industries. For this, the Whole

Effluent Assessment (WEA) concept of the OSPAR expert group has been applied (OSPAR Hazardous Substances Committee 2007). Here, the wastewater samples are assessed with regard to persistency (P), presence of potentially bio-accumulative substances (B) and toxicity (T). Within the Integrated Pollution Prevention and Control Directive (IPPC, 2008/1/EC), the WEA concept has been included as a suitable monitoring tool on effluent in several Best Available Techniques Reference Documents. One consequence of the IPPC Directive is that for direct dischargers as well as for indirect dischargers, the same best available techniques should be applied. Within the study, a systematic approach for determining persistent toxicity of indirectly discharged wastewater was applied.

3.2 Materials and methods

3.2.1 Paper mill wastewater samples

In total, 13 paper mills from several parts of Germany representing different types of raw materials used (groundwood pulp, cellulose, recovered paper with/without deinking, chemicals for special papers, etc.) have been analysed. Twelve paper mills directly discharge their wastewater after passing a biological treatment plant of their own and one paper mill indirectly discharges to a municipal treatment plant. All factories (except one indirectly discharging) use a primary chemical–physical treatment of the wastewater followed by a single or multi-stage biological treatment. Most paper mills use the activated sludge process, sometimes coupled with percolating filters upstream, which are also used for cooling purposes. In three factories, the first biological stage is anaerobic treatment (Table 3.1).

Table 3.1: Characteristics of the paper mills investigated

Type of discharge	Production	Raw material	Waste-water	WWTP	
	t/a		1000 m ³ /a		
P1	D	140,000 woodfree and 140,000 special papers	90% cellulose 10% recycling paper	545	Sedimentation, bio-filter
P2	D	555,000 from recycling paper with deinking and 25,124 from recycling paper without deinking	2% cellulose 9% groundwood pulp 89% recycling paper	4937	Sedimentation, activated sludge, final clarifier, ozonisation, bio-filter
P3	D	148,000 decor -, special -, and carbon paper	96% ECF cellulose 4% TCF cellulose	1404	Sedimentation, moving bed reactor, activated sludge, final clarifier
P4	D	180,000 from recycling paper without deinking	100% A recycling paper	1110	Precipitation, flotation, high load moving bed reactor, low load activated sludge, final clarifier
P5	D	378,000 woody coated paper	25% cellulose 75% groundwood pulp (TMP)	5200	Chemical-mechanical pre-treatment, flotation, cooling sprinkling filter, moving bed reactor, activated sludge, final clarifier, biofiltration.
P6	D	583,000 from recycling paper without deinking	100% recycling paper	1580	Thermomechanical pulp (TMP) treatment with sodium dithionite Mechanical pre-treatment (disk filter), anaerobic treatment, cooling trickling filter, aerobic treatment (3 cascades), final clarifier
P7	D	125,000 from recycling paper with deinking	100% recycling paper	1600	Sedimentation, moving bed reactor, activated sludge, final clarifier, flotation, sand filtration
P8	D	240,000 woody coated paper	33% cellulose 69% groundwood pulp	4143	No mechanical pre-treatment, cooling trickling filter, activated sludge, final clarifier
P9	D	660,000 uncoated woodfree and woody paper	8% cellulose 32% groundwood pulp 60% recycling paper	7069	Chemical-physical pre-treatment (multi-disk clarifier, flotation, turbocirculator), cooling trickling filter, moving bed reactor, activated sludge, final clarifier
P10	D	342,000 from recycling paper with deinking	100% recycling paper	3820	Sedimentation, cooling trickling filter, anaerobic treatment, activated sludge, final clarifier
P11	D	1,192,000 woodfree coated paper	100% cellulose	3772	Sedimentation, bio-trickling filter, activated sludge, final clarifier
P12	I	40,000 paper board	95% recycling paper without deinking 5% TMP cellulose	350	Sedimentation, indirectly discharged to municipal WWTP (20% of hydraulic load)
P13	D	295,000 from recycling paper with deinking	100% recycling paper	2700	Flotation, anaerobic treatment, activated sludge, final clarifier, sand filtration

P: Paper mill

D: Direct discharger

I: Indirect discharger

Raw material: ECF=elemental-chlorine-free, TCF=total-chlorine-free, TMP= thermomechanical pulp

WWTP: Wastewater treatment plant

3.2.2 Testing strategy

The testing strategy and WEA principles have been described in the WEA Guidance document (OSPAR Hazardous Substances Committee, 2007). In principle, the same persistency, bio-accumulation and toxicity criteria used for identifying priority substances in water policy are applied with native wastewater samples. The overall test concept

consists in coupling the effect-based tests from a “toolbox” with biodegradation tests. For indirectly discharged effluents, the Zahn–Wellens test (adopted from OECD 302 B) has been suggested as a suitable tool for determining the behaviour in wastewater treatment plants and for discriminating persistent toxicity from nonpersistent toxicity caused e.g. by ammonium or readily biodegradable compounds. Therefore, in this study, all indirectly discharged wastewater samples have been biologically pretreated in the Zahn–Wellens test with activated sludge (1 g dry solids per litre) from the respective municipal treatment plants which received the wastewater and afterwards tested concerning their ecotoxicity (Figure 3.1, Table 3.2).

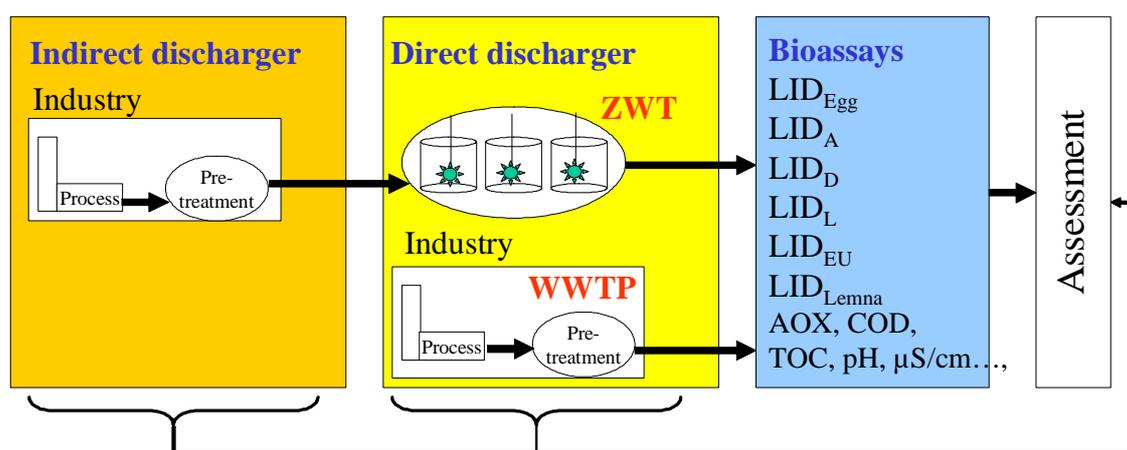


Figure 3.1: Testing strategy for direct and indirect dischargers

Table 3.2: Test methods used from the wastewater ordinance

Waste-water Ordinance	Method	Standard	Test-organism	Test-duration	Criteria
No. 401	Zebrafish embryo assay LID _{Egg} -value	DIN 38415-6: 2003 ISO 15088: 2007	<i>Danio rerio</i>	48	Development of embryos (coagulated eggs, heart beat, somites and tail differentiation) < 10%
No. 402	Daphnia acute toxicity LID _D -value	DIN 38412-30: 1989	<i>Daphnia magna</i>	24 h and 48 h	90% of Daphnia maintain ability to swim
No. 403	Alga growth inhibition test LID _A -value	DIN 38412-33: 1991	<i>Scenedesmus subspicatus</i>	72 h	Inhibition of biomass production < 20%
No. 404	Fluorescent bacteria test LID _{fb} -value Duckweed growth inhibition test LID _{DW} -value	DIN 38412-34: 1997 EN ISO 11348-2: 1998 ISO 20079: 2006	<i>Vibrio fischeri</i> <i>Lemna minor</i>	30 min 7 d	Inhibition of light emission < 20% Inhibition of growth based on fronds number and area < 10%
No. 407 / 408	Zahn-Wellens test	DIN EN ISO 9888: 1999	Activated sludge	2-7 d	COD/DOC-elimination
No. 410	Genotoxic potential <i>umu</i> test, LID _{EU} -value	DIN 38415-3: 1996 ISO 13829: 2000	<i>Salmonella typhimurium</i> TA1535/pSK1002	2 h	Induction rate < 1.5

Toxicity of wastewater might be caused by salts. In the German Wastewater Ordinance (2004), this is considered by a correction factor, which takes into account that the salt concentration increases when the water cycles are closed, which is appreciated from an environmental point of view. For the salt correction factor, the sum of chloride and sulphate ion concentrations (in g L^{-1}) is divided by an organism-specific value (3 for fish eggs, 2 for daphnia, 0.7 for algae and 15 for luminescent bacteria) and subtracted from the lowest ineffective dilution (LID). Hereby as a first approximation, a sum of 1 g L^{-1} chloride and sulphate (in equal proportions) corresponds to a conductivity of $5,000 \mu\text{S L}^{-1}$. For example, if the waste water permit requires a LID_{Egg} of 2, a value of $\text{LID}_{\text{Egg}} = 3$ is considered being acceptable if the wastewater contains more than 3 g L^{-1} chloride and sulphate (in this case $\text{LID}_{\text{Egg}} 3 - 3/3 = 2$).

3.2.3 Biodegradability/Treatability

Two vessels with 4,000 mL each of all indirectly discharged wastewater samples have been biodegraded in the Zahn–Wellens test (DIN EN ISO 9888) in order to provide sufficient material for subsequent ecotoxicity testing. The wastewater samples were supplemented with an inorganic nutrient solution and continuously stirred and aerated with an aquarium pump. The pH was adjusted to pH7–8 each working day. Chemical oxygen demand (COD) determination was done using ready to use cuvette tests from Hach-Lange, Germany. The activated sludge used as inoculum was obtained from the municipal sewage treatment plants to which the respective wastewater is discharged. The bio-elimination extents were referred to the expected initial start concentration calculated from the COD of the original sample and the dilution by adding mineral medium and activated sludge (less than 20% of total volume). In parallel, an abiotic control without inoculum but with addition of copper sulphate (final copper concentration 20 mg L^{-1}) for reducing biological degradation is tested to determine non-biological elimination such as stripping or adsorption. Synthetic wastewater made up of peptone, yeast extract and urea, according to DIN 38412-26 (1994), has been used as reference substance for a functional control. After treatment for 7 days, the activated sludge was allowed to settle for about 1 h, and the supernatant was decanted, split in 100-ml polyethylene bottles, stored at -18°C and used for ecotoxicity testing with bioassays.

3.2.4 Ecotoxicity and genotoxicity testing

All tests have been carried out according to DIN or ISO standards (Table 3.2). As far as possible, the original wastewater samples have been tested after pH adjustment with hydrochloric acid or sodium hydroxide solution to 7.0 ± 0.2 without any further pre-treatment. Where suspended particles might have an influence on the test results by mechanically interfering with the test organisms (*Daphnia*) or by light absorbance (algae, luminescent bacteria test), the solids were allowed to settle for 1 to 2 h immediately before starting the incubation period. In parallel to the wastewater samples, one concentration of suitable reference compounds (ecotoxicity: 3,4-dichloroaniline or potassium dichromate; genotoxicity: 2-aminoanthracene, nitrofurantoin, 4-nitro-1,2-phenylenediamine, 4-nitroquinolineoxide) has been tested according to the Analytical Quality Assurance bulletin of the German Working Group of the Federal States on water issues (LAWA 2009).

In Germany, for wastewater evaluation, the acute fish toxicity test with *Leuciscus idus* was replaced in 2004 by the short-term fish-egg assay with zebrafish (*Danio rerio*, also called fish embryo assay) for animal protection considerations. The test is classified as a sub-organism test because the central nervous system of fish embryos is not fully developed (Oberemm 2000). The fish were cultivated at 26°C and 14:10 h light/dark cycle and were fed daily with TetraMIN® flakes and two times per week with newly hatched brine shrimps (*Artemia* sp.) The fertilised eggs were collected in a rectangular glass spawning box, covered by a stainless steel mesh and artificial plants and were separated manually from unfertilised eggs using an inverted microscope. The eggs were incubated over 48 h, which covers the time from the blastula to the stage with fully developed blood circulation. The test performance consists in exposing 10 fertilised eggs for each concentration in 24-well cell culture plates (2 ml each).

The *Daphnia* toxicity test was performed using the clone 5 of *Daphnia magna* STRAUS of the German Federal Environment Agency. *Daphnia* were held in Elendt M4 medium and were fed daily with living algae cells (*Desmodesmus subspicatus* CHODAT, formerly called *Scenedesmus subspicatus*). Each concentration (dilution) was tested in two replicates with five *daphnia* each and incubated at 20°C in the dark. The test was evaluated after 24 h (DIN 38412-30).

For the algae growth inhibition test *D. subspicatus*, a planktonic fresh-water alga was used. After adding an algal nutrient solution, the vessels were inoculated with 10^4 algae per ml and incubated under defined light conditions ($135 \mu\text{E m}^{-2}\text{s}^{-1}$ photosynthetically active

radiation) at $23\pm 1^\circ\text{C}$. Each concentration (dilution) was tested in two replicates, the control vessels in five replicates. At the beginning of the incubation period and after 72 h, the chlorophyll fluorescence (excitation wavelength 465 nm, emission wavelength 670 nm) has been measured for quantifying the biomass increase (TECAN Infinite 200F, Tecan, Switzerland). The luminescent bacteria toxicity test with the marine bacteria *Vibrio fischeri* was performed using the LUMIStox system of the company Hach–Lange, Düsseldorf with liquid-dried bacteria of the strain *V. fischeri* NRRL-B-11177. The wastewater samples were tested after salinising with sufficient sodium chloride to obtain a 2% solution with two replicates at an incubation temperature of $15\pm 1^\circ\text{C}$ after 30-min contact time. The duckweed *Lemna minor* represents freshwater aquatic plants. The growth inhibition was determined by both determining the frond numbers and the frond area after an incubation time of 7 days at defined light conditions ($85\text{--}135\mu\text{E m}^{-2}\text{s}^{-1}$ photosynthetically active radiation) at $24\pm 2^\circ\text{C}$ with an image analysis system (Scanalyzer, LemnaTec, Germany). Each concentration (dilution) was tested in three replicates, the control vessels in six replicates. For the testing of dark-coloured test solutions compared to the algae growth inhibition test, the Lemna test has the advantage of light absorption and thereby resulting growth inhibition is irrelevant. As test result, the more sensitive of the two endpoints (frond numbers and frond area) is reported.

The umu test is a genotoxicity test with the biotechnologically modified bacterial strain *Salmonella typhimurium* TA1535/pSK1002. Gene toxins induce the umuC-gene, which belongs to the SOS-repair system of the cell. By coupling of the umuC-gene promoter with the lacZ-gene for β -galactosidase, the activation of the umuC-gene can be indirectly measured spectrophotometrically at 420 nm through the formation of a coloured product from the β galactosidase substrate o-nitrophenyl-galactopyranoside (ONPG). The bacteria are exposed for 4 h to the wastewater with and without metabolic activation using microplates, and the genotoxin-dependent induction of the umuC-gene is compared to the spontaneous activation of the control culture. Each concentration has been tested three-fold in 96-well microtiter plates (Greiner Bio-One, Frickenhausen, Germany). The induction rate corresponds to the increase of the extinction at 420 nm relative to the negative control. Bacterial growth and inhibition are determined turbidimetrically from the optical density at 600 nm. For growth factors below 0.5 (50% growth inhibition), the results are not evaluated. The result given is the smallest dilution step at which an induction rate <1.5 is measured. All samples have been tested in at least four concentrations. Subsequently, toxic or genotoxic samples have been further analysed until no growth inhibition or induction of

genotoxicity was determined. Samples which are toxic at higher concentrations but non-genotoxic at growth factors >0.5 have been designated as “toxic”.

All results of ecotoxicity and genotoxicity testing are given as the LID, which is defined as the reciprocal volume fraction of the wastewater sample at which only effects not exceeding the test-specific variability are observed (ISO 5667-16 1998, Annex A). This corresponds to the lowest dilution level (threshold level) where effects do not exceed the test-specific variability. The following thresholds effect levels are given in the respective standards: a mortality or inhibitory effect or an immobilisation of $\leq 10\%$ (Fish-egg test, Daphnia test, Lemna test), an inhibitory effect $\leq 20\%$ (luminescent bacteria test, algae test), an induction rate ≤ 1.5 (umu test).

3.2.5 Potentially bio-accumulative substances

The potentially bio-accumulating substances (PBS) were determined by solid-phase microextraction (SPME) according to a protocol adapted by Leslie and Leonards (2005) for the OSPAR WEA group. Briefly, a 1-cm long quartz glass fibre coated with $100\mu\text{m}$ polydimethylsiloxan (PDMS) from Supelco (Bellafonte, CA, USA) was exposed at room temperature to 250 mL wastewater which was continuously stirred at 500 rpm over 24 h. The Erlenmeyer flask used was nearly headspace-freely filled with the sample and wrapped with aluminium foil during SPME. Gas chromatographic analysis was performed after thermodesorption of SPME fibre in the GC injector (in splitless mode) using a CP 9001 (Chrompack, Frankfurt a. M.) with flameionisation detector and a capillary column OPTIMA-1 (10 m long, 0.25 mm I.D., $0.1\mu\text{m}$ film thickness) from Macherey–Nagel (Düren, Germany). The whole chromatogram was integrated between the retention times of nonane (n-C9) and octa-triacontane (n-C38). The obtained peak areas were normalised to the peak of the reference compound 2,3-dimethylnaphthalene (DMN; $\log K_{ow} = 4.4$) which was injected separately from a standard solution. The results (PBS concentrations) are expressed as mmol L^{-1} DMN equivalents. Note that this concentration does not mean a PBS concentration in the water sample extracted but expresses per convention the PBS amount (as DMN equivalent) per volume of PDMS coating, the extracting phase. Additionally, two blank values from two PE bottles filled with distilled water (one new, one used before) were determined according to the same procedure.

3.2.6 Accompanying chemical analysis

Along with the biological tests, also physicochemical parameters of the Wastewater Ordinance such as pH, conductivity, COD, total organic carbon (TOC), total phosphate, ammonium and heavy metals have been determined, and here are only partly documented because of the limited space available.

3.3 Results with paper mill wastewater samples

3.3.1 Overview

Altogether, 20 wastewater samples from 13 different paper mills have been analysed in the research programme. One or two independent samples per paper mill have been taken. A repetition of sampling at the same site is designated as a “B” sample. The results are shown in Table 3.3. The COD of directly discharged paper mill effluents was between 24 and 498 mg L⁻¹; the respective TOC was between 7 and 136 mg L⁻¹. The inorganic nitrogen compounds (as sum of ammonium, nitrite and nitrate) with one exception (P6-B: N_{total} = 14.8 mg L⁻¹) were below the requirements of the Wastewater Ordinance (N_{total} = 10 mg L⁻¹). The limit values concerning total phosphate of 2 mg L⁻¹ were slightly exceeded by two samples (maximum P6-B: P_{total} = 3.9 mg L⁻¹). The maximum conductivity of the samples was 3370 μS cm⁻¹, thus not indicating a toxicity caused by salts. AOX values were only available from five samples, and these were not elevated (maximum P11: AOX = 0.213 mg L⁻¹).

Table 3.3: Results with wastewater from the paper manufacturing industry

	COD	Duck-weed assay	Algae assay	Daphnia test	Fish-egg test	Luminescent bacteria test	Umu assay	Conductivity	Potentially bio-accumulating substances
	mg L ⁻¹	LID _{DW}	LID _A	LID _D	LID _{Egg}	LID _{lb}	LID _{EU}	μS cm ⁻¹	mmol L ⁻¹
P1	43	1	1 S	1	1	3	1.5	1540	8.21
P2	210	2	3	1	1	≤ 2	1.5	3040	7.24
P3	24	2	1 S	1	1	≤ 2	1.5	1031	8.15
P3-B	32	2	1 S	1	1	≤ 2	1.5	1281	2.92
P4	146	1	1 S	1	1	≤ 2	1.5	1075	7.88
P4-B	125	4	1	1	1	≤ 2	1.5	1779	2.03
P5	250	4	64	1	1	≤ 2	1.5	1475	14.61
P5-B	244	3	128	1	1	4	1.5	1278	1.40
P6	233	2	1 S	1	1	8	1.5	2920	6.91
P6-B	237	3	1 S	1	1	3	1.5	3370	1.12
P7	133	2	1 S	1	1	≤ 2	1.5	2570	6.03
P7-B	102	1	1 S	1	1	3	1.5	2080	0.89
P8	116	1	2	1	1	≤ 2	1.5	1951	3.36
P9	446	3	16	1	1	6	1.5	1693	4.28
P9-B	498	8	16	1	1	4	1.5	2390	1.94
P10	247	8	6	1	1	≤ 2	1.5	2472	7.50
P10-B	191	4	6	1	1	3	1.5	2040	1.30
P11	43,0	2	1 S	1	1	3	1.5	1283	3.18
P12 after ZW	88	3	1 S	1	1	6	1.5	1293	0.78
P13	278	6	12	1	1	4	1.5	2760	0.86

LID: Lowest ineffective dilution

P: Paper mill

ZW: Zahn-Wellens test

S: Stimulation of algae growth

The results show no toxicity at all in the daphnia and fish-egg tests. No sample was genotoxic in the umu assay. However, the wastewater of four paper mills demonstrated an elevated or high algae toxicity while many others in contrast stimulated the growth of algae. This is in line with the result observed with the Lemna test, which mostly was less sensitive than the algae. With some wastewater samples, the Lemna test revealed slight effects not detected with the algae test. In the luminescent bacteria test, half of the samples were inconspicuous while the other samples showed a moderate toxicity (up to LID_{lb} = 6). The only indirectly discharged wastewater sample of paper mill P12 was biodegraded in the Zahn-Wellens test by 96% (see Figure 3.2) and was not toxic after treatment.

Considering the sum parameter PBS, wastewater from the paper manufacturing industry exhibited low levels of PBS ($0.78\text{--}14.61\text{ mmol L}^{-1}$, median 3.27 mmol L^{-1}).

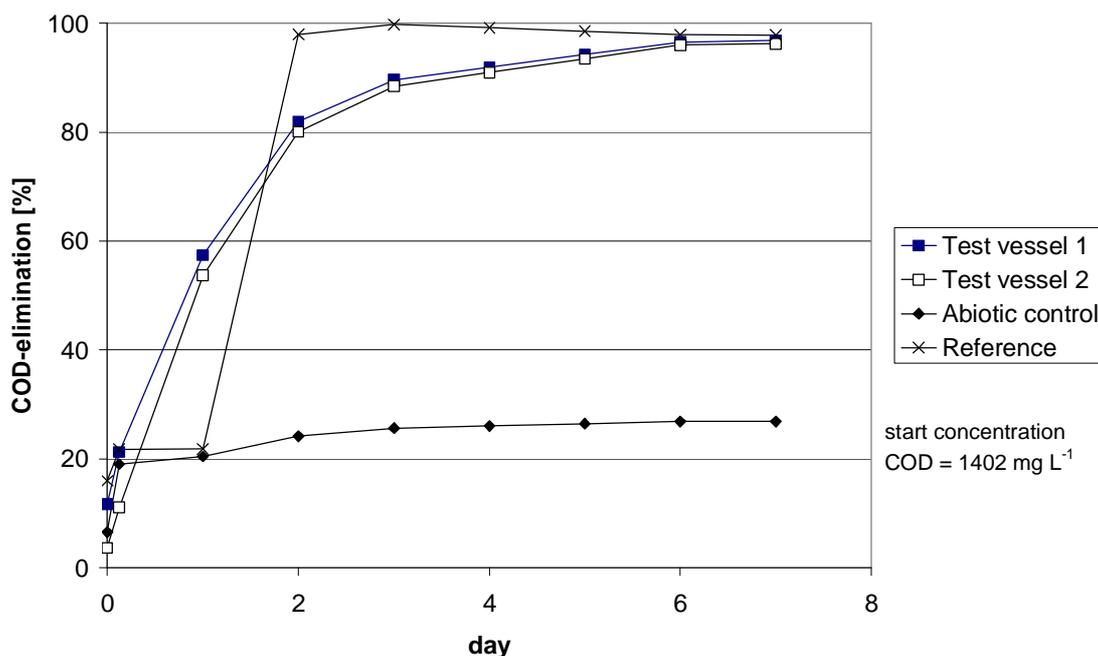


Figure 3.2: COD-elimination of paper mill wastewater P12 in the Zahn-Wellens test

3.3.2 Origin of algae toxicity

Wastewater from paper mills often did not show a definite dose–response relationship in the algae test or even stimulated algae growth. Repeatability of algae tests has been studied on wastewater from paper mill P5. Figure 3.3 shows that all four independent tests consistently indicated considerable algae toxicity of P5. However, within the range of dilution factor 16 and 192, a flat decline of algae growth inhibition was observed, sometimes combined with an increase at higher dilution factors. As the LID_A is defined as the lowest dilution where for the first time the inhibition is below the threshold of 20%, the overall results fluctuate between LID_A 64 and LID_A 192. In the first trial, the threshold is even touched at a dilution factor of 32.

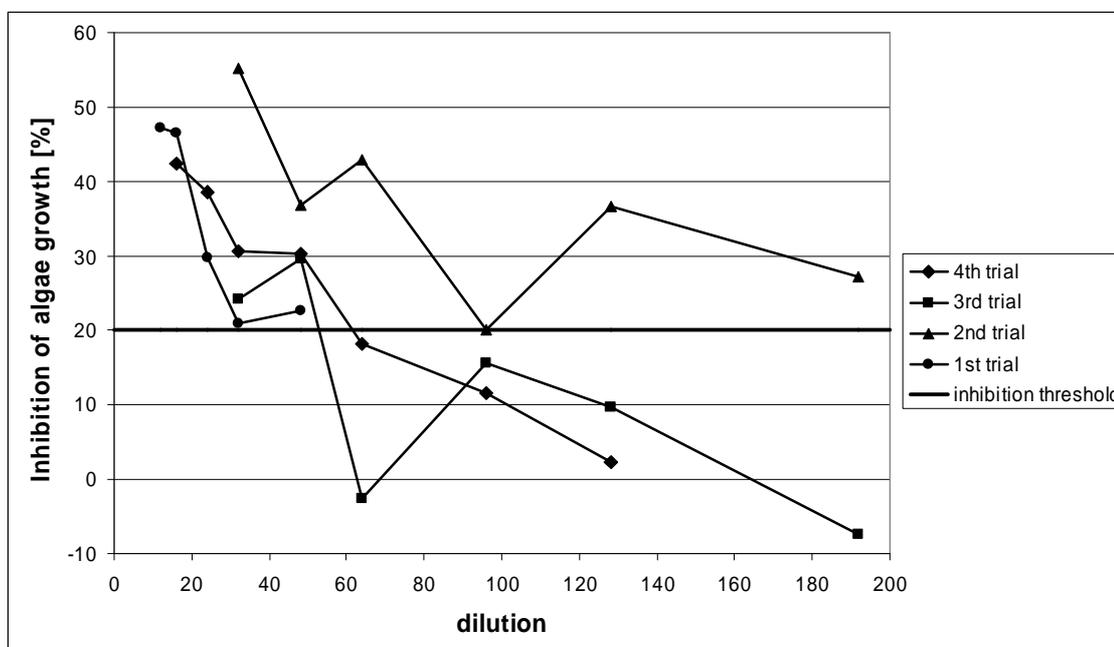


Figure 3.3: Algae toxicity of P5 in four independent tests

The results demonstrate that obviously several effects like inhibition, light absorbance and growth promotion interact. It is known that coloured samples might reduce photosynthetic efficiency and, therefore, inhibit algae growth. Paper mill wastewater often is considerably coloured because of the lignin fraction present in the water, which is not completely removable even not through bleaching processes. In order to determine the influence of colouration on testing results, all paper mill wastewater samples have been photometrically measured in the visible range (Figure 3.4).

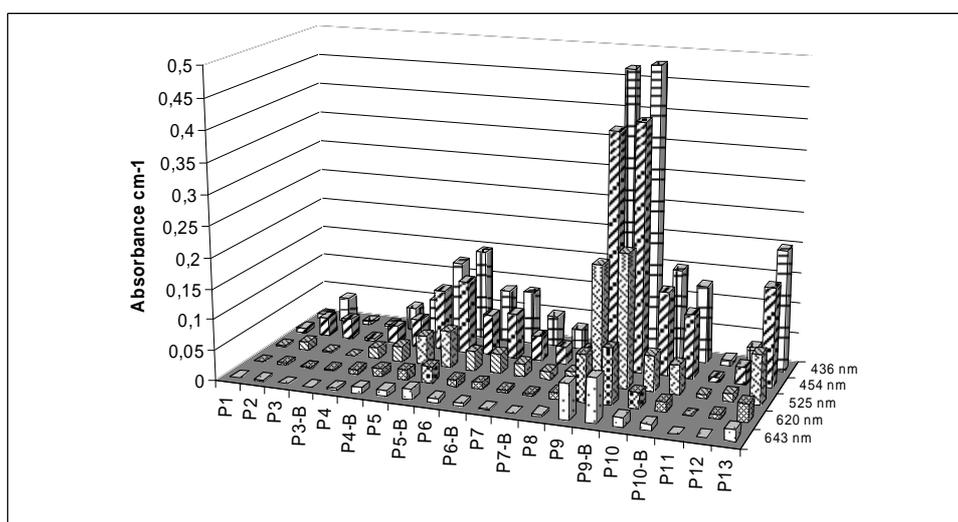


Figure 3.4: Light absorbance of paper mill wastewater samples

The light absorption maxima of the chlorophyll from the algae used are 440 and 680 nm. In particular, both samples from paper mill P9 most strongly absorbed light in the whole visible range. However, these samples were only moderately toxic in the algae test. The outstanding samples with highest algae toxicity from factory P5 were not particularly notable with regard to their colouration. Also, the results with the Lemna test, which mostly were in the same direction as those with the algae test, gave an indication against the hypothesis that algae toxicity is mainly caused by colouration of the samples. As duck weeds swim at the water surface, their photosynthetic efficiency is not influenced by colouration. Therefore, attention was turned to other potential influencing factors. It was known that in factory P5 mainly TMP is used as raw material. Hereby, the external part of decorticated log wood rich in lignin, which is provided from a sawmill, is decomposed under heat and pressure. The resulting wastewater has a high COD of up to 5,000 mg L⁻¹ and is biodegraded by around 90% in the wastewater treatment plant. For determining whether algae toxicity of P5 is caused by the TMP, a partial stream of the TMP wastewater was taken and at first degraded in the Zahn–Wellens test. The COD elimination reached 85% in 7 days (Figure 3.5). Afterwards, algae and luminescent bacteria toxicity of the pretreated wastewater was determined. The results demonstrate that the sample was rather unpolluted. The luminescent toxicity was $LID_{lb} = 3$, the algae toxicity $LID_A \leq 4$. Therefore, the ecotoxicity found in wastewater from paper mill P5 cannot be explained with this partial stream. Presumably other raw materials such as biocides might be the source of algae toxicity. It is known that the successful closing of water circuits in paper mills which led to a reduction of the specific water consumption per tonnage produced from around 50 m³·year⁻¹ in the 1970s to about 10 m³·year⁻¹ increased microbiological problems in the circuits which subsequently were combated by biocides (European Commission 2001).

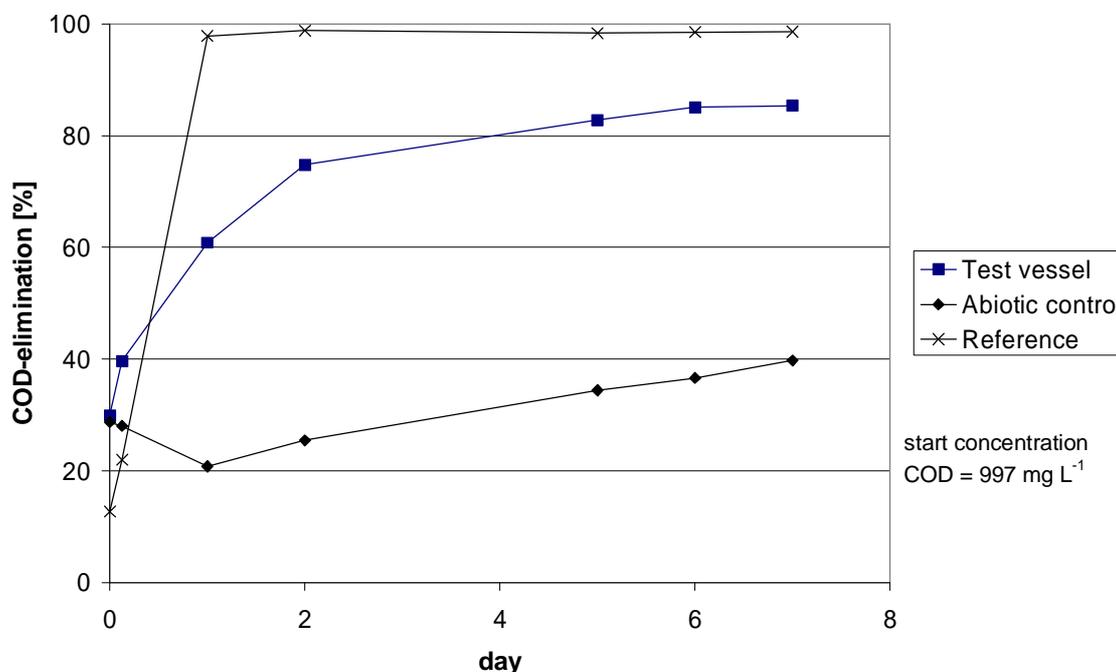


Figure 3.5: COD-elimination of TMP groundwood pulp P5-C in the Zahn-Wellens test

3.4 Discussion

In Germany, the application of bioassays in wastewater surveillance by local authorities has a long tradition. Several surveys on results with bioassays in different wastewater sectors have been elaborated, considering distinct timeframes (1993–1996, 1997–2000, 2001–2007). These comparative data of Diehl and Hagendorf (Diehl and Hagendorf 1998; Diehl et al. 2003) and Gartiser et al. (2008) presented in Table 3.4 confirm the range of ecotoxicity observed in the study in hand. Wastewaters from paper mills generally have no or a moderate ecotoxicity (median LID 1–2) while the maximum LID values especially for the algae and daphnia test are considerably elevated (LID_A up to 128, LID_D up to 48; it should be mentioned that all data of Table 3.4 from the period from 2001–2007 and most data of the previous periods refer to direct dischargers). The maximum value of the fish-egg test was not conspicuous (LID_{Egg} = 3) but is based on relatively few data. However, historic data with the acute fish test, which was replaced in 2004 by the fish-egg test, also demonstrated elevated fish toxicity (LID_F up to 48).

Table 3.4: Effect-based data from paper mill wastewater surveillance by German authorities

Period	No. of tests (No. of paper mills)	Maximum					Median					90% Percentile				
		LID _A	LID _D	LID _{Egg}	LID _F	LID _{lb}	LID _A	LID _D	LID _{Egg}	LID _F	LID _{lb}	LID _A	LID _D	LID _{Egg}	LID _F	LID _{lb}
1993-1996	324 (20)	8	8		16	64	2	1		2	2	4	2		2	12
1997-2000	30 (3)	2	1		2	2	2	1		2	2					
2001-2007	380 (16)	128	48	3	48	8	2	1	2	2	2	6	2	2	6	3

LID_{A, D, Egg, F, L}: Lowest ineffective dilution for algae, daphnia, fish eggs, fish, and luminescent bacteria

Sources: 1993 – 1996: Diehl et al. (1998) ; 1997-2000: Diehl et al. (2003), 2001-2007: Gartiser et al. (2008)

Data from 2001-2007 exclusively, data from 1993 – 2000 mainly belong to direct dischargers

In a former WEA practical approach with another directly discharged paper mill wastewater sample, no toxicity or genotoxicity at all was observed in the algae, daphnia, fish-egg and luminescent bacteria tests, as well as in the umu assay and Ames test (Gartiser et al. 2009).

Literature data suggest that the discharge of pulp and paper mill effluents negatively affect light transmission through the content of lignosulphonates. However, the impact of colouration on phytoplankton development cannot be distinguished from inhibitory toxic effects on the phytoplankton (Karrasch et al. 2006). A survey of 12 pulp and paper effluents in Canada even found that effluent treatment using aerated stabilisation basins leads to average increases in colour of 20–40% (Milestone et al. 2004).

The application of bioassays for surveillance and wastewater permits in the pulp and paper sector is very common. An overview about national limit values is given by OECD (1999). For example, no acute toxicity to rainbow trout or *D. magna* is allowed in wastewater from kraft mills in Canada ($LC_{50} \geq 50$ vol.%). Numerous studies on the effluent quality of the paper mill industry have been published which confirm that short-term and chronic effects on organisms may occur (OSPAR Hazardous Substances Committee HSC 2000; Kovacs and Ferguson 1990; Robinson et al. 1994; Hall et al. 2009). However, often, other test organisms have been applied or the effluents contained both pulp and paper partial streams so that the results are not directly comparable.

Most PBS values were in the same range as the blanks; thus, only minor potentially bio-accumulating substances are present. An interlaboratory study has proposed the following classification of water samples when using SPME as a screening method for PBS (Leslie 2006):

-
- <5 mmol L⁻¹ PBS very low level of PBS (clean) effluent
 - 5–20 mmol L⁻¹ PBS low level PBS effluent
 - >20 mmol L⁻¹ PBS high level PBS effluent
 - <40 mmol L⁻¹ PBS narcotic toxicity expected on this level

Thus, all wastewater samples analysed were in very low or low level respective pollution with PBS. It should be noted again that PBS concentrations refer to the volume of the extracting fibre and not to the water phase (because it is in contrast to the exhaustive solvent extraction a negligible depletive, biomimetic extraction).

3.5 Conclusions

Wastewater from paper mills generally was low to moderate ecotoxic to aquatic organisms in acute toxicity tests. Some samples showed effects in the chronic algae growth inhibition test which cannot be explained exclusively with colouration of the samples. The origin of elevated algae ecotoxicity could not be determined. In the algae test, often, flat dose–response relationships and growth promotion at higher dilution factors have been observed, indicating that several effects are overlapping.

3.6 Recommendations and perspectives

It is recommended to include at least one bioassay in routine wastewater control of paper mills. Although the algae test was the most sensitive test, it might not be the most appropriate test because of the complex relationship of colouration and inhibition and the smooth dose–effect relationship or even promotion of algae growth often observed. The Lemna test would be a suitable method which also detects inhibitors of photosynthesis and is not disturbed by wastewater colouration. Because the paper manufacturing industry is among the most water-consuming industrial sectors, also the fish-egg test, which is used for the determination of wastewater charges, could be a useful parameter.

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4 CHAPTER 4

Whole effluent assessment of industrial wastewater for determination of BAT compliance Part 2: metal surface treatment industry

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Abstract

Background, aim and scope Toxicity testing has become a suitable tool for wastewater evaluation included in several reference documents on best available techniques of the Integrated Pollution Prevention and Control (IPPC) Directive. The IPPC Directive requires that for direct dischargers as well as for indirect dischargers, the same best available techniques should be applied. Within the study, the whole effluent assessment approach of OSPAR has been applied for determining persistent toxicity of indirectly discharged wastewater from the metal surface treatment industry.

Materials and methods Twenty wastewater samples from the printed circuit board and electroplating industries which indirectly discharged their wastewater to municipal wastewater treatment plants (WWTP) have been considered in the study. In all factories, the wastewater partial flows were separated in collecting tanks and physicochemically treated in-house. For assessing the behaviour of the wastewater samples in WWTPs, all samples were biologically pretreated for 7 days in the Zahn–Wellens test before ecotoxicity testing. Thus, persistent toxicity could be discriminated from non-persistent toxicity caused, e.g. by ammonium or readily biodegradable compounds. The fish egg test with *Danio rerio*, the *Daphnia magna* acute toxicity test, the algae test with *Desmodesmus subspicatus*, the *Vibrio fischeri* assay and the plant growth test with *Lemna minor* have been applied. All tests have been carried out according to well-established DIN or ISO standards and the lowest ineffective dilution (LID) concept. Additionally, genotoxicity was tested in the umu assay. The potential bioaccumulating substances (PBS) were determined by solid-phase micro-extraction and referred to the reference compound 2,3-dimethylnaphthalene.

Results The chemical oxygen demand (COD) and total organic carbon (TOC) values of the effluents were in the range of 30–2,850 mg L⁻¹ (COD) and 2–614 mg L⁻¹ (TOC). With respect to the metal concentrations, all samples were not heavily polluted. The maximum conductivity of the samples was 43,700 μS cm⁻¹ and indicates that salts might contribute to the overall toxicity. Half of the wastewater samples proved to be biologically well treatable in the Zahn–Wellens test with COD elimination above 80%, whilst the others were insufficiently biodegraded (COD elimination 28–74%). After the pre-treatment in the Zahn–Wellens test, wastewater samples from four (out of ten) companies were extremely ecotoxic especially to algae (maximum LID_A = 16,384). Three wastewater samples were genotoxic in the umu test. Applying the rules for salt correction of test results as allowed in

the German Wastewater Ordinance, only a small part of toxicity could be attributed to salts. Considering the PBS, wastewater from the metal surface treatment industry exhibited very low levels of PBS. In one factory, the origin of ecotoxicity has been attributed to the organosulphide dimethyldithiocarbamate (DMDTC) used as a water treatment chemical for metal precipitation. The assumption based on rough calculation of input of the organosulphide into the wastewater was confirmed in practice by testing its ecotoxicity at the corresponding dilution ratio after pre-treatment in the Zahn–Wellens test. Whilst the COD elimination of DMDTC was only 32% in 7 days, the pretreated sample exhibited a high ecotoxicity to algae ($LID_A = 1,536$) and luminescent bacteria ($LID_{lb} = 256$).

Discussion Comparative data from wastewater surveillance by authorities (data from 1993 to 2007) confirmed the range of ecotoxicity observed in the study. Whilst wastewater from the metal surface treatment industry usually did not exhibit ecotoxicity (median LID 1–2), the maximum LID values reported for the algae, daphnia and luminescent bacteria tests were very high (LID_A up to 3,072, LID_D up to 512 and LID_{lb} up to 2,048). DMDTC was found to be one important source of ecotoxicity in galvanic wastewater. DMDTC is added in surplus, and according to the supplier, the amount in excess should be detoxified with ferric chloride or iron sulphate. The operator of one electroplating company had not envisaged a separate treatment of the organosulphide wastewater but was assuming that excess organosulphide would be bound by other heavy metals in the sewer. DMDTC degrades via hydrolysis to carbon disulfide (which is also toxic to animals and aquatic organisms), carbonyl sulphide, hydrogen sulphide and dimethylamine, but forms complexes with metals which stabilise the compound with respect to transformation. Although no impact on the WWTP is expected, the question arises whether the organosulphide is completely degraded during the passage of the WWTP.

Conclusions and recommendations The results show that the organic load of wastewater from the electroplating industry has been underestimated by focussing on inorganic parameters such heavy metals, sulphide, cyanide, etc. Bioassays are a suitable tool for assessing the ecotoxicological relevance of these complex organic mixtures. The proof of biodegradability of the organic load (and its toxicity) can be provided by the Zahn–Wellens test. The environmental safety of water treatment chemicals should be better considered. The combination of the Zahn–Wellens test followed by the performance of ecotoxicity tests turned out to be a cost-efficient suitable instrument for the evaluation of indirect dischargers and considers the requirements of the IPPC Directive.

Keywords Wastewater ordinance, Metal surface treatment, Printed circuit board industry, Electroplating industry, Ecotoxicity, Genotoxicity, Algae test, *Vibrio fischeri* assay, Daphnia test, umuC assay, Fish egg test, Lemna test, Zahn–Wellens test, Potential bioaccumulating substances, Organosulphides, Dimethyldithiocarbamate, Whole effluent assessment, WEA, OSPAR

4.1 Introduction: Background, aim and scope

Wastewater discharges from metal surface treatment are regulated in Annex 40 of the German Wastewater Ordinance. One of the most important processes applied in Annex 40 is electroplating in galvanic baths. In Germany, there exist about 2,050 electroplating companies with 48,000 employees (Anonymous 2008). Most companies belong to medium-sized industry and discharge their wastewater indirectly to municipal wastewater treatment plants. Also, in the printed circuit boards industry, electroplating processes are applied. Around 35 bigger and 200 small- and medium-sized companies produce printed circuit boards in Germany (Achterbosch and Brune 1996). Within the Integrated Pollution Prevention and Control Directive (IPPC, 2008/1/EC), the whole effluent assessment (WEA) concept has been included as a suitable monitoring tool on effluents in several best available techniques reference documents (BREFs). One consequence of the IPPC Directive is that the same best available techniques should be applied for direct dischargers as well as for indirect dischargers. Whilst in Germany bioassays are routinely applied for wastewater control of directly discharged effluents, they are not used for wastewater discharged to municipal treatment plants, although indirect discharges represent an important part of industrial emissions. Within the study, a systematic approach for determining persistent toxicity of indirectly discharged wastewater was applied.

4.2 Materials and methods

4.2.1 Wastewater samples from the metal surface treatment industry

Two factories producing printed circuit boards and eight electroplating companies have been considered in the study. All companies are situated in the southwest of Germany and discharge their wastewater indirectly to municipal treatment plants after a physicochemical

in-house pre-treatment for metal separation. The physicochemical wastewater pre-treatment stages are organised in a similar way in all factories. Usually, the different wastewater partial flows (acidic, alkaline, containing/ not containing chromium, complexing agents or cyanides) are separated in collecting tanks and treated individually (Table 4.1).

Detoxification of cyanides In total, five factories used cyanides as complexing agents for the stabilisation of alkaline metal-containing electrolytes (mainly copper, silver or gold). Most companies detoxified these cyanides by oxidation with sodium hypochlorite, one company used hydrogen peroxide, and another disposed of these liquids as hazardous waste.

Detoxification of chromium The reduction of toxic chromium(VI) by sodium dithionite or sodium bisulfite is a common process mainly followed by hydroxide precipitation

Detoxification of nitrite Only one company indicated that nitrite-containing wastewater is oxidised by sodium peroxide. Here, nitrite is used as complexing agent for acidic electroless nickel plating of steel.

Hydroxide precipitation For precipitation of soluble metals from acidic dissolutions, mainly hydrated calcium oxide (lime) is used. The metals were transformed in their hardly soluble hydroxides, whereas the bivalent calcium supports the precipitation, which partly is improved through the addition of flocculation aids. Afterwards, the sludge is usually separated in chamber filter presses and disposed of.

Sulphide precipitation Many metal sulphides have a considerable lower solubility than their respective hydroxides. However, this process was not applied in the factories investigated.

Organosulphide precipitation Organosulphides form even more poorly soluble compounds with heavy metals than the respective metal sulphides. In five factories, organosulphides from the carbamate group have been added for precipitation of heavy metals in the presence of complexing agents. Usually, the concentration of the organosulphide added in excess is measured by a rapid test (colouration after addition of copper sulphate) after treatment and then is detoxified by other metal-containing wastewater, iron chloride or hydrogen peroxide. Only one company discharged organosulphide-containing wastewater indirectly, assuming that it would be inactivated by other heavy metals present in the sewer network.

All factories apply water-saving flushing techniques and many recycle their water by means of anionic and cationic ion exchangers. As a consequence, the volume of wastewater indirectly discharged is relatively low (1,200 to 71,000 m³/year) and salt content can be high. Cationic exchangers sometimes are also used as final safety filters at the outflow into the sewer.

Table 4.1: Characteristics of the metal surface treatment factories investigated

	Production (% of total production)	Raw material	Waste- water	pre-treatment of wastewater					Municipal WWTP
				Cromium VI reduction 1)	Hydroxide precipitation 2)	Precipitation with organosulphide 3)	Cyanide oxidation Nitrite oxidation 4)	Ion exchange 5)	
	Material	Amount y ⁻¹	m ³ y ⁻¹						
L1	Printed circuit boards	180 t Cu (acidic) Au, Sn, Ni	54.000		LW	Cu	H	F	41.000
L2	Printed circuit boards	110 t Cu (acidic)	71.000		LW Fe SS			F	71.000
G1	Electroplating (100%)	16 t Ni, 8 t chromic acid, 13 t Cu (acidic and alkaline cyanidic), 12 t Zn (acidic)	34.000	SBS	LW		N	F	3800
G2	Electroplating (92%)	35 t Ni, 40 t Cu (acidic), Ni/Cr, Cr(VI)	43.000	SBS	LW B	WW		C	100.000
G3	Electroplating (60%)	100 kg Ni, 12 t Cr(VI), 50 kg Cu (alkaline cyanidic)	1.233	SBS	LW	H	H	C	160.000
G4	Electroplating (95%)	1.100 kg Ni 500 kg Cr(VI)	1571	SBS	LW	nl		C F	13.000
G5	Electroplating (100%)	Ni/Cr Cr (VI)	1832	SDT SBS	LW			C F	160.000
G6	Electroplating (60%)	3 t Ni, 0,1 t chromic acid, 2 t Cu (alkaline cyanidic), 6 t Zn (acidic), 60 kg Ag (cyanidic)	7500	SDT SBS	LW	WW	N	C	75.000
G7	Electroplating (100%)	2,5 t Ni, 40 t Cr(VI)	1975	SDT			NH	F	47.000
G8	Electroplating (100%)	100 kg Ni, 20 kg Cu (alkaline cyanidic), 32 kg Ag + 36 kg Au (cyanidic) 10 kg tin	1400		LW		N AC	C	16.500

L: Printed circuit boards, G: Electroplating

1) SDT = sodium dithionite, SBS = sodium bisulfite

2) LW = lime water; SS= sodium sulfite, Fe= Fe(III)Cl₂; B= bentonite

3) Cu=surplus bound with CuCl₂; WW=surplus bound with further wastewater; H=surplus destroyed with H₂O₂, nl=no inactivation

4) H=H₂O₂; N=NaOCl; W=to waste; NH: nitrite oxidation with H₂O₂

5) C: in water circuit; F: as final safety filter

4.2.2 Testing strategy for biodegradation, ecotoxicity and potentially bioaccumulating substances

The testing strategy and methods applied have been described in part 1 of this paper in detail. In principle, the test concept for indirect dischargers consists in coupling the effect-based tests with the Zahn–Wellens test as a suitable tool for determining the behaviour in wastewater treatment plants (WWTP) and for discriminating persistent toxicity from non-persistent toxicity caused, e.g. by ammonium or readily biodegradable compounds. The test methods applied corresponded to those considered in the German Wastewater Ordinance for routine measurements based on DIN or ISO standards (fish egg test with *Danio rerio*, *Daphnia magna* acute toxicity test, algae test with *Desmodesmus subspicatus*, *Vibrio fischeri* assay, plant growth test with *Lemna minor* and genotoxicity in the umu assay).

4.3 Results with wastewater from the metal surface treatment industry

4.3.1 Overview

In total, 20 wastewater samples from ten metal surface treatment factories have been analysed, among them two producing printed circuit boards and eight electroplating factories. As a rule, two samples have been taken from all companies. A repetition of sampling at the same site is designated as a “B” sample; sampling at different sites of the same company is numbered consecutively (e.g. G2-1, G2-2; see Table 4.2).

Table 4.2: Results with wastewater from the metal surface treatment industry

	Zahn-Wellens test	Duckweed assay	Alga-assay	Daphnia-test	Fish egg test	Luminescent bacteria test	umu-assay	Conductivity	Potentially bioaccumulating substances	
	COD	Elimination after 7 d [%]	LID _{DW}	LID _A	LID _D	LID _{Egg}	LIF _{ib}	LID _{EU}	$\mu\text{S cm}^{-1}$	mmol L^{-1}
L1	978	91	8	16	1	3	4	1,5	9660	1,93
L1-B	968	68	6	8	1	2	6	6	8620	2,36
L2	678	95	3	2	1	4	3	1,5	11050	2,37
L2-B	787	80	6	4	3	4	3	1,5	19380	0,93
G1	757	70	8	6	2	3	3	1,5	18900	4,27
G1-B	163	87	8	32	2	2	2	1,5	13220	3,65
G2-1	93,8	100	6	2	1	1	≤ 2	1,5	5200	4,41
G2-2	956	95	6	3	1	2	≤ 2	1,5	8330	2,36
G3	1811	65	96	16	3	4	24	1,5	24400	1,41
G3-B	2854	74	48	8	12	3	6	6	18820	2,45
G4-1	2684	28	384	16384	64	1024	1536	toxic	39400	4,54
G4-2	923	58	384	1024	16	64	192	toxic	24400	3,47
G4-3	1665	55	96	256	128	32	96	6	28800	3,97
G5	108	100	2	48	1	1	≤ 2	1,5	2700	1,21
G5-B	94,3	100	2	12	1	1	≤ 2	1,5	1580	0,53
G6	276	69	64	512	4	96	128	toxic	11780	1,14
G6-B	194	72	64	4	1	2	4	1,5	10840	0,44
G7	168	70	6	64	1	2	4	1,5	7800	2,41
G8	34,5	100	3	1	1	1	3	1,5	1972	3,69
G8-B	30,8	100	2	1	1	1	≤ 2	1,5	3190	1,68

LID: Lowest ineffective dilution

L: Printed circuit boards

G: Electroplating

The chemical oxygen demand (COD) of indirectly discharged printed circuit board and electroplating effluents was between 30 and 2,850 mg L^{-1} ; the respective TOC was between 2 and 614 mg L^{-1} . The requirements of the German Wastewater Ordinance concerning heavy metal concentrations (before mixing with other wastewater partial streams) were mainly fulfilled (chromium, copper, nickel $\leq 0.5 \text{ mg L}^{-1}$ each, chromium(VI) $\leq 0.1 \text{ mg L}^{-1}$, zinc $\leq 2 \text{ mg L}^{-1}$). In one sample each, the zinc and chromium(VI) concentrations marginally exceeded the limit values (maximum zinc G4-1 = 4.57 mg L^{-1} , maximum chromium(VI) G6 = 0.116 mg L^{-1}).

With respect to the other metal concentrations, all samples were inconspicuous. The maximum conductivity of the samples was $43,700 \mu\text{S cm}^{-1}$. Thus, a contribution of salts to the overall toxicity could not be excluded. About half of the wastewater samples proved to be biologically well treatable in the Zahn–Wellens test (COD elimination $>80\%$; see Figure 4.1).

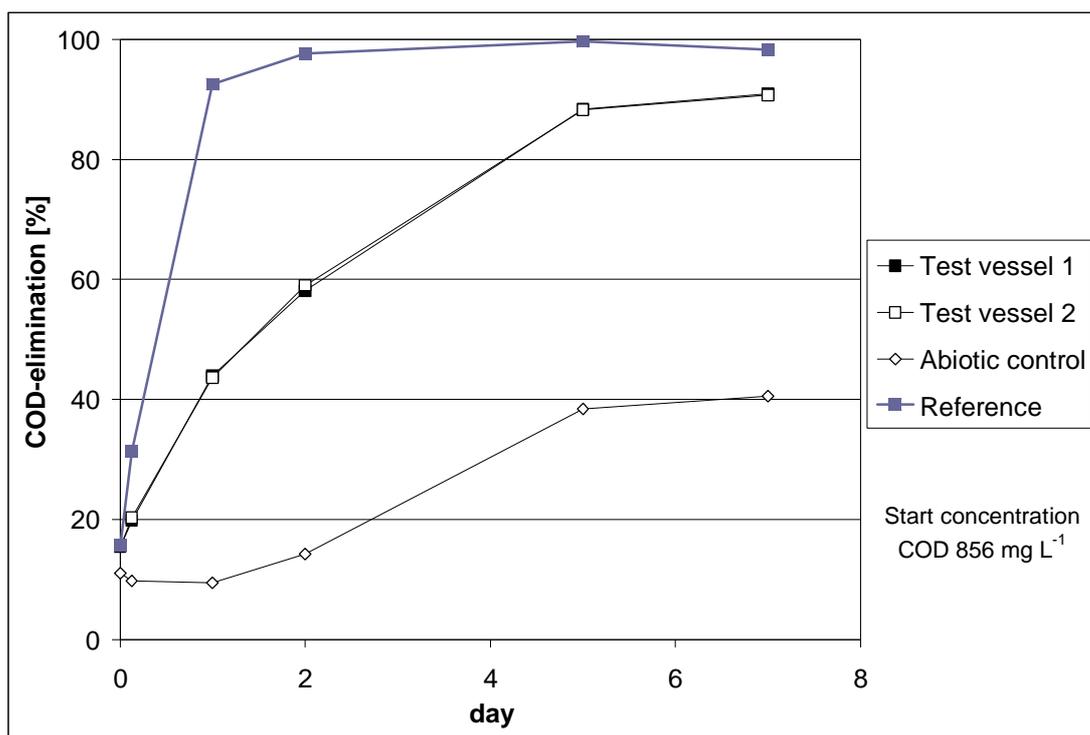


Figure 4.1: COD-elimination of wastewater L1 in the Zahn-Wellens test

However, the COD of some individual wastewater samples was insufficiently removed (28–74%, see Table 4.3 and Figure 4.2).

Table 4.3: Effect-based data from metal surface treatment wastewater surveillance by German authorities

Period	No. of tests (No. of factories)	Maximum					Median					90% Percentile				
		LID _A	LID _D	LID _{Egg}	LID _F	LID _{lb}	LID _A	LID _D	LID _{Egg}	LID _F	LID _{lb}	LID _A	LID _D	LID _{Egg}	LID _F	LID _{lb}
1993 -	1235															
1996	(112)	3072	256		64	256	2	1		2	2	12	6		3	12
1997 -	2022															
2000	(126)	1024	512		512	2048	2	2		2	2	24	16		6	48
2001 -	940															
2007	(58)	128	128	12	200	96	1	1	1	2	2	16	6	4	4	8

LID_{A, D, Egg, F, L}: Lowest ineffective dilution for algae, daphnia, fish-eggs, fish, and luminescent bacteria
 Sources: 1993 – 1996: Diehl et al (1998) ; 1997-2000: Diehl et al. (2003), 2001-2007: Gartiser et al. (2008)
 Data from 2001-2007 exclusively, data from 1993 – 2000 mainly belong to direct dischargers

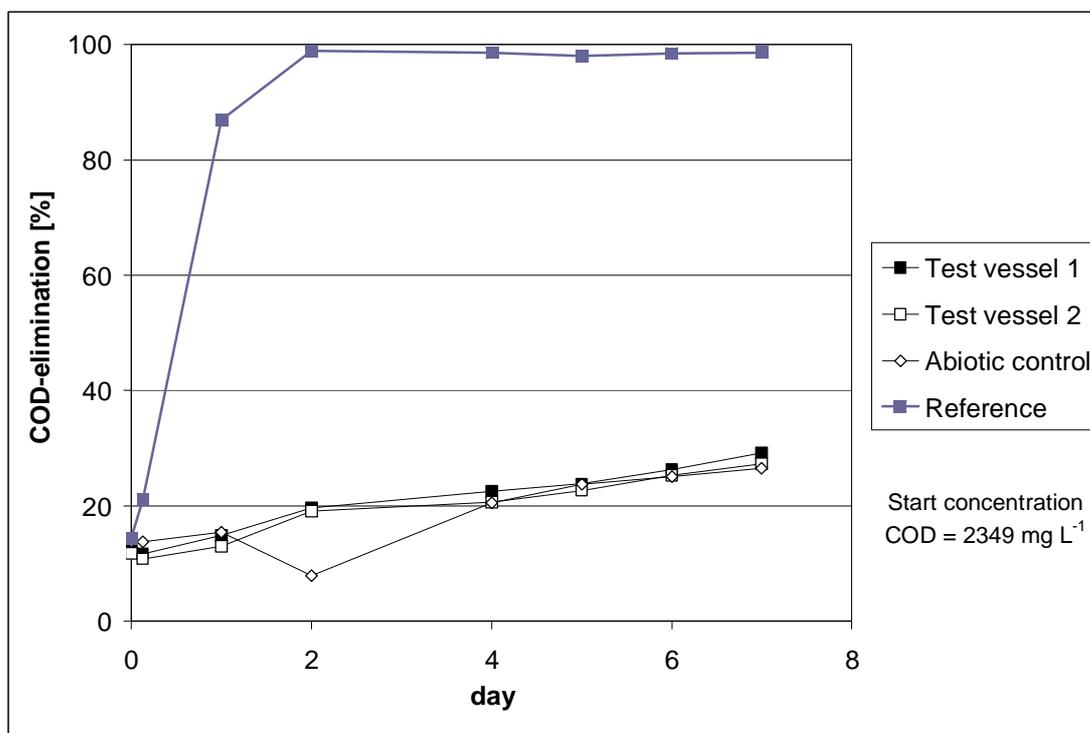


Figure 4.2: COD-elimination of wastewater G4-1 in the Zahn-Wellens test

In principle, at high TOC concentrations, an inhibition of the activated sludge cannot be excluded completely. In order to assess the influence of the test concentration on the results in the Zahn-Wellens test, sample G4-1 was tested nearly undiluted (COD 2,349 mg L⁻¹) and in a follow-up trial more diluted (COD 1,007 mg L⁻¹). Although biodegradability of this sample increased from 28% after 7 days to 63%, it remained below the critical value of 75% which is referred to for wastewater from other industrial sectors. The COD/TOC relation of up to 15 (median 4.8) indicates that wastewater from the electroplating industry contains reduced inorganic compounds and/or high chloride concentrations (above 1,000 mg L⁻¹) which disturb the COD analysis. The COD has therefore only a limited informational value with regard to the organic pollution. In some wastewater samples, extremely high ecotoxicity was determined (Table 4.3). However, compared with the existing limit value for the fish egg test in Germany of $LID_{Egg} = 6$, this value was exceeded by only four out of 20 wastewater samples from two companies out of ten (G4 and G6). The highest ecotoxicity was measured in the algae test where LID_A values up to several thousands (maximum 16,384) have been determined. Those samples most heavily polluted reacted with all bioassays. The results of the Lemna test were mainly in the same direction as the algae test, but partly also showed slightly higher toxicity than the algae test at lower dilution levels.

Wastewater samples L1-B, G3-B and G4-3 proved to be genotoxic in the umu test without S9 activation at the highest concentration (test result was $LID_{EU} = 6$). With some samples, no conclusion concerning genotoxicity could be drawn at higher concentrations because of bacteria toxicity.

The conductivity of the samples of up to $43,700 \mu\text{S cm}^{-1}$ indicates that salts might contribute to the overall toxicity. In order to assess this influence, a salt correction of the toxicity data has been performed (for methodology, see part 1 of this publication). However, it was demonstrated that also whilst applying the conservative rules for salt correction, some wastewater samples were highly ecotoxic. For only three wastewater samples (G1, G3 and G3-B), a salt correction results in another evaluation of the samples because the LID_{Egg} values of 3–4 (measured) decreased to $LID_{Egg} = 2$ (salt-corrected), which according to the German Wastewater Ordinance is not regarded as a toxic effect. Concerning the other bioassays, a salt correction resulted in a certain improvement of the LIDs, but toxic samples were still detected as such.

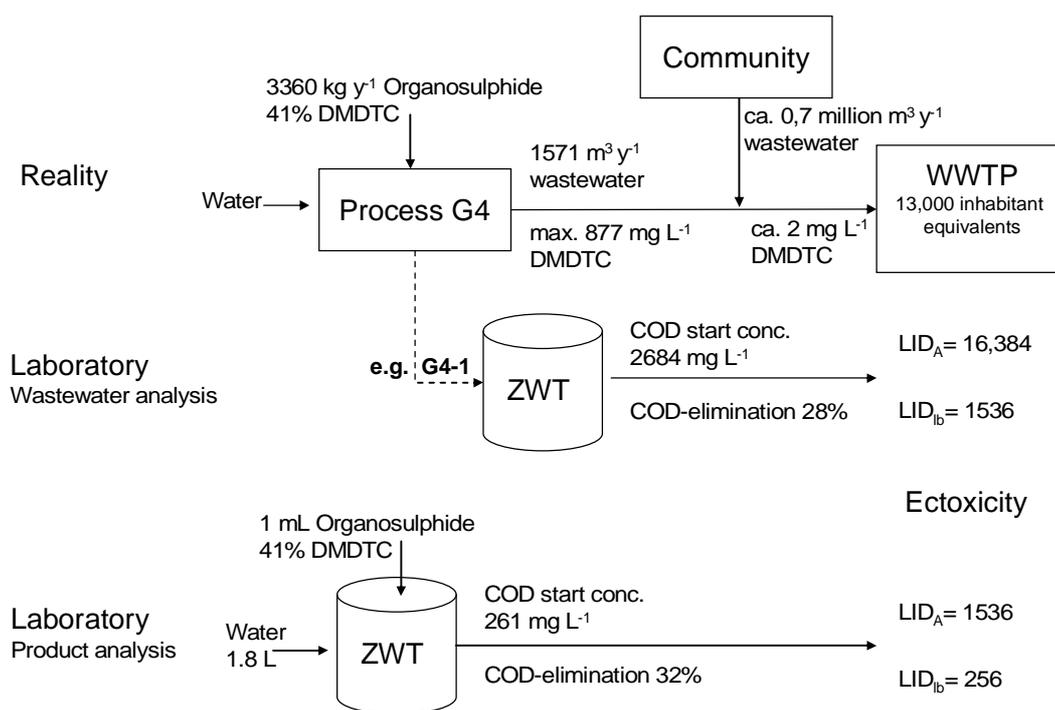
Considering the sum parameter potentially bioaccumulating substances (PBS), wastewater from the metal surface treatment industry exhibited very low levels of PBS ($0.44\text{--}4.54 \text{ mmol L}^{-1}$, median 2.37 mmol L^{-1} ; see part 1).

4.3.2 Water treatment chemicals as a source of elevated ecotoxicity

Among the wastewater samples, in particular factory G4 had an exceptionally high algae toxicity after treatment in the Zahn–Wellens test. In total, three samples have been investigated: Sample G4-1 contained complexing agents from electroless nickel plating, G4-2 did not contain complexing agents, and G4-3 was a mixture (~ equal parts) of both partial wastewater streams from another sampling day. As in other companies, organosulphides have been added for metal precipitation in the area of wastewater containing complexing agents. Here, organosulphides are an alternative to the use of sodium sulphide and a supplement to the hydroxide precipitation. During the analytical determination of sulphide in wastewater, organosulphides are not detected. Whilst the German Wastewater Ordinance sets a limit value for sulphide of 1 mg L^{-1} , no requirements for organosulphides are described. According to the safety data sheet of the organosulphides, the product contains sodium dimethyldithiocarbamate (DMDTC) as active substance at a concentration of 41%. According to Directive 67/458/EEC, this substance is classified as “very toxic to aquatic organisms” (R50) and “may cause longterm adverse effects in the aquatic environment” (R53). The compound and related

others are used as plant protection products (e.g. Ziram) and biocides (product types 9–12 of Directive 98/8/EC). DMDTC is added as a precipitation agent in excess and precipitates heavy metals even in the presence of strong complexing agents such as EDTA. In water, DMDTC slowly hydrolyses to toxic carbon disulphide (Rether 2002). Literature data confirm that this substance is ecotoxic in the lower parts per million range (IUCLID 2000). From the annual consumption of the organosulphide and the wastewater volume per year, a mean wastewater concentration of 877 mg L^{-1} DMDTC is calculated at the outlet of the factory without consideration of the elimination through the precipitation process and the hydrolysis.

For confirming the hypothesis, in practice, the organosulphide was treated at the corresponding dilution ratio (1:1,800 mL) in the Zahn–Wellens test. The resulting COD elimination was 2% after 3 h and 32% after 7 days. Following this pre-treatment, the ecotoxicity in the luminescent bacteria test was $\text{LID}_{\text{bc}} = 256$, and in the algae test, it was $\text{LID}_{\text{A}} = 1,536$. From this, it could be unambiguously derived that the ecotoxicity in the wastewater of company G4 can be explained by the use of organosulphides (Figure 4.3).



ZWT: Zahn-Wellens test

Figure 4.3: Use of precipitation aid dimethyldithiocarbamate as source of elevated ecotoxicity

The question of the source of toxicity from wastewater sample G4-2 remained open because here, no organosulphides are applied. An evaluation of the safety data sheets revealed that in this area, mainly chemicals for degreasing like alcohol ethoxylates, sodium metasilicate, fatty alcohol polyglycol ether, quaternary fatty amines and ethoxylates are added and contribute to the main part of the COD. Therefore, it is a complex wastewater whose toxicity at present cannot be attributed to a distinct substance group. According to the operator of the electroplating process, it also cannot be excluded that in the collection tank of G4-2, residues of complexing agents containing wastewater with organosulphides were present. The documentation of specific processes in the different factories revealed that also in other companies, organosulphides on the basis of DMDTC are used (Table 4.1).

However, only with sample G6 were toxicities comparable to those of factory G4 determined. Further inquiries at other electroplating factories revealed that in two companies (G2 and G6), the surplus of the organosulphide is bound with other heavy metal-containing wastewater. In one company (L1), the surplus organosulphide is precipitated with copper chloride.

According to the product documents of the supplier, for the precipitation of 1 g heavy metal, between 1.6 and 4.1 g DMDTC is required. After the precipitation, the concentration should be determined by a rapid test (colouration after addition of copper sulphate), and the amount in excess should be detoxified with ferric chloride or iron sulphate. The operator of the electroplating company G4 had not envisaged a separate treatment of the organosulphide wastewater but rather was assuming that excess organosulphide would be bound by other heavy metals in the sewer. From the relatively small municipal wastewater treatment plant (13,000 inhabitant equivalents), no disturbances are reported. From the dilution ratio, an annual mean concentration of 2 mg L⁻¹ active substance in the influent flow of the WWTP can be calculated. According to data on bacteria toxicity, no disturbance of biological processes in the treatment plant is expected. However, the question arises whether the organosulphide is completely degraded during the passage of the WWTP. Whilst degradation data are available from its use as plant protection product, no degradability data in WWTP are available. However, the substance at present is being risk-assessed within the review programme of the Biocidal Product Directive.

4.4 Discussion

Comparative data from wastewater surveillance by authorities confirm the range of ecotoxicity observed in the study in hand (Table 4.3). Whilst wastewater from the metal surface treatment industry usually did not exhibit ecotoxicity (median LID 1–2), the maximum LID values for the algae, daphnia and luminescent bacteria test were very high (LID_A up to 3,072, LID_D up to 512 and LID_{lb} up to 2,048; it should be considered that for the data presented in Table 4.3, all data of the period 2001–2007 and most data of the previous periods refer to direct dischargers). As the fish egg test was only recently introduced for compliance testing as substitute for the acute fish toxicity test, only few data are available so far. However, the maximum of the fish egg test (and formerly of the acute fish toxicity) also revealed elevated toxicity.

In the literature, only a few papers referring to toxicity of effluents from the metal surface treatment industry can be found. In a broad-scale Canadian study, the results obtained by whole effluent toxicity testing (WET) were correlated with the presence of priority substances. In total, 45 industrial sites have been included in the study, among them 15 paper mills and three metal surface treatment companies. The luminescent bacteria test, an algae growth inhibition test and a 7-day crustacean assay with *Ceriodaphnia dubia* have been applied. The results have been used for calculating a WET factor based on toxic loads (thus considering the wastewater volume) which has been compared with a chemical-based toxicity factor that was derived from concentrations of priority chemicals and from published chemical toxicity data. When toxicity was corrected for bioavailable metal and ion concentrations, 43% of the variability in measured toxicity was explained by the priority substances analysed (Sarakinos et al. 2000). This study underlines the added value of a whole effluent assessment. The toxic effects of a mixture of seven heavy metals contained in electroplating wastewater on *L. minor* have been studied by Horvat et al. (2007). The original electroplating wastewater strongly suppressed plant growth which was mainly attributed to the content of zinc and ferrous in a toxic unit approach. After treatment with ferrous sulphate and wood fly ash as coagulant, no toxicity was observed. DMDTC was found to be one important source of ecotoxicity in galvanic wastewater. The BREF document on surface treatment of metals and plastics does not specifically refer to the toxicity of DMDTC but refers to its capacity of reducing the concentration of soluble transition metals to below 0.1 mg L⁻¹ (European Commission

2006). In another sector, the DMDTCs are mentioned as potential substitutes for other biocides because of their low persistency and toxicity levels (European Commission 2003). DMDTCs degrade via acid-catalysed hydrolysis to form carbon disulfide (which is also toxic to animals and aquatic organisms) and can also decompose to carbonyl sulphide (OCS), hydrogen sulphide (H_2S) and dimethylamine in aqueous environments. The half-life of DMDTC at 25°C is estimated to range from 2 h at pH 6 to 10 days at pH 8. However, DMDTC forms complexes with metals (which is the reason for its use as a precipitation aid), which stabilises the compounds with respect to transformation reactions.

Experiments show that especially the affinity of Cu(II) for DMDTC is very high (Weissmahr and Sedlak 2000). Carbon disulfide is resistant to hydrolysis in water within pH 4–10, with a hydrolysis half-life extrapolated to 1.1 years. Its predicted rate of biodegradation in water is negligible compared with its rate of volatilization from surface water (WHO 2005).

According to the operator of the electroplating company, G4 several options to substitute and/or eliminate the organosulphides are envisaged, e.g. the elimination of excess organosulphides with ferrous chloride or destruction of complexing agents with ozone or hydrogen peroxide followed by hydroxide precipitation.

4.5 Conclusions

The results point out that the organic load of wastewater from the electroplating industry has been underestimated. So far, wastewater surveillance focussed on inorganic parameters (heavy metals, sulphide, cyanide, etc.). Even the operators of the facilities often do not know the substances contained in the products of their suppliers. In light of this observation, the unspecific COD requirement of 400 mg L^{-1} for direct dischargers seems to be quite high. The proof of biodegradability of that organic load can be provided by the Zahn–Wellens test. Because the COD does not allow any conclusion about the toxicity of the wastewater, the application of bioassays for wastewater surveillance would be an appropriate tool. However, the possibility of salt toxicity contributing to overall toxicity in wastewater from the metal surface treatment industry should be taken into account.

The environmental safety of water treatment chemicals should be better considered. Already in 1997, a study of the German Federal Environment Agency revealed that dithiocarbamates exhibit the highest toxicity of all chemicals used for wastewater

treatment in the daphnia and algae test. The EC50s of the active substance were determined to be 0.1 mg L⁻¹ (daphnia), 0.2 mg L⁻¹ (algae) and 3.6 mg L⁻¹ (luminescent bacteria). A biodegradability test with laboratory flow-through treatment plants resulted in a breakdown of the biology at 40 mg L⁻¹, whilst the (short-term) activated sludge respiration inhibition test did not show effects at this concentration (Schumann et al. 1997). German water authorities recommended reducing the use of organosulphides to the minimum extent necessary and optimising dosing (Anonymous 2005).

The combination of the Zahn–Wellens test followed by the performance of ecotoxicity tests turned out to be a cost-efficient and suitable instrument for the evaluation of indirect dischargers. Meanwhile there exist sufficient results to introduce this combination, which is also recommended in OSPAR's strategy on WEA for other indirect dischargers (Gartiser et al. 1997, 2009; OSPAR 2005). This would also take into consideration the requirements of the IPPC Directive to apply the same level of requirements concerning best available techniques for direct and indirect dischargers.

Regarding the concentration at which the wastewater should be tested in the Zahn–Wellens test, a compromise is needed. In principle, the samples should be tested without dilution because this would reduce the sensitivity in the subsequent ecotoxicity tests. However, higher concentrations might cause inhibitory effects to the activated sludge which would reduce biodegradability. It is suggested to maintain the test concentration given in DIN EN ISO 9888 (COD, 100–1,000 mg L⁻¹; DOC 50–400 mg L⁻¹).

4.6 Recommendations and perspectives

The importance of the organic load of wastewater from the electroplating industry has been underestimated so far. Bioassays are a suitable tool for detecting impacts of complex chemical mixtures and should therefore be applied for routine wastewater surveillance. The combination of the Zahn–Wellens test followed by the performance of ecotoxicity tests is a cost-efficient and suitable instrument for the evaluation of indirect dischargers.

The environmental impact of water treatment chemicals should be better addressed. It is contradictory that the same active substances which have to pass an approval and authorisation before being applied as a plant protection or biocidal products can be used in huge amounts for water treatment without being risk-assessed.

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5 GENERAL DISCUSSION

5.1 Prospects of wastewater evaluation with bioassays

The application of bioassays for wastewater evaluation follows distinct objectives such as screening of discharges for effluent toxicity, characterising the toxic hazards of effluents as part of a risk assessment process; assessing the toxic impact of point source discharges on the receiving water environment; identifying the cause(s) and source(s) of final effluent toxicity; and monitoring compliance against a toxicity reduction target (Johnson et al. 2004). Figure 5.1 shows how these different objectives interact.

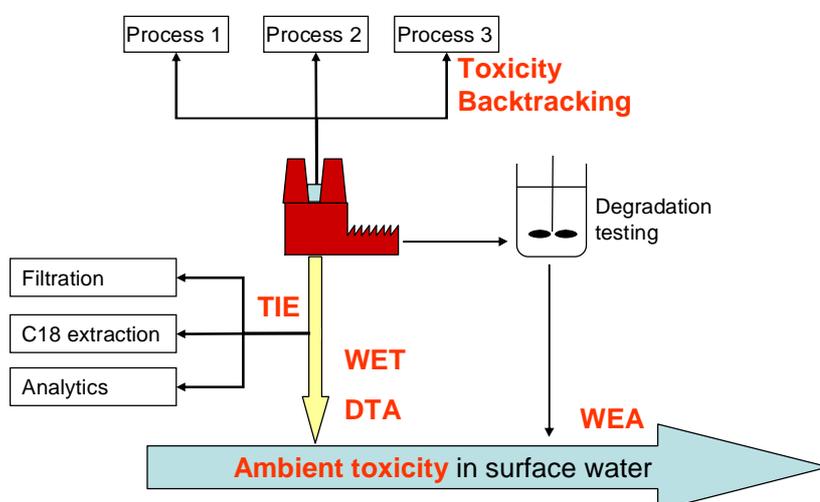


Figure 5.1: Objectives of the application of bioassays for wastewater assessment

The screening of effluent toxicity and monitoring of compliance to the discharge limits follow the emission-based approach, which mainly focuses on the hazard of effluents. The “Whole Effluent Toxicity“ (WET) and the “Direct Toxicity Assessment“ (DTA) approach are examples of this objective. “Whole Effluent Assessment” (WEA) can be regarded as an amplification of the hazard-based approach by combining the determination of toxicity with degradation and bioaccumulation data (PBT criteria). Ambient toxicity testing has the objective of assessing the toxic impact of point source discharges on the receiving water environment and clearly focuses on risks. The DTA approach is a combination of both the hazard and risk-based approaches.

Often it is useful to identify the origin of toxicity detected in effluents. Here the objective is to reduce the toxicity e.g. by modification of the process or substitution of certain input chemicals in conformance with BAT. The methodology applied might follow a “Toxicity identification evaluation” (TIE) process or might be driven by testing distinct partial wastewater streams from different processes. TIE is often used to identify the causative agents for toxicity and has been developed e.g. by the US EPA (1992, 1993a, 1993b). The TIE approach consists in a systematic pre-treatment of the wastewater samples. Filtration of the sample might indicate whether the toxicants are adsorbed to particles, the addition of EDTA might reduce toxicity due to dissolved heavy metals, removal of toxicity through aeration might indicate volatilization and/or oxidation of the toxicants. Solid-phase extraction of wastewater samples through C18 columns allows a separation of toxicants by their hydrophobic properties. If the toxicant is relatively polar, it remains in the aqueous phase, whereas nonpolar toxicants will be retained on the column. Also chemical analysis of known toxicants may identify an obvious cause of ecotoxicity. Another methodology often applied is “toxicity backtracking”. If an effluent shows a substantial toxicity, the origin might be identified by testing partial wastewater streams from different processes which contribute to the mixed effluent. This might be accompanied by an analysis of the input chemicals or processes.

This outline of the TIE and toxicity approaches shows that the methodology followed in this thesis was a combination of the emission-based approach and an abbreviated TIE and toxicity backtracking approach. In the OSPAR’s practical studies all wastewater samples were screened for toxicity in a defined test battery before and after a degradation step, which can be considered as part of the TIE. The origin of outstanding toxicity was assessed by backtracking the processes and/or chemical analysis (chapter 2). Also the WEA studies with the paper manufacturing and metal surface treatment industries followed this approach, with the exception that only indirectly discharged effluents were biodegraded (see chapters 3 and 4).

Whole effluent toxicity testing has been proven as a practical instrument for surveillance of wastewater in numerous studies. Several countries have included bioassays in their research programmes and/or in their legislation for wastewater control. However, while the development of suitable bioassay methods for wastewater evaluation is going on, obviously their application in regulatory practice is lagging behind. The *Lemma* sp. growth inhibition test according to DIN EN ISO 20079 (2006), the Ames test with *Salmonella thyphimurium* with tester strains TA98 and TA100 according to DIN 38415-4 and ISO

16240 (2005), the Ames fluctuation assay according to ISO/DIS 11350 (2009) or the V79 *in vitro* micronucleus test according to EN ISO 21427-2 (2006) are examples of test methods specifically designed for effluent analysis which have not been used in routine wastewater analysis so far in Germany.

Table 5.1 shows the requirements for different industrial sectors concerning bioassays in Germany (Wastewater Ordinance, 2004). The fish egg assay with *Danio rerio* is the most often applied test, which has a historical reason because for animal protection this assay has substituted for the acute fish toxicity test, which has been applied since the 1980s.

Table 5.1: Regulatory practice including bioassays in Germany

Annex	Sector	<i>Danio rerio</i> [LID]	<i>Daphnia magna</i> [LID]	<i>Desmodes mus subsp.</i> [LID]	<i>Vibrio fischeri</i> [LID]	UmuC [LID]	Elimi- nation
22	Chemical and pharmaceutical	2 (DD)	8 (DD)	16 DD)	32 (DD)	1,5 (DD)	80% - 95% TOC (DD, ID)
23	Biological treatment of wastes	2 (DD, ID after treatment)	8 (ID after treatment)		4 (ID after treatment)		75% (ID)
25	Leather and fur	2-4 (DD)					90% COD 98% BOD 75% (ID)
27	Chemical-physical treatment of wastes	2 (DD, ID after treatment)	4 DC, (ID after treatment)		4 (DD, ID after treatment)		
29	Ferrous and steel production	2-6 (DD depending on sub-sector)					
31	Cooling water				12 (DD)		
39	Non-ferrous metal production	4 (DD)					
40	Metal processing	2-6 (DD depending on sub-sector)					
51	Landfill leachate	2 (DD, ID after treatment)	4 (ID after treatment)		4 (ID after treatment)		75% DOC (ID)
56	Printing industry	4 (DD)					
57	Raw wool washing	2 (DD)	2 (DD, ID after treatment)				

LID: lowest ineffective dilution

DD: direct discharge to a receiving water

ID: indirect discharge via public sewers to a wastewater treatment plant

For the following Annexes of the Wastewater Ordinance only one bioassay, the fish egg test, has been implemented with a limit value of LID = 2: Coating materials/Paint resins (Annex 9), Wood fibre board (13), Pulp, paper, cardboard (19), Iron and steel (24),

Processing of natural and synthetic rubber, lattices (32), Flue gas scrubbing from waste incineration (33), Inorganic pigments (37), Textiles (38), Alkali chloride electrolysis (42), Man-made fibres/Foils (43; Coal (46), Flue gas scrubbing (47), Use of certain hazardous substances (48), and Semiconductors (Annex 54).

With the notable exception wastewater from waste treatment and landfill leachate all limit values exclusively refer to directly discharged wastewater. Köppke (2009) suggested that the same requirements concerning bioassays should apply for both directly and indirectly discharged wastewater. The limit values for indirectly discharged wastewater from waste treatment or landfill leachate (Annexes 23, 27, and 51 of the Wastewater Ordinance) concerning fish egg, daphnia and bacteria toxicity may be obtained after treatment in a laboratory flow through WWTP. The primary focus of this pre-treatment is to remove ammonium toxicity by nitrification. The effort for this combination is considerable and the Zahn-Wellens test offers a lower cost alternative which, according to experience from routine laboratories, is accepted by most authorities. However wastewater surveillance of indirectly discharged wastewater with bioassays in Germany has not been put into regulatory practice so far. For indirectly discharged wastewater the Zahn-Wellens test is exclusively applied for determining the recalcitrant DOC after biological treatment, which means, it is used as a test for treatability.

Obviously, according to table 5.1 the requirements concerning bioassays in different sectors are fragmentary. The *umu* test for determining genotoxicity and the algae tests are only required for wastewater from one sector (chemical and pharmaceutical industry). It should be noted that in some federal states, notable North Rhine-Westphalia, wastewater from far more sectors is routinely monitored bioassays (see evaluation of biotest data in wastewater sectors by Diehl et al. 1998, 2003).

For some industrial sectors also requirements for the input auxiliary aids have been defined. For example, only detergents/sizes for processing of textiles or separating agents for rubber processing that meet a degradability of 80% within 7 days in the Zahn-Wellens test are allowed for use.

The bioassays applied are “fit for purpose” and it is now up to the authorities to include them in regulatory practice. While acute toxicity tests are widely applied for effluent assessment there is a lack of adopted chronic tests in Europe. Therefore the next generation of tests should consider longer-term exposures. The WET methods of the US EPA (2010)

provide an initial point for chronic toxicity testing. Further on, other biological end-points such as mutagenicity and endocrine disruption should be considered (e.g. Wharfe 2004).

5.2 Prospects of Whole Effluent Assessment

Whole Effluent Assessment (WEA) can be considered as an extension of the Whole Effluent Toxicity (WET) approach. Within WEA toxicity results do not stand alone but are combined with persistency and liability to bioaccumulation to obtain an overall evaluation of the hazardousness of all ingredients comparable to the approach for prioritising hazardous substances. WEA can be considered as a safety net for all the substances of concern not included in hazardous substances lists due to the lack of knowledge.

Within the two practical programmes of the OSPAR WEA expert group in total 17 and 25 effluents have been tested all over Europe (OSPAR 2005, 2007b). Among the results it has been stated that WEA is particularly useful for complex effluents, where it has an added value to the substance-by-substance approach. In this context complex effluents are those which contain complex mixtures of chemicals or require very detailed process-specific knowledge (OSPAR 2007a). As OSPAR focuses on the protection of the marine environment, WEA should preferably be linked to long-term adverse effects, that means that the focus should be on substances that are persistent, liable to bio-accumulate and toxic. OSPAR specifically recommends the application of chronic tests but agrees that these are less developed for effluent evaluation and more effortful compared to the acute toxicity tests. Because as a rule chronic tests are more sensitive than their corresponding acute tests any observed acute effect with native wastewater samples should be interpreted as a warning signal.

Taking into account the OSPAR Strategy with regard to Hazardous Substances and the benefits of using WEA as an additional tool for BAT determination, OSPAR WEA should focus on hazard. This means that the main objective of the WEA approach is its use for the identification and surveillance of hazardous effluents.

While the importance of determining potential bioaccumulating substances (PBS) present in effluents is being recognised by authorities (e.g. De Maagd 2000) this parameter has not been considered in routine wastewater surveillance in Germany so far. For single substances bioaccumulation is tested in living biota (often fish) but this approach is not suitable for complex mixtures. Therefore different options for assessing the bioaccumulation potential of effluents as a sum parameter have been analysed in several research

projects. One trend focused on liquid extraction with solvents followed by quantification of the organic fraction by TOC or weight measurements (Reemtsma et al. 2001, Stenz et al. 2002). The other trend focused on biometric solid phase micro extraction (SPME) followed by thermodesorption in a GC and referring the GC integral to that of reference compounds with corresponding octanol water partition coefficients (Paschke, 2003, 2004). The last approach was followed in this thesis following the recommendations of the OSPAR WEA group (Leslie 2006, OSPAR 2007a). Low levels of PBS were determined in the effluents considered in this thesis after biological treatment (see chapter 2-4). However, the database is too small to allow general conclusions about the interpretation of this parameter.

According to the OSPAR WEA recommendations endocrine disruption and mutagenity tests represent specific modes of action that are of relevance to the marine environment. In the WEA strategy these tests have been proposed tailor-made for specific situations where these modes of actions may play a role. Thus, these tests have been included in the optional tool box (OSPAR 2007a).

With regard to the degradability step the following conclusions have been drawn in the practical WEA study (OSPAR 2005):

- Although the effluents with a high organic load displayed higher toxicity, no clear relationships could be distinguished between organic carbon content and acute toxicity.
- The organic carbon content removal was higher than toxicity removal after a biodegradation test. In some effluents a slight increase in toxicity was found after performing a biodegradation test.
- For the biologically treated effluents a biodegradation step had no added value, since hardly any decrease in toxicity took place.

The last mentioned statement was the reason why in the WEA study with directly discharged effluents from the paper manufacturing industry was not submitted to a biodegradation step, namely because they all had passed through a biological treatment plant before.

After concluding OSPAR's work on WEA, for the first time a European-wide harmonized concept for the evaluation of wastewater through effect-based tests is available. This concept has already been considered in the description of the best available techniques in BREF-documents to several sectors of the IPPC Directive 2008/1/EC (see chapter 1.2).

However, WEA has not been considered as a monitoring instrument in river basin management plans within the Water Framework Directive 2000/60/EC or in the Marine Strategy Framework Directive 2008/56/EC. Both focus on monitoring programmes of the ecological status and the chemical status of the water bodies. River invertebrate data provide useful information on impacted sites and for general water quality classification. However, Wharfe (2004) concluded that biological responses that are predictive of potential damage before it happens can provide better protection. This gap could be filled with the WEA methodology. According to the WFD all discharges into surface waters should be controlled according to the combined approach, which consists in emission controls based on BAT or in emission limit values. Additionally, for diffuse impacts “best environmental practices” might be defined (Article 10 WFD). To date WEA is mainly used in the context of BAT but not for establishing emission limit values. For the chemical status, the selection of priority substances, the elaboration of emission controls and of quality standards are based on the same intrinsic properties of hazardous substances (PBT) as within OSPARs strategy. This means that WEA also has the potential to contribute to the WFD targets for priority substances. As to the ecological status, applying WEA on effluents may help to understand or maybe to predict the effects on the ecological situation (OSPAR 2005).

OSPAR work on WEA has finished with the publication of the WEA Guidance document (OSPAR 2007a). In 2009 OSPAR started a survey on the implementation of the OSPAR WEA strategy among member states and on the progress obtained three years later. However, no results of this survey are available so far.

2009 was the start-up year for a research project “Control of hazardous substances in the Baltic Sea region” (COHIBA), which intends to identify the sources and inputs of the 11 hazardous substances and develop measures to reduce these substances. Additionally the project aims to evaluate the ecotoxicity of complex effluents throughout the Baltic Sea region, based on the Whole Effluent Assessment (WEA) approach. Here, the OSPAR WEA concept serves as a starting point. The outcome will be used to possibly establish PBT (persistent, bioaccumulating, toxic) discharge limit values based on the WEA approach. In principle, well-treated effluents should not be acutely toxic and toxicity threshold values might be applied by HELCOM (Helsinki Commission on Baltic Marine Environment Protection, www.helcom.fi) in a set of standardized tests to limit effluent toxicity (<http://www.cohiba-project.net>).

5.3 Further development of biodegradation assessments of wastewater

In this thesis in compliance with the OSPAR WEA approach two degradation tests have been applied: The indirectly discharged wastewater samples have been pretreated with the Zahn-Wellens test according to OECD 302 B test to simulate elimination in municipal treatment plant. A part of the wastewater samples directly discharged to surface water has been biodegraded in the DOC Die away assay according to OECD 301 A to simulate surface water. The following parameters may influence the results:

Test duration

The test duration has a decisive influence on overall results. In the ECETOC report on WEA it has been suggested that the wastewater should be biodegraded usually for several weeks before toxicity and bioaccumulation testing (ECETOC 2004). According to the TGD usually a degradation extent of more than 70% (bio)degradation within 28 days in the Zahn-Wellens test indicates that the substance is inherently biodegradable. However, extrapolation of the results of the inherent tests should be done with great caution because of the strongly favourable conditions for biodegradation that are present in this test. Therefore, no degradation in WWTPs is predicted from the results of the Zahn-Wellens test unless the pass level of 70% biodegradation is reached within 7 days while the log-phase is not longer than 3 days, and the percentage removal in the test before biodegradation occurs is below 15% (European Commission 2003). Consequently, the standard test duration in the German Wastewater Ordinance has been set to 7 days and this duration has also been assigned to the Zahn-Wellens test carried out in this thesis.

Guhl et al. (2006) analysed the comparability of different biodegradation test methods of a large number of chemical substances. Results from the Zahn-Wellens test (OECD 302B) representing a screening test with high sludge concentration, were shown to be useful for the prediction of the organic carbon removal under WWTPs conditions. When comparing the elimination in the Zahn-Wellens test after 28 days with that in the Continuous Activated Sludge (CAS) test (Coupled Units test according to OECD 303A), congruent results were obtained in 82% of the 65 chemicals included in the analysis. In almost 11% the results from the CAS model were higher than the predictions by the Zahn-Wellens test, only in 8% the Zahn-Wellens test data did not deliver a sufficiently conservative C-removal prognosis for WWTPs. The database for a comparison of the Zahn-Wellens test results after 7 days with the CAS test was too small to obtain reliable conclusions. However, results from the 7-day Zahn-Wellens fitted very well with those of ready

biodegradability tests (87% congruent results), leading to the conclusion that high C-removals in the 7-day Zahn–Wellens test (excluding the C-removals in the first 3 h of the test) are indicative for a pass of the ready biodegradability threshold values. Pagga (1995) also presented comparative data of degradation extents in the Zahn-Wellens test and real WWTPs and found a high level of congruent results (<10% deviation).

Concentration of the effluent

Degradability testing of wastewater should be preferentially performed within the range recommended in OECD 302 B (DOC: 50 mg L⁻¹ to 400 mg L⁻¹, COD: 100 mg L⁻¹ to 1000 mg L⁻¹). The lower limit has practical reasons, because assuming e.g. 80% degradation of 50 mg L⁻¹ results in a residual DOC of 10 mg L⁻¹ which is near the blank value in parallel vessels. If subsequent toxicity testing is planned preferably the undiluted sample should be tested (see below). On the other hand, it must also be ensured that no toxic effects of the wastewater to the activated sludge inoculum occur, which would inhibit its degradability. Thus, a compromise of an optimal dilution must be found depending on the kind of wastewater. The concentration of the effluent at the start has a decisive influence on the time required for the adaptation of the activated sludge (which usually is below three days). Thus, the Zahn-Wellens test cannot be used to determine the minimum hydraulic retention time in real WWTPs (Pagga 1995). In principle, the activated sludge is relatively robust to toxicants. Also the degradation kinetics depend on the starting test concentration and on the adaptation of the activated sludge to the effluent. Inhibitory effects often have an influence on the lag time required for an adaptation with the result that the plateau phase is reached at a later point in time. A complete inhibition of the activated sludge's activity in the Zahn-Wellens test is hardly found. Thus, the overall result, represented by the degradation extent at the plateau, is relatively independent of the starting concentration (Gartiser et al 1996). If a plateau has been reached within the test duration, this is a good indicator that the degradation extent is representative. Stucki (2000) stated that in batch tests the growth rate of (adapted) bacteria is very important, whereas in real WWTPs the effluents are more diluted and thus the growth rate is of less importance for achieving a high removal rate. In some cases it might therefore be useful to prolong the test beyond the standard test duration of 7 days (Pagga, 1995, Gartiser et al. 1996, Stucki 2000). However, a prolongation of the test duration beyond 7 - 14 days should rather be used to assess the long term biodegradation potential of the wastewater than to predict the elimination in real WWTPs (Gartiser 2009).

End points

One disadvantage of the Zahn-Wellens test is that it does not distinguish between the elimination factors biodegradation, adsorption and volatilisation. Therefore, as a somewhat arbitrary attempt the elimination occurring during the first three hours is attributed to adsorption and that in additionally abiotic uninoculated control vessels to volatilization. No conclusion about the fate and behaviour of the fraction adsorbed to the activated sludge inoculum can be drawn from results obtained in the Zahn-Wellens test. It might be biodegraded or might be carried together with the surplus sludge to a subsequent treatment step (most often to the anaerobic treatment in digesters). The CO₂ evolution method gives the most direct evidence of oxidation of organic carbon during biodegradation; the removal of DOC can be due to processes other than biodegradation, and the uptake of oxygen is only an indirect measurement for assessing biodegradability. Thus several authors suggested to determine ultimate biodegradation under inherent test type conditions by combining CO₂ and DOC measurement within one test (Strotmann et al. 1995, Baumann et al, 1996, Gartiser et al. 1997). This concept has been transferred for the first time to the assessment of biodegradability of real wastewater samples within the study on paper making and metal working effluents under inherent conditions. By determining both DOC elimination and ultimate biodegradability (mineralization) of wastewater samples in the Zahn-Wellens test the mineralization to CO₂ can unambiguously be differentiated from other elimination factors such as adsorption or volatilization (Gartiser 2009). To this end gas wash bottles used as reactors were aerated with CO₂-free air and the CO₂ produced in the reactors was absorbed in sodium hydroxide gas wash bottles connected in series and quantified. The method is suitable for wastewater samples with TOC above about 100 mg/L. If the inorganic carbon of the wastewater, consisting of CO₂, hydrogen carbonate and carbonate, is higher than about 1/3 of the TOC, the procedure reaches its limits. The effort for the method proposed is insignificantly higher than the standard procedure for performing the Zahn-Wellens test in so far as no abiotic control is included (Gartiser 2009).

Concerning the suitability of testing degradability of effluents, industry agrees that persistency is an important parameter for assessing the potential for long-term exposure and adverse effects in remote areas. However, the criteria for persistence are based on half-lives of single substances in the environmental compartment and the tests were not designed for mixtures. Many scientists state that it is incorrect to refer to the “persistence of effluents” (ECETOC 2004). The author of this thesis agrees that this statement is true in

principle. The dilemma is that persistence cannot be measured directly, but only be derived from degradability data. Results from simulation tests using ^{14}C labelled test substances (e.g. OECD 309: 2004) provide the best alternative to determine the phase distribution and ultimate biodegradability of the test item. The best approach of persistence estimation of effluents would be knowledge of all different compounds and their biodegradability. Obviously it is not possible to apply these principles to complex mixtures. Thus, degradability tests are the only meaningful instrument for assessing persistency of effluents. In chemical risk assessment usually degradation rate constants are attributed to the results in screening tests; thus there is a link between screening tests and half-life estimation, and several approaches have been undertaken with the aim to improve the assessment of biodegradation kinetics from screening tests (European Commission 2003).

Interpretation of results and limit values:

The question, what degradability for indirectly discharged industrial wastewater should be required is controversial. According to industry and the OSPAR WEA expert group approach, the persistency of effluent should not be assessed as a separate parameter but should be combined with biological end points (ECETOC 2004, OSPAR 2007a). In contrast, the BREFs elaborated under the IPPC refer to the Zahn-Wellens test for treatability testing of industrial effluents in terms of refractory COD load (e.g. BREF on Common waste water and waste gas in the chemical sector, see chapter 1.2). In Germany usually a COD elimination in the Zahn-Wellens test of 75% in 7 days is considered as pass level for treatability in WWTPs. Other authors suggest a pass level of 80% for each partial wastewater stream before mixing (Köppke, 2009). A pass level of 70%-80% is consistent with the requirements of the TGD for predicting elimination in WWTPs from Zahn-Wellens test results (70% COD-elimination in 7 days). Another reference is Directive 91/271/EEC concerning urban waste-water treatment. Here, requirements for discharges from municipal treatment have been prescribed in terms of the COD concentration (≤ 125 mg/l) or for the percentage of COD reduction ($\geq 75\%$). It could be argued that biological treatment of wastewater which contains high concentrations of refractory or “hard” COD is not BAT. Thus, it can be concluded that when the Zahn-Wellens test is used for assessing treatability in WWTPs a COD elimination of about 75% is a convenient pass level.

5.4 Further development of coupling of degradation tests with ecotoxicity tests

Within WEA persistency of effluents does not stand alone but is combined with bioaccumulation and toxicity. The assessment of toxicity biodegradation in effluents has

been suggested by several authors. Nyholm (1996) proposed a long-term (one to three month) stabilization (ageing) of the effluent by diluting it with at least an equal volume of mineral medium containing inoculum (e.g. 30 ppm activated sludge) to distinguish between degradable and persistent toxicity. The author noted that no agreed scheme has yet been developed for biodegradability assessment of industrial effluents. Thus various laboratory or pilot scale treatability tests (mostly non-standardised) are used. While Nyholm agreed that biodegradability of complex mixtures has technical and conceptual drawbacks, the alternative of testing the degradability of all individual compounds would not be practical. Therefore Nyholm proposed to combine biodegradation testing of the whole effluent with bioassays to obtain a functional characterisation of the effluent in terms of toxicity reduction. However, only a few publications are available where this principle has been put into practice (see chapter 1.5). Within the OSPAR practical studies (see chapter 2) and the study on effluents from the paper making and metal surface treatment industries (see chapters 3 and 4) this approach has been routinely applied and can be considered as a cost efficient instrument for assessing indirectly discharged wastewater. Besides these investigations very few publications have developed similar approaches. Bierbaum (2008) analysed the efficiency of ozone pre-treatment of paper manufacturing effluents by determining its influence on DOC degradation in the Zahn-Wellens test followed by ecotoxicity testing.

From a practical point of view, when only testing DOC-elimination of effluents in the Zahn-Wellens test the test concentration will follow the level required for analytical detection. If the test is coupled with ecotoxicity tests the primary objective is to test undiluted effluents in order not to dilute toxicants below the sensitivity level of the test organisms. As described above there are some limitations of this concept due to the interaction of the test concentration with the test duration and adaptation as well as the potential appearance of inhibitory effects to the activated sludge.

The results with the Zahn-Wellens test show that the nitrification capacity of the test after two days is comparable to that of laboratory flow through treatment plants according to OECD 303. Thus the Zahn-Wellens test can be used to remove ammonium toxicity for detecting non-degradable (persistent) ecotoxicity (Gartiser et al. 1996).

The OSPAR practical studies from 2003 and 2005 demonstrated that no strong relationship exists between DOC removal and the remaining acute toxicity. The reason is

that high DOC removal might be caused by only a few non-toxic substances, while other, more toxic or bio-accumulative substances may not be affected (OSPAR 2005, 2007b).

For the testing strategy of coupling degradation tests with ecotoxicity tests the following principles can be derived:

- Degradability testing of wastewater should be preferentially performed with the undiluted sample in order to assure that subsequent toxicity testing is not affected by dilution. If the sample has to be diluted before degradability testing for practical reasons (e.g. too high TOC), the dilution factor should be considered in the interpretation of the results (OSPAR 2007a).
- The DOC removal is no direct indication of toxicity removal. Thus ecotoxicity testing after biodegradation might have further implications on the test design respective the start concentrations and the test duration other than testing for DOC elimination. There might be a need to optimise the test strategy for specific types of effluents.
- In a few cases the toxicity increased slightly after the degradation step (especially for the algae test), but at an overall low level. While in principle this could be interpreted as a result of the formation of metabolites with higher toxicity, other reasons such as the addition of mineral medium to the degradation test should be considered. In the standard version of the Zahn-Wellens test a phosphorus buffer is used as mineral medium ($\sim 100 \text{ mg L}^{-1}$ phosphorus). It is known that algae have an optimum in their phosphorus supply. Depending on the species, a phosphorus concentrations above 20 mg L^{-1} is considered inhibitory to algae (Duncan et al. 1974).
- It should be kept in mind that removal by adsorption to the activated sludge might cause cross-media effects when the surplus sludge is disposed on landfills sites or organic fertilizer on soils. Including a degradation step means going one step behind the precautionary principle. If one expects only minor or medium toxicity or bioaccumulation potential in the raw effluent an assessment could be done with the original sample without the degradation step. If high toxicity or bioaccumulation potential has been observed, then the behaviour after the degradation step gives additional information whether the observed effects or values might be acceptable or not.

5.5 Transferability from the laboratory to the field

The progress in wastewater treatment technology in Germany indubitably led to a recuperation of water quality in many rivers in the last decades. In this context, the question arises, what contribution surveillance of wastewater with chemical and biological methods has. Experience shows that innovation in water treatment and integrated measures (e.g. substitution of hazardous chemicals) often has been orientated on the parameter being controlled (COD, heavy metal, chloroorganic compounds, AOX, since the 1970s also bioassays). Therefore, the selection of the parameters for wastewater surveillance and their ecological relevance for water quality is of high importance. In a literature survey of the US-EPA it was analysed whether toxicity results with single species are good indicators for predicting impacts on aquatic biocoenoses (Vlaming und Norberg-King 1999). The background of this survey was that the US-EPA has included toxicity tests in wastewater permits for preventing and controlling impacts on surface water quality through effluents. This was criticised because the ecological relevance of the test results with single species and their transferability on aquatic biocoenoses was questioned. It was demonstrated that results of single species tests qualitatively correlated quite well with the results from biomonitoring (e.g. macroinvertebrates, fish population) when several test series were available. In about 70% of the case studies both approaches went into the same direction. Therefore, screening tests with single species have an early warning function for predicting potential impacts on aquatic biocoenoses before these become manifest. However, mainly chronic test systems have been included in the comparison (often the 7 day reproduction toxicity test with *Ceriodaphnia dubia*), while in Germany mainly acute ecotoxicity tests are applied.

Concerning the testing strategy, standards designed to protect the quality of the receiving environment and standards designed to reduce emissions to the environment can be distinguished (Power et al. 2004). The first focuses on a site-specific risk assessment. That means that it may take the sensitivity of the receiving environment or the available dilution into account (immission-based approach). The second focuses on emission limit values or load values and is designed to promote the use of 'best available technology' for a specific industry sector, regardless of the receiving environment. The objective is to reduce releases to the environment by controlling point emission sources (emission or hazard-based approach). The OSPAR WEA concept clearly focuses on the hazard-based approach and this is in line with the overall objective of OSPAR and the WFD to reduce the emission of hazardous substances close to zero. The objective of WEA is to identify

complex effluents that contain hazardous ingredients which might cause adverse effects in surface water. However, WEA could also be a tool for detecting possible causes of a poor ecological quality of surface water by directly measuring the effects on organisms and thus form a link between chemistry and ecology. It must be recognised that good ecological status of the surface water - on the other hand – does not guarantee the absence of hazardous substances (OSPAR 2007a).

It should be noted that when WEA is applied according to the water quality or risk-based approach by assessing the impact to the receiving water quality, as suggested e.g. by the US EPA (2010), SETAC (2004) or ECETOC (2004), the acute tests usually applied have a low discriminative power. Acute toxicity testing of effluents from WWTPs are cost and time efficient but are less informative compared to sub-lethal end-points such as reproduction or growth (Petla et al. 2009). Consequently the US EPA WET test methods include several chronic tests. The algae growth test is the only test of this category routinely applied in Germany.

Applying the risk or immission-based principle with PEC/PNEC-comparison the dilution of the waste water is being considered. In the OSPAR WEA Background document (OSPAR 2000) several examples have been documented where the results of bioassays have been used to argue that there is no risk in surface water following the principle "dilution as solution". However, according to the author of this thesis in this case the same principles applied for the setting of chemical quality standards according to the Water Framework Directive (WFD) should be used (figure 5.2).

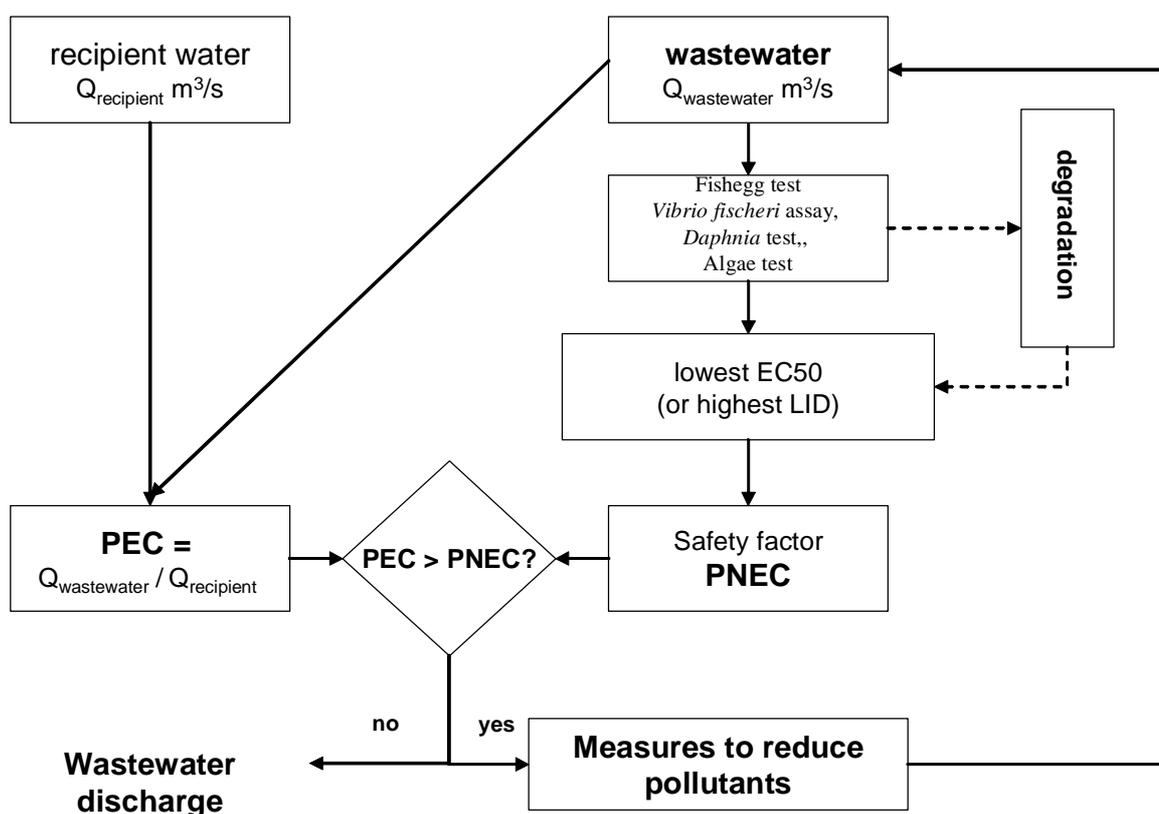


Figure 5.2: Prediction of impacts in surface water

That means that the following principles should be applied:

- A test battery of representative organisms of different trophic levels should be applied.
- A statistical evaluation of the ecotoxicity tests is required for deriving the EC50. Alternatively the reciprocal value of the highest LID (which corresponds to the wastewater fraction below which no significant toxicity is observed) could be used for the evaluation.
- Safety factors should consider the number of results with different organisms available as well as the type of the test system (acute/chronic). As in Germany usually only acute tests are applied for effluent testing a safety factor of 1000 seems appropriate (see table 5.2).
- The PEC-evaluation should be referred to the low-flow rate of the receiving waters where dilution is minimized.

Table 5.2: Safety factors for setting environmental quality standards

	Safety factor
At least one acute L(E)C ₅₀ from each of three trophic levels of the basic set	1 000
One chronic NOEC (either fish or daphnia or a representative organism for saline waters)	100
Two chronic NOECs from species representing two trophic levels (fish and/or daphnia or a representative organism for saline waters and/or algae)	50
Chronic NOECs from at least three species (normally fish, daphnia or a representative organism for saline waters and algae) representing three trophic levels	10
Other cases, including field data or model ecosystems, which allow more precise safety factors to be calculated and applied	Case-by-case assessment

Source: 2000/60/EC, Annex V, 1.2.6

The following example aims to illustrate this conservative approach: It is assumed that the mean river flow is $800 \text{ m}^3 \text{ sec}^{-1}$, the low river flow is $400 \text{ m}^3 \text{ sec}^{-1}$ and the wastewater flow is $50 \text{ litre sec}^{-1}$. Additionally, it is assumed that the ecotoxicity results with fish eggs, daphnia and luminescent bacteria show values of $\text{LID}_{\text{Egg}} = 2$, $\text{LID}_{\text{D}} = 4$ and $\text{LID}_{\text{lb}} = 6$. Further evaluation of data revealed that the EC50 of luminescent bacteria toxicity corresponds to a fraction of wastewater of 45%.

$$\hat{e} \text{ PEC [\% wastewater]} = 100 [\%] * 0.05 [\text{m}^3 \text{ sec}^{-1}] / 400 [\text{m}^3 \text{ sec}^{-1}] = 0,013 [\%]$$

$$\hat{e} \text{ PNEC [\% wastewater]} = 45 [\%] / \text{safety factor } 1000 = 0,045 [\%]$$

$$\text{PEC} < \text{PNEC} \hat{e} \text{ discharge acceptable}$$

As the PEC is lower than the PNEC, the discharge would be acceptable. Assuming a wastewater flow above $180 \text{ litre sec}^{-1}$, impacts to the surface water could not be excluded. It should be noted that applying safety factors for effluent assessment has not been included in WEA strategies so far.

In the test scheme proposed the degradation step is optionally included. One might look at the persistency of measured effects and the elimination might be included in deriving the PNEC, but that depends on the data measured with the original sample.

Direct toxicity testing of the ambient water has the same drawbacks as the risk or immission-based approach described above, when only acute test systems are used. Whitehouse et al. (2004) stated that the absence of measured toxicity does not mean that there is no risk to the receiving waters, especially when the result is based on acute tests alone. However, it can reduce some of the uncertainty associated with the assessment of

the potential of discharges to cause harm to the receiving water. Alternatively, toxicity could be measured in concentrated water samples.

Durand et al. (2009) suggested a concentration procedure with a synthetic XAD resin followed by elution with an organic solvent for obtaining concentrated water extracts from wastewater samples. Testing these extracts in acute tests can be an alternative to chronic tests even if no acute effects can be found in the original sample. However, any concentration procedure might cause a preselection of contaminants due to different adsorption/desorption behaviour.

It can be concluded that the major benefit of the use of bioassays for wastewater evaluation is hazard-based, which means that hazardous effluents are identified as such and treated according to BAT. The results of the wastewater investigations considered in this thesis demonstrate that the OSPAR WEA concept provides a suitable tool for detecting hazardous effluents and the origin of sources which contributes to the description of BAT for the respective industrial sectors. The Zahn-Wellens test is a suitable test system for assessing the treatability of wastewater in WWTPs. Combining the Zahn-Wellens test with ecotoxicity tests offers a tool for assessing the effluent quality after biological treatment. From a regulatory point of view the results can be used for determining whether biological treatment of such effluents corresponds to BAT or whether another pre-treatment of the effluent is required. Meanwhile, the concept is widely accepted and proposed to be included in future wastewater legislation for adequately addressing the quality of indirectly discharged effluents (Köppke 2009). The approach is fit for this purpose and is an appropriate instrument for assessing indirectly discharged industrial wastewater according to the IPPC Directive.

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6 SUMMARY

Chapter 1 of this thesis gives a general introduction to the application of bioassays for the assessment of effluents. With bioassays the effects of all compounds present in a complex sample as well as any synergistic or antagonistic interactions of bioavailable substances, including their degradation products, are accounted for. The “emission-based” approach focuses on fixed emission limits applied to all effluents within an industry sector following the precautionary principle. The “immission-based” approach takes the volume, nature and use of the receiving waters into account and is considered “risk-based”.

In 1999 a whole effluent assessment (WEA) expert group was established within the OSPAR convention for the protection of the North-East Atlantic (Oslo-Paris-Commission). The OSPAR WEA strategy developed in this expert group is based on the analysis of the persistency (P), the bioaccumulation potential (B), and the toxicity (T) of complex effluents. That means that the same PBT-criteria that are used within OSPAR’s Hazardous Substances Strategy for identifying priority substances are applied to the entire effluent.

The focus of this thesis is on the assessment of the degradability of indirectly discharged wastewater in municipal treatment plants and on assessing indirectly discharged effluents by coupling the Zahn-Wellens test with effect-based bioassays. With this approach persistent toxicity of an indirectly discharged effluent can be detected and attributed to the respective emission source. The objective of the studies presented in the thesis was to elaborate a new proposal for integrating persistency and bioaccumulation in the present methodology with the main focus on indirectly discharged effluents. Another objective was to identify the primary toxicants present in the wastewater and to link the effects measured in the effluents to the respective processes. For this the studies were accompanied by substance-specific analysis and/or an analysis of the main input chemicals and processes.

Chapter 2 presents the results of a Whole Effluent Assessment (WEA) of 8 wastewater samples from different industrial sectors as the German contribution to the practical WEA studies of OSPAR. All wastewater samples have been tested before and after a biodegradation test in order to assess the elimination of ecotoxic and genotoxic effects through biological treatment in municipal treatment plants (indirect dischargers) or the persistence of effects in surface water (direct dischargers). Generally, low to moderate ecotoxic effects of wastewater samples have been determined with maximum values of

$LID_A = 8$ in the algae test, $LID_{lb} = 24$ in the luminescent bacteria test and $LID_{Egg} = 6$ in the fish egg test. Low levels of PBS were determined in the effluents after biological treatment. The textile wastewater analysed was mutagenic in the Ames test and genotoxic in the umu test. After treatment in the Zahn-Wellens test the mutagenicity in the Ames test was eliminated completely while in the umu test genotoxicity could still be observed. Another wastewater sample from chemical industry was mutagenic before and after the performance of the DOC Die-Away test. Considering elevated algae toxicity occasionally observed at the same site after treatment in the WWTP, the first suspicion was that production of the antimicrobial triclosan and its derivatives might cause these effects. However a partial wastewater stream from triclosan production which was treated in the Zahn-Wellens test over 14 days showed no or only moderate ecotoxicity to daphnia, fish eggs, algae, and luminescent bacteria. No clear correlation was found between algae toxicity of total wastewater samples and the triclosan concentration measured by analytical means. The mutagenicity this wastewater sample from chemical industry was investigated by additional chemical analysis and backtracking. A nitro-aromatic compound (2-methoxy-4-nitroaniline) used for batchwise azo dye synthesis and its transformation products are the probable cause for the mutagenic effects analysed. Testing the mother liquor from dye production containing 100 mg L^{-1} 2-methoxy-4-nitroaniline, confirmed that this partial wastewater stream was mutagenic in the Ames test.

The Zahn-Wellens test proved to be a suitable screening tool for the biological treatability of wastewater samples by avoiding the much higher effort needed for laboratory flow through activated sludge simulation tests.

In **Chapter 3** the applicability of the WEA concept for the proof of compliance with the “best available techniques” (BAT) has been analysed with twenty paper mill wastewater samples from 13 paper mills in Germany covering different types of cellulose fibres as raw materials. No toxicity or genotoxicity at all was detected in the acute daphnia and fish egg as well as in the umu assay. In the luminescent bacteria test, moderate toxicity (up to $LID_{lb} = 6$) was observed. Wastewater of four paper mills demonstrated elevated or high algae toxicity (up to $LID_A = 128$), which was in line with the results of the Lemna test, which mostly was less sensitive than the algae test (up to $LID_{DW} = 8$). One indirectly discharged wastewater sample was biodegraded in the Zahn-Wellens test by 96% and was not toxic after treatment. Low levels of PBS have been detected (median 3.27 mmol L^{-1}). The colouration of the wastewater samples in the visible band did not correlate with algae toxicity and thus is not considered as its primary origin. The algae toxicity in wastewater of

the respective paper factory could also not be explained with the thermomechanically produced groundwood pulp (TMP) partial stream. Presumably other raw materials such as biocides might be the source of algae toxicity. In the algae test, often flat dose–response relationships and growth promotion at higher dilution factors have been observed, indicating that several effects are overlapping. The Lemna test turned out to be a suitable method which also detects inhibitors of photosynthesis and is not disturbed by wastewater colouration.

Chapter 4 presents the results of a WEA approach applied for determining persistent toxicity of indirectly discharged wastewater from the metal surface treatment industry. Twenty wastewater samples from the printed circuit board and electroplating industries which indirectly discharged their wastewater to municipal wastewater treatment plants (WWTPs) have been considered in the study. In all factories, the wastewater partial flows were separated in collecting tanks and physicochemically treated in-house. All samples were biologically pretreated for 7 days in the Zahn–Wellens test before ecotoxicity testing. Thus, persistent toxicity could be discriminated from non-persistent toxicity caused, e.g. by ammonium or readily biodegradable compounds. With respect to the metal concentrations, all samples were not heavily polluted. The maximum conductivity of the samples was $43,700 \mu\text{S cm}^{-1}$ and indicates that salts might contribute to the overall toxicity. Half of the wastewater samples proved to be biologically well treatable in the Zahn–Wellens test with COD elimination above 80%, whilst the others were insufficiently biodegraded (COD elimination 28–74%). After the pre-treatment in the Zahn–Wellens test, wastewater samples from four (out of ten) companies were extremely ecotoxic especially to algae (maximum $\text{LID}_A = 16,384$). Three wastewater samples were genotoxic in the umu test. Applying the rules for salt correction to the test results as allowed in the German Wastewater Ordinance, only a small part of toxicity could be attributed to salts. Considering the PBS, wastewater from the metal surface treatment industry exhibited very low levels of PBS. In one factory, the origin of ecotoxicity has been attributed to the organosulphide dimethyldithiocarbamate (DMDTC) used as a water treatment chemical for metal precipitation. The assumption, based on rough calculation of input of the organosulphide into the wastewater, was confirmed in practice by testing its ecotoxicity at the corresponding dilution ratio after pre-treatment in the Zahn–Wellens test.

The results show that the organic load of wastewater from the electroplating industry has been underestimated by focusing on inorganic parameters such as heavy metals, sulphide, cyanide, etc. Bioassays are a suitable tool for assessing the ecotoxicological

relevance of these complex organic mixtures. The combination of the Zahn–Wellens test followed by the performance of ecotoxicity tests turned out to be a cost-efficient suitable instrument for the evaluation of indirect dischargers and considers the requirements of the IPPC Directive.

In **Chapter 5** the prospects of wastewater evaluation with bioassays and the applicability of the OSPAR WEA concept is discussed in a broader context. The application of bioassays for wastewater evaluation follows distinct objectives such as screening of discharges for effluent toxicity, characterising the toxic hazards of effluents as part of a risk assessment process, assessing the toxic impact of point source discharges on the receiving water environment. Additionally, the causes and sources of toxicity in final effluent might be analysed by a “toxicity identification evaluation” (TIE) approach. This consists in a systematic pre-treatment of the wastewater samples. Another approach is “toxicity backtracking” where the origin of ecotoxicity is identified by testing partial wastewater streams from different processes which contribute to the mixed effluent. The methodology followed in this thesis was a combination of the emission-based approach and an abbreviated TIE and toxicity backtracking approach.

Whole Effluent Assessment (WEA) can be considered as an extension of the Whole Effluent Toxicity (WET) approach. WEA can be considered as a safety net for all the substances of concern not included in hazardous substances lists due to the lack of knowledge. As the WEA approach focuses on hazard, the main objective is its use for identification and surveillance of hazardous effluents.

Three years after the work of OSPAR on WEA has been finished with the publication of the WEA Guidance document a survey on the implementation of the WEA concept among the contracting parties started.

Many scientists suggest that it is incorrect to refer to the “persistence of effluents” due to the fact that persistency is defined from the half life of individual substances. However, degradability tests are the only meaningful instrument for assessing persistency of complex effluents also with respect to non-degradable ecotoxicity and content of PBS. Comparing different regulatory requirements a COD elimination of about 75% in 7 to 14 days is a convenient pass level when the Zahn-Wellens test is used for assessing treatability in WWTPs. For coupling degradation tests with ecotoxicity tests preferentially the undiluted sample should be analysed in order to assure that subsequent toxicity testing is not affected by dilution. If the sample has to be diluted before degradability testing for practical reasons

(e.g. too high TOC), the dilution factor should be considered in the interpretation of results. As the DOC removal is no direct indication of toxicity removal, the test conditions respective the start concentrations and the test duration might need to be optimised for specific types of effluents. It should be kept in mind that removal by adsorption to the activated sludge might cause cross-media effects when the surplus sludge is disposed of on landfills sites or as organic fertilizer on soils.

The OSPAR WEA concept clearly focuses on the hazard-based approach and this is in line with the overall objective of OSPAR and the WFD to reduce the emission of hazardous substances close to zero. The objective of WEA is to identify complex effluents that contain hazardous ingredients which might cause adverse effects in surface water. There are approaches where WEA is used to assess the impact of effluents to the receiving water quality, which corresponds to a water quality or risk-based approach. In this case it should be kept in mind that the acute tests usually applied have a low discriminative power after dilution of the effluent in the receiving water. Applying the risk or immission-based principle with PEC/PNEC-comparison the dilution of the waste water is being considered. In this case the same principles applied for the setting of chemical quality standards according to the Water Framework Directive (WFD) should be used. Direct toxicity testing of the ambient water has the same drawbacks as the risk or immission-based approach described above, when only acute test systems are used. It can be concluded that the major benefit of the application of bioassays for wastewater evaluation is hazard-based, which means that hazardous effluents are identified as such and treated according to BAT. The results of the wastewater investigations considered in this thesis demonstrate that the OSPAR WEA concept provides a suitable tool for detecting hazardous effluents and the origin of sources which contributes to the description of BAT for the respective industrial sectors. The Zahn-Wellens test is a suitable test system for assessing the treatability of wastewater in WWTPs. Combining the Zahn-Wellens test with ecotoxicity tests offers a tool for assessing the effluent quality after biological treatment. From a regulatory point of view the results can be used for determining whether biological treatment of such effluents corresponds to BAT or whether another pre-treatment of the effluent is required. This approach is suitable for this purpose and an appropriate instrument for assessing indirectly discharged industrial wastewater according to the IPPC Directive.

7 ZUSAMMENFASSUNG

Kapitel 1 dieser Dissertation gibt eine allgemeine Einführung in die Anwendung von Biotests für die Abwasserbewertung. Mit Biotests werden die Wirkungen aller in einer komplexen Probe enthaltenen Verbindungen, alle synergistischen oder antagonistischen Wechselwirkungen bioverfügbarer Substanzen sowie deren Abbauprodukte erfasst. Der “emissionsbezogene” Ansatz setzt einen Schwerpunkt auf feste Emissionsgrenzwerte, die auf alle Abwässer eines industriellen Sektors angewandt werden und folgt dem Vorsorgeprinzip. Der “immissionsbezogene” Ansatz berücksichtigt auch das Volumen, die Beschaffenheit und die Nutzung des Vorfluters und entspricht einer risikobasierten Herangehensweise.

Im Jahr 1999 wurde eine Expertengruppe zur Gesamtabwasserbewertung (“*whole effluent assessment*”, WEA) innerhalb des Meeresschutzabkommens für den Nordost-Atlantik eingerichtet (Oslo-Paris-Konvention). Die OSPAR WEA Strategie, die in dieser Expertengruppe erarbeitet wurde, basiert auf der Untersuchung der Persistenz (P), des Bioakkumulationspotentials (B) und der Toxizität (T) komplexer Abwässer. Dies bedeutet, dass dieselben PBT-Kriterien, die in der „OSPAR-Strategie zu gefährlichen Substanzen“ zur Identifizierung prioritärer Stoffe dienen, direkt für Abwasserproben eingesetzt werden.

Der Schwerpunkt dieser Dissertation liegt auf der Bewertung der Abbaubarkeit indirekt über kommunale Abwasserreinigungsanlagen eingeleiteter Abwasserproben und in der Bewertung dieser Abwässer durch Verknüpfung des Zahn-Wellens-Tests mit wirkungsbasierten Biotests. Mit diesem Ansatz lässt sich die persistente Toxizität indirekt abgeleiteter Abwässer bestimmen und der jeweiligen Emissionsquelle zuordnen. Ziel der in dieser Dissertation vorgestellten Arbeiten war es, einen neuen Vorschlag zur Integration von Persistenz und Bioakkumulation in die bestehende Methodik zu erarbeiten, wobei das Hauptaugenmerk auf indirekt eingeleiteten Abwasserproben lag. Als weiteres Ziel sollten die in den Abwasserproben enthaltenen wesentlichen toxischen Stoffe identifiziert und mit den jeweilig zugehörigen Prozessen verknüpft werden. Daher wurden die Studien mit substanzspezifischer Analytik und/oder mit einer Analyse der wichtigsten eingesetzten Chemikalien und Prozesse ergänzt.

In **Kapitel 2** werden die Ergebnisse einer WEA-Studie mit 8 Abwasserproben aus verschiedenen Industriesektoren als deutscher Beitrag zur OSPAR-WEA-Praxisstudie präsentiert. Alle Abwasserproben wurden vor und nach einem Abbautest untersucht, um

die Elimination ökotoxischer und gentoxischer Effekte durch die biologische Behandlung in kommunalen Abwasserreinigungsanlagen (Indirekteinleiter) oder die Persistenz von Wirkungen im Oberflächenwasser (Direkteinleiter) zu bestimmen. Im Allgemeinen wurden geringe bis moderate ökotoxische Effekte in den Abwasserproben bestimmt. Die maximalen Verdünnungsstufen (G-Werte) lagen bei $G_A = 8$ im Algentest, $G_L = 24$ im Leuchtbakterientest und $G_{Ei} = 6$ im Fischeitest. Nach der biologischen Behandlung wurden geringe Mengen potentiell bioakkumulierbarer Substanzen (PBS) in den Abwasserproben bestimmt. Die untersuchte Textilabwasserprobe war im Ames-Test mutagen und im umu-Test gentoxisch. Nach der Behandlung im Zahn-Wellens-Test war die Mutagenität im Ames-Test vollständig eliminiert, während im umu-Test noch Gentoxizität beobachtet wurde. Eine andere Abwasserprobe aus der chemischen Industrie war vor und nach der Durchführung des DOC-Die-Away-Tests mutagen. In Anbetracht der bei demselben Einleiter gelegentlich bestimmten erhöhten Algentoxizität nach Behandlung in der Abwasserreinigungsanlage wurde zuerst vermutet, dass diese Effekte aus der Produktion des Biozids Triclosan and seiner Nebenprodukte stammen könnten. Allerdings erwies sich eine Teilstromprobe aus der Triclosan-Produktion nach 14-tägiger Behandlung im Zahn-Wellens-Test als nicht bzw. nur gering ökotoxisch gegenüber Daphnien, Fischeiern, Algen und Leuchtbakterien. Es bestand keine eindeutige Korrelation zwischen der Algentoxizität der Gesamtabwasserproben und den analytisch bestimmten Triclosankonzentrationen. Die Herkunft der Mutagenität dieser Abwasserprobe aus der chemischen Industrie wurde durch weitergehende chemische Analyse und Zurückverfolgung („backtracking“) untersucht. Eine nitroaromatische Verbindung (2-Methoxy-4-nitroanilin), die für die chargenweise Synthese von Azofarbstoffen verwendet wird und weitere Transformationsprodukte erwiesen sich als wahrscheinliche Quelle der mutagenen Effekte. Durch Untersuchung der Mutterlauge aus der Farbstoffherstellung mit 100 mg L^{-1} 2-Methoxy-4-nitroanilin wurde bestätigt, dass dieser Abwasserteilstrom im Ames-Test mutagen ist.

Der Zahn-Wellens-Test erwies sich als geeignete Screeningmethode zur Bestimmung der biologischen Behandelbarkeit von Abwasserproben, mit der der weit höhere Aufwand für den Betrieb von Durchfluss-Laborkläranlagen (Belebtschlamm-Simulationstest) vermieden wird.

In **Kapitel 3** wird die Anwendbarkeit des WEA-Konzepts als Nachweis der Einhaltung der “Besten verfügbaren Techniken” (BVT, in Deutschland meist mit „Stand der Technik“ übersetzt) mit 20 Abwasserproben von 13 Papierfabriken untersucht, die verschiedene Rohstoffe als Cellulosefasern einsetzen. Im akuten Daphnien- und Fischeitest sowie im

umu-Test wurde keine Toxizität bzw. Gtoxizität bestimmt. Im Leuchtbakterientest wurden moderate Toxizitäten (bis $G_L = 6$) beobachtet. Die Abwässer von vier Papierfabriken zeigten eine erhöhte bis hohe Algentoxizität (bis zu $G_A = 128$), die mit den Ergebnissen des Lemna-Tests übereinstimmte. Dieser war meist weniger sensitiv als der Algentest (bis zu $G_{DW} = 8$). Eine indirekt eingeleitete Abwasserprobe war im Zahn-Wellens-Test zu 96% abbaubar und nach biologischer Behandlung nicht toxisch. Insgesamt wurden geringe Mengen an PBS bestimmt (Median $3,27 \text{ mmol L}^{-1}$). Die Färbung der Abwasserproben im sichtbaren Bereich korrelierte nicht mit der Algentoxizität und wird daher nicht als deren ursächliche Quelle angesehen. Die Algentoxizität im Abwasser der betreffenden Papierfabrik konnte auch nicht mit einem Abwasserteilstrom aus der thermomechanischen Herstellung von Zellstoff aus Holzschliff erklärt werden. Vermutlich sind andere Hilfsstoffe wie Biozide als Quelle für die Algentoxizität verantwortlich. Im Algentest wurden oftmals flache Dosis-Wirkungskurven und eine Förderung des Algenwachstums bei höheren Verdünnungsstufen beobachtet. Dies deutet darauf hin, dass sich hier verschiedene Effekte überlagern. Der Lemna-Test erwies sich als geeignete Methode, mit der ebenfalls Photosyntheseinhibitoren erfasst werden und die nicht durch die Färbung der Abwässer gestört wird.

In **Kapitel 4** werden die Ergebnisse der Anwendung des WEA-Konzepts zur Bestimmung der persistenten Toxizität indirekt eingeleiteter Abwasserproben aus der Oberflächenbearbeitung von Metallen präsentiert. In der Studie wurden zwanzig Abwasserproben aus der Herstellung von Leiterplatten und der galvanischen Industrie, die ihr Abwasser indirekt über kommunale Kläranlagen einleiten, untersucht. In allen Fabriken werden die Abwasserteilströme getrennt in Tanks gesammelt und physikochemisch vor Ort behandelt. Alle Proben wurden biologisch für 7 Tage im Zahn-Wellens-Test vorbehandelt und anschließend die Ökotoxizität bestimmt. So konnte die persistente Toxizität von nicht-persistenter Toxizität, die beispielsweise durch Ammonium oder leicht abbaubare Inhaltsstoffe verursacht wird, unterschieden werden. In Hinsicht auf die Schwermetallkonzentrationen waren alle Abwasserproben nur wenig belastet. Die maximale Leitfähigkeit der Proben lag bei $43.700 \mu\text{S cm}^{-1}$ und weist darauf hin, dass Salze zur Gesamttoxizität beitragen könnten. Die Hälfte der Abwasserproben war im Zahn-Wellens-Test mit CSB-Eliminationen über 80% gut biologisch behandelbar, während die anderen Proben nur unzureichend biologisch abgebaut wurden (CSB-Elimination 28% – 74%). Nach der Vorbehandlung im Zahn-Wellens-Test wurde im Abwasser von vier (von zehn) Betrieben extrem hohe Ökotoxizitäten insbesondere gegenüber Algen bestimmt

(Maximum $G_A = 16.384$). Drei Abwasserproben erwiesen sich im umu-Test als gentoxisch. Bei Anwendung der in der Abwasserverordnung vorgesehenen Salzkorrektur der Ergebnisse konnte nur ein kleiner Anteil der Toxizität mit einem Salzeinfluss erklärt werden. In Hinblick auf die PBS waren die Abwässer der Metalloberflächenbehandlung nur sehr gering belastet. In einer Fabrik wurde das als Wasserbehandlungsmittel zur Metallfällung eingesetzte Organosulfid Dimethyldithiocarbamat (DMDTC) als ursächliche Quelle der Ökotoxizität nachgewiesen. Diese, auf einer überschlägigen Kalkulation der Organosulfideinträge in das Abwasser getroffene Annahme, wurde durch Testung der Ökotoxizität des Organosulfids in einer entsprechenden Verdünnung nach Behandlung im Zahn-Wellens-Test in der Praxis bestätigt.

Die Ergebnisse zeigen, dass die organischen Bestandteile von Abwasser der galvanischen Industrie bisher unterschätzt wurden, indem der Hauptaugenmerk auf anorganische Parameter wie Schwermetalle, Sulfide, Cyanide etc. gelegt wurde. Biotests sind geeignete Werkzeuge, um die ökotoxikologische Relevanz dieser komplexen organischen Mischungen zu beurteilen. Die Kombination des Zahn-Wellens-Tests mit der nachfolgenden Bestimmung der Ökotoxizität erwies sich als geeignetes und kosteneffizientes Instrument zur Beurteilung von Indirekteinleitern und berücksichtigt die Anforderungen der IVU-Richtlinie.

In **Kapitel 5** werden die Möglichkeiten der Abwasserbewertung mit Biotests und die Anwendbarkeit des OSPAR-WEA-Konzepts in einem breiteren Kontext diskutiert. Die Anwendung von Biotests zur Abwasserbewertung verfolgt verschiedene Ziele: Das Screening von Abwassereinleitungen bezüglich Toxizität, die Charakterisierung der Abwassertoxizität als Teil der Risikobewertung oder die Bewertung toxischer Auswirkungen von Abwasser als Punktquelle auf den Vorfluter. Zudem können Ursachen und Quellen von Toxizität im Gesamtabwasser durch die Methode der *“toxicity identification evaluation”* (TIE) untersucht werden. Diese besteht in einer systematischen Vorbehandlung der Abwasserprobe. Eine andere Herangehensweise besteht in der Rückverfolgung der Toxizität (*„toxicity backtracking“*). Hierbei wird der Quelle der Ökotoxizität durch Testung der Abwasserteilströme aus verschiedenen Prozessen, die zum Mischabwasser beitragen, untersucht. Die in der vorliegenden Dissertation verfolgte Methode entspricht einer Kombination des emissionsbezogenen Ansatzes mit einem abgekürzten *“TIE”* und *“toxicity backtracking”* Ansatz.

Das WEA-Konzept kann als Teil des “*Whole Effluent Toxicity*” (WET) Ansatzes angesehen werden. WEA ist hierbei ein Sicherheitsnetz für all die bedenklichen Substanzen, die aufgrund von Wissenslücken nicht in Listen gefährlicher Stoffe aufgenommen wurden. Da das WEA-Konzept auf Gefahrenmomente hinzielt, wird es überwiegend für die Identifizierung und Überwachung gefährlicher Abwässer eingesetzt.

Drei Jahre nachdem die OSPAR-Arbeiten zu WEA mit der Veröffentlichung eines WEA-Leitfadens abgeschlossen wurden, startete eine Umfrage zur Umsetzung von WEA in den OSPAR-Vertragsstaaten.

Viele Wissenschaftler weisen drauf hin, dass es inkorrekt sei, von der “Persistenz von Abwasser” zu sprechen, angesichts der Tatsache, dass Persistenz als Halbwertszeit einzelner Substanzen definiert ist. Allerdings sind Abbautests das einzige aussagekräftige Instrument, um die Persistenz komplexer Abwasserproben, auch in Hinblick auf die nicht-abbaubare Ökotoxizität und die PBS, zu bestimmen. Durch Vergleich verschiedener gesetzlicher Anforderungen kann eine CSB-Elimination von etwa 75% in 7 bis 14 Tagen als angemessener Schwellenwert angesehen werden, wenn der Zahn-Wellens-Test zur Bewertung der Behandelbarkeit von Abwässern in kommunalen Kläranlagen eingesetzt wird. Für die Kopplung von Abbautests mit Ökotoxizitätstests sollte vorzugsweise die unverdünnte Abwasserprobe verwendet werden, um sicherzustellen, dass die Aussagekraft der nachfolgenden Ökotoxizitätsbestimmung nicht durch die Verdünnung beeinträchtigt wird. Wenn die Probe vor der Abbauprüfung aus praktischen Erwägungen verdünnt werden muss (z.B. wegen eines zu hohen TOC), sollte der Verdünnungsfaktor bei der Interpretation der Ergebnisse mit berücksichtigt werden. Da die DOC-Elimination keinen direkten Hinweis für die Abnahme der Ökotoxizität gibt, müssen die Versuchsbedingungen bezüglich der Startkonzentration und der Testdauer für spezifische Abwassertypen optimiert werden. Es sollte bedacht werden, dass die Elimination durch Adsorption an den Belebtschlamm medienübergreifende Effekte mit sich bringen kann, wenn der Überschussschlamm auf Deponien oder als Dünger auf Böden abgelagert wird.

Das OSPAR-WEA-Konzept fokussiert eindeutig auf dem gefahrenbasierten Ansatz und entspricht der Gesamtstrategie von OSPAR und der Wasserrahmenrichtlinie, die Emission von Schadstoffen nahezu vollständig zu reduzieren. Ziel des WEA-Konzepts ist die Identifizierung von komplexen, Schadstoffe enthaltenden Abwässern, die negative Auswirkungen auf Oberflächengewässer haben könnten. Es gibt Ansätze, bei denen WEA verwendet wird, um die Auswirkung von Abwassereinleitungen auf die Beschaffenheit der

Vorfluter zu bewerten, was einem Wasserqualität- oder risikobezogenen Ansatz entspricht. In diesem Fall sollte bedacht werden, dass die üblicherweise eingesetzten akuten Tests bei den im Vorfluter vorliegenden hohen Verdünnungen eine geringe Aussagekraft haben. Bei Anwendung des risiko- oder immissionbasierten Prinzips mit einem PEC/PNEC-Vergleich wird die Verdünnung der Abwasserprobe im Vorfluter berücksichtigt. In diesem Fall sollten dieselben Regeln, die für die Ableitung von Umweltqualitätsnormen für chemische Stoffe nach Wasserrahmenrichtlinie gelten, angewandt werden. Bei direkter Testung von Umweltproben des Vorfluters treten dieselben Nachteile auf, wie sie oben für den risiko- oder immissionsbezogenen Ansatz beschrieben werden, wenn lediglich akute Testsysteme eingesetzt werden. Daraus folgt, dass der Hauptnutzen der Anwendung von Biotests mit Abwasserproben gefahrenbezogen ist. Das bedeutet, dass gefährliche Abwässer als solche identifiziert und einer Behandlung gemäß BVT zugeführt werden. Die Ergebnisse der Abwasseruntersuchungen, die in vorliegender Dissertation berücksichtigt wurden, zeigen deutlich, dass das OSPAR-WEA-Konzept ein geeignetes Instrument ist, um gefährliche Abwässer und die Herkunft der ursächlichen Quellen zu identifizieren und somit einen Beitrag zur Beschreibung von BVT in den betreffenden industriellen Bereichen zu leisten. Der Zahn-Wellens-Test ist ein geeignetes Testsystem zur Bestimmung der Behandelbarkeit von Abwasser in kommunalen Kläranlagen. Die Koppelung des Zahn-Wellens-Tests mit Ökotoxizitätstests ist ein Instrument zur Bewertung der Abwasserbeschaffenheit nach biologischer Behandlung. Unter regulatorischen Gesichtspunkten können die Ergebnisse verwendet werden, um abzuklären, ob die biologische Behandlung solcher Abwässer den BVT entspricht oder ob andere Verfahren zur Abwasserbehandlung erforderlich sind. Der gewählte Ansatz ist für diesen Zweck geeignet und ein brauchbares Instrument zur Bewertung indirekt eingeleiteter industrieller Abwasserproben in Anlehnung an die Vorgaben der IVU-Richtlinie.

Als Grundlage für die kumulative Dissertation dienen folgende rezensierte in internationalen Fachzeitschriften erschienene Arbeiten:

- Gartiser, S., Hafner, C., Oeking, S., Paschke, A. 2009: Results of a "Whole Effluent Assessment" study from different industrial sectors in Germany according to OSPAR's WEA strategy. *J. Environ. Monit.* 11, p. 359–369.
- Gartiser, S., Hafner, C., Hercher, C., Kronenberger-Schäfer, K., Paschke, A. 2010: Whole Effluent Assessment of Industrial Wastewater for Determination of BAT Compliance Part 1: Paper Manufacturing Industry. *Environ. Sci. Pollut. Res.* 11, p. 359–369

- Gartiser, S., Hafner, C., Hercher, C., Kronenberger-Schäfer, K., Paschke, A. 2010: Whole Effluent Assessment of Industrial Wastewater for Determination of BAT Compliance Part 2: Metal Surface Treatment Industry. Environ. Sci. Pollut. Res. 17, p. 856-865

In rezensierten deutschsprachigen Zeitschriften sind folgende Publikationen zum selben Thema erschienen:

- Gartiser, S., Hafner, C., Oeking, S., Paschke, A., Maue, G.: Einsatz wirkungsbezogener Methoden zur Bewertung und Kontrolle von Industrieabwässern im Rahmen von OSPAR-Aktivitäten. KA Abwasser Abfall 54 (2007), H. 11, S. 1146-1155
- Gartiser, S., Hafner, C., Hercher, C., Kronenberger-Schäfer, K.: Bewertung von Direktleinleitern mit Biotests. Teil 1: Abwasser in der Papierindustrie. Korrespondenz Abwasser, Abfall 56, 2009, S. 915-920
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- Gartiser, S.: Bestimmung der Mineralisierung und DOC-Elimination von Abwasserproben im Zahn-Wellens-Test. gwf-Wasser-Abwasser 150, 9, 2009, S. 700-710.

Zudem wurden die folgenden Poster auf internationalen Tagungen vorgestellt:

- Gartiser, S., Hafner, C., Paschke, A.: Einsatz wirkungsbezogener Methoden zur Bewertung und Kontrolle von Industrieabwässern im Rahmen von OSPAR Aktivitäten. SETAC German Language Branch - 10. Jahrestagung in Basel, Schweiz, 28. - 30. September 2005
- Gartiser, S., Hafner, C., Oeking, S., Paschke, A.: Results of a Practical Whole Effluent Assessment Study in Germany. SETAC Europe 16th Annual Meeting in The Hague, The Netherlands, 7 - 11 May 2006
- Gartiser, S., Hafner, C., Hercher, C., Kronenberger-Schäfer, K., Paschke, A.: Effect-related Evaluation of Paper Manufacturing and Metal Surface Treatment Wastewater. SETAC Europe, 18th Annual Meeting in Göteborg, Schweden, 31. Mai - 4. Juni 2009

Die Poster können auf der Webseite der Hydrotox GmbH www.hydrotox.de heruntergeladen werden.

8 CURRICULUM VITAE

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	1984	Berufspraktikum am Instituto de Ingenieria de la U.N.A.M., Mexiko City.
	1987	Abschluss mit Diplomhauptprüfung in den Prüfungsfächern Hydrologie, Limnologie, Bodenkunde und Geologie. Thema der Diplomarbeit: <i>„Erosionsproblematik, Speicherverlandung und Austrag von Schwebstoffen, dargestellt am Beispiel des Stausees „Contzio“ in Michoacán, Mexiko“.</i>
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Freiburg, den 12.07.2010

Stefan Gartiser

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Ich erkläre hiermit an Eides Statt, dass ich die vorgelegte Dissertation über

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