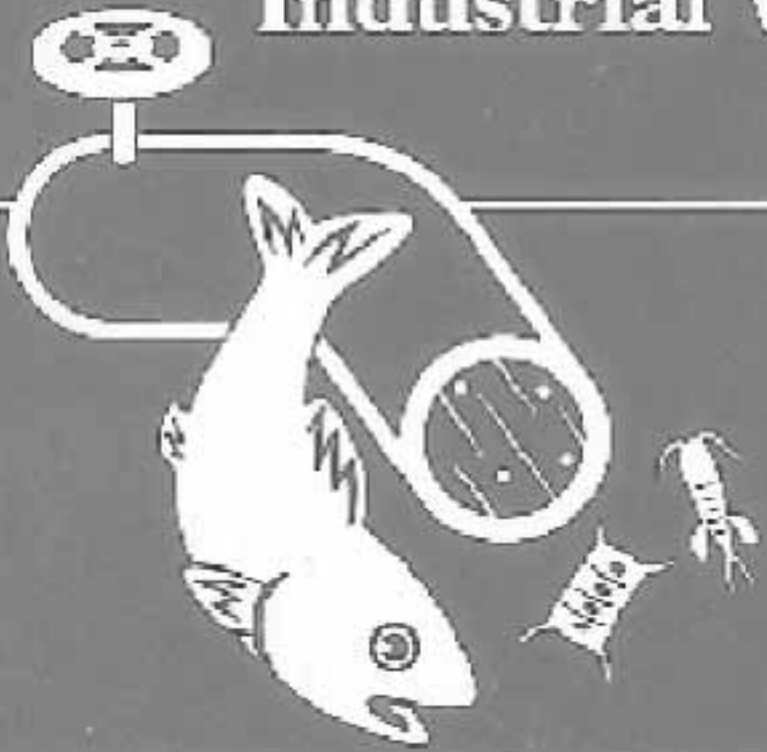
Miljøprojekt nr. 254

1994

Ecotoxicological Evaluation of Industrial Wastewater



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Ecotoxicological Evaluation of Industrial Wastewater

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1. Introduction

The present report is an English version of the report "Økotoksikologisk vurdering af industrispildevand" published in 1992 by the Danish Environmental Protection Agency (Environmental Report No. 188). The report has been translated into English due to the intention of the Danish Environmental Protection Agency to initiate and inspire the discussions in the EEC, the OECD, the East European Countries, CEN, etc. on common guidance on methods for investigation of complex industrial wastewater and chemicals contained therein.

The report is a technical background report describing the present Danish approach to investigate, characterize and evaluate the ecotoxicological properties of industrial wastewater based on the experiences obtained during the latest decade both in Denmark and abroad. The work will be followed by a guidance document on evaluation of environmental impact of industrial wastewater and criteria setting for industrial discharges.

In the first part of the present report (chapter 2) international and Danish strategies for investigation of industrial wastewater are described.

In part two methods to characterize and evaluate industrial effluents are described. Chapter 3 discusses methods to assess exposure concentrations of chemicals and complex wastewater in the receiving water taking variability of effluents, dispersion and distribution in the environment, and degradability and bioaccumulation into account. Chapter 4 describes ecotoxicological test methods, and extrapolation methods for determination of environmental no-effect-concentrations based on results from laboratory tests.

Finally, in part three (chapter 5) existing principles for environmental hazard and risk assessment of chemicals and complex mixtures are described. Also a concept for a step-wise investigation programme, where an integrated evaluation after each step is carried out, is proposed.

The report has been prepared by Preben Kristensen, Henrik Wenzel Christensen and Axel Damborg, the Water Quality Institute (VKI) and Finn Pedersen, the Danish Environmental Protection Agency. The report has been discussed in detail in a working group during the writing process. The working group consisted of:

Christian Bastholm	(Cheminova A/S)
Flemming Møhlenberg	(National Environmental Research Institute)
Preben Kristensen	(Water Quality Institute, ATV)
Axel Damborg	(Water Quality Institute, ATV)
Finn Pedersen	(Danish Environmental Protection Agency)

Moreover, the work has been followed and discussed in a steering group consisting of:

Niels Juul Jensen	(Danish Environmental Protection Agency)
Christian Bastholm	(Cheminova A/S)

T. Schjødt Jensen	(Novo Nordisk A/S)
Ejvind Thorsen	(Grindsted Products A/S)
Søren Vogelsang	(Lundbeck A/S)
Per Eichner	(Kemira Danmark A/S)
Erik Degn	(Ringkjøbing Amt)
Hans Løkke	(National Environmental Research Institute)
Flemming Møhlenberg	(National Environmental Research Institute)
Preben Kristensen	(Water Quality Institute)
Axel Damborg	(Water Quality Institute)
Lydia Frost	(Danish Environmental Protection Agency)
Ulla Ringbæk	(Danish Environmental Protection Agency)
Finn Pedersen	(Danish Environmental Protection Agency)

The comments from and the discussions in the working group and the steering group are gratefully acknowledged.

The work was initiated and financed by a number of major Danish chemical industries (Cheminova A/S, Grindsted Products A/S, Lundbeck A/S, Novo-Nordisk A/S) and the Danish Environmental Protection Agency.

The report has been translated into English by Mike Robson, VKI. Note that some of the described ongoing initiatives may have been finalized since the Danish version in 1992 and that new projects, not described in the report, may have been initiated. Many of the data sources cited are only available in Danish and these references have therefore not been translated into English.

2. Investigative strategies

This section presents existing experience and strategies relating to the investigation and evaluation of complex industrial wastewater. The first part describes the strategies used in a number of countries for investigating and evaluating industrial wastewater. The next part presents ways in which wastewater from Danish industries has been examined and evaluated, and describes the experience gained from this.

2.1 Strategies in other countries

Although many countries have set requirements for examination, evaluation and environmental approval of industrial wastewater discharges, only a few have developed and published formal strategies for the evaluation of the environmental risk posed by a discharge. The working group has collected information about the following, more or less different, investigative and decision-making strategies:

2.1.1 The European Community

There are no European Community directives prescribing standards for ecotoxicological effects of complex wastewater. However, the Community has adopted Directive 76/464/EEC on pollution caused by certain dangerous substances discharged into the aquatic environment of the Community /1/. In order to eliminate pollution by a number of substances (list I), primarily chosen because of their toxicity, persistency or bioaccumulative potential, the directive lays down discharge limits and water quality objectives (limits for concentrations in a recipient water body). The Commission's proposed List I includes 132 substances or groups of substances /2/. Furthermore, member states are required to develop programmes, for example recipient water quality programmes, for substances which are included in list II of the Directive because of their harmful effect on the environment within limited areas.

Further the Community has developed criteria for environmental hazard classification of chemical substances, based on data on the inherent properties of the substances. The required data set corresponds to the data that must be submitted to the authorities in connection with registration of new chemical substances (the Minimum Pre-marketing Data Set, MPD). Thus the environmental classification corresponds to a hazard identification of chemical substances, and one must expect that this type of information will be widely used in future, also for evaluation of industrial discharges.

In addition, the Community has also developed methods for hazard evaluation of new chemical substances using the MPD set together with information on the expected usage pattern, so that before the substance is marketed, decisions can be made about the need for restrictions on use. Similar methods are expected to be developed in connection with the Commission regulation on existing chemical substances.

2.1.2 OECD

The Environment Directorate of the OECD has collected information about experience with analysis of industrial wastewater in OECD countries. This information formed the basis for the report "The use of biological tests for water pollution assessment and control", which was discussed at an expert group meeting in June 1986. The report was revised on the basis of the group's discussions and conclusions /9/, but because of disagreement between OECD countries the report could not be finalized and published as the official description of OECD policy on this topic.

The report's proposals for investigative and evaluatory strategies are heavily influenced by the strategy adopted in the USA (see section 2.1.3), working with up to three levels of increasing complexity. The report also refers to the different approaches adopted in several countries for using the results of wastewater analyses for reaching regulatory decisions. This applies to the definition of discharge criteria, development of monitoring programmes, requirements for reduction of wastewater toxicity, prioritising of identified toxic discharges and affected receiving waters, and the evaluation of the short and long term effects of discharges. Some of these topics will be referred to in the following sections.

2.1.3 USA

Throughout the 80's, the US Environmental Protection Agency (EPA) has worked to develop a policy and strategy for the investigation and assessment of complex wastewater mixtures as a basis for setting discharge criteria. The overall policy is described in the document "Development of Water Quality-Based Permit Limitations for Toxic Pollutants; National Policy" /3/. The strategy focuses primarily on toxicity, while other characteristics such as bioaccumulative potential, persistence, and genotoxicity have only sporadically been treated so far. The strategy is described in "Permit Writers Guide to Water Quality-Based Permitting for Toxic Pollutants" /5/ and in "Technical Support Document for Water Quality-based Toxics Control" /12/ (Revised 1990 /4/).

The investigation and assessment strategy builds on the generally applied requirement to use "best available technology" (BAT), and it comes into play whenever BAT can not ensure satisfactory water quality in the recipient. The individual states lay down criteria for water quality, both with respect to individual toxic substances by means of specific water quality criteria for the substance (recipient standards), and also in more general terms through requirements such as "no content of toxic materials in toxic quantities" /3/. The water quality-based strategy is thus twin-tracked, since it comprises investigations and assessments both of identified chemical substances and of complex effluent mixtures, so that the two aspects complement and supplement each other, and rarely can stand alone /3,8/. A third method of evaluation is also being developed, since US-EPA has proposed that each state should develop and use biological criteria, i.e. a numeric or qualitative description of the biological integrity of the recipient /4/.

The advantages of investigating complex wastewater mixtures in their entirety are:

- that the total toxicity of the wastewater is measured,
- that this gives an indication of the content of bioavailable toxic materials, and
- that any synergistic or antagonistic effects are taken into account.

The advantages of investigating the individual substances are:

- that treatment plants can more easily be designed to treat individual substances,
- that environmental administrations are more used to regulating individual substances,
- that substances with special properties (such as persistence) are difficult to detect in effluent mixtures /8/
- that the toxicity of problematical substances is often better documented than the toxicity of complex effluent mixtures, and finally
- that it is also possible to evaluate human exposure and the possible effects.

Biological investigations in the receiving water body can give the most precise descriptions of the total pollution load on the receiving water and its general status, providing that sufficiently precise methods are available.

However, none of the methods can stand alone, nor can one be recommended at the expense of others, and US-EPA therefore recommends that the method used in any given wastewater evaluation exercise should be the method giving the highest degree of protection /4/.

On the basis of the local requirements regarding receiving water quality a total acceptable load should be established ("Total Daily Maximum Load"). If necessary this can be split up into subsidiary contributions from several wastewater sources ("Wasteload Allocation") and a contribution from diffuse sources ("Load Allocation") /4/.

Investigation of the wastewater is split up into several stages. At the screening stage the objective is to distinguish between the discharges which are not expected to cause effects in the receiving water, and the discharges which possibly or definitely cause damage. The latter must then be investigated more closely in the subsequent stages in order to generate adequate data for setting permissible discharge levels.

The starting point for determining which wastewater investigations should be carried out is a calculation of the dilution capacity of the recipient /4/. If the potential dilution factor is greater than 1000 at the minimum water flow, acute toxicity tests on three types of test organism (plant, invertebrate and vertebrate) are recommended, and these should be used to set a maximum concentration (Criteria Maxi-

mum Concentration, CMC) which, after initial dilution, must not be exceeded as a mean over a 1 hour period.

If the dilution factor is between 100 and 1000 at minimum water flow, tests for either acute or chronic toxicity are recommended, and these should be used to calculate an average concentration (Criteria Continuous Concentration, CCC), which must not be exceeded as a mean over a 4-day period.

If the expected dilution factor at minimum water flow is below 100, chronic tests are recommended as the basis for calculating the CCC.

The general discharge policy of US-EPA is that effluents must not cause acute toxic effects in the mixing zone. This is the basis for the recommendation that the effluent concentration, measured as a 1-hour mean, must not exceed the CMC, which is defined as 0.3 times the lowest LC-50 value (acute toxicity) for at least three test species, and that the concentration, measured as a 4-day (96-hour) mean, must not exceed the CCC, which is defined as the lowest EC-50 value (chronic toxicity) for a similar number of species. These concentrations must not be exceeded more than once every 3 years /4,5/.

EPA also recommends that substances with characteristics which are particularly hazardous for the environment (such as bioaccumulating substances) should be regulated individually /9/.

If the toxicity tests show that the effluent is unacceptably toxic and that the discharge criteria can not be met, the local authorities are entitled to ask for a "Toxicity Reduction Evaluation" (TRE) /4,5/. This exercise identifies the fractions and/or contributors to the wastewater which are responsible for the overall toxicity, and provides a basis for initiatives in order to reduce the wastewater toxicity - for example by substitution of chemicals or by process modifications. To this end US-EPA has developed methods /13,14/ which describe a stepwise investigation programme.

2.1.4 The Netherlands

The Netherlands focuses primarily on evaluating and limiting the environmental effects of the use and discharge of individual substances. One part of the strategy is to reduce the pollution at source, for example by the use of cleaner technology or by the application of best practicable technology /6,7/. The second part of the strategy is to define water quality criteria for a large number of substances. The evaluation system is based on international experience and requires tests using at least one species of algae or aquatic plant, one species of crustacean, and one species of fish. Water quality criteria are defined on the basis of chronic toxicity tests or of field investigations in such a way that 95% of the species in the ecosystem in question are protected /6/. No criteria have been published for complex whole effluents apart from overall parameters such as oxygen demand, nutrients, heavy metals, and organic micropollutants /7/.

2.1.5 Germany

In Germany the regulations applying to wastewater discharges are different depending on whether the discharge goes via public sewers

to a wastewater treatment plant, or directly to a receiving water body.

For discharges to public sewers, the regulations of the individual Länder apply /11/. These require registration of the discharge and monitoring of certain individual substances in the wastewater: heavy metals, chlorinated organic compounds (AOX), chlorinated solvents (1,1,1-trichloroethane, trichloroethylene, tetrachloroethylene, trichloromethane) and reactive chlorine. Discharge limits are set for the individual substances. In some Länder the list is extended to cover additional metals, cyanide, nitrite, sulphide, sulphite, sulphate, hydrocarbons and phenol /11/. Only Baden-Württemberg sets requirements to wastewater with regard to inhibition of activated sludge or other relevant effects on treatment plant operation.

Discharges to surface waters (rivers) are regulated by a federal protocol which lays down minimum requirements with regard to chemical characterisation and prescribes discharge standards /11/. Requirements apply branchwise, which means that the analytical requirements are aimed specifically at the substances that may be expected to be present on the basis of knowledge of the chemical use patterns etc. within the branch in question.

2.1.6 United Kingdom

The United Kingdom has developed a proposal for a strategy for monitoring and control of discharges from point sources (see figure 2.1.1) /9/. It will be seen that the point of departure is the quality of the receiving water. If this is acceptable, no further investigations are made. Only if the water quality is unacceptable and only if the discharge causes acute toxic effects in the recipient is there cause for more detailed investigations or for limitation of the discharge.

2.1.7 Ireland

Discharge permits are based on 96-hour LC50 values in such a way that different types of industry can be allowed to discharge different amounts of toxicity (see table 2.1.1) /9/. The importance of dilution for toxicity is also taken into account, and a dilution factor of 20 is required within the immediate vicinity of the discharge for each toxic unit (TU - see below) discharged.

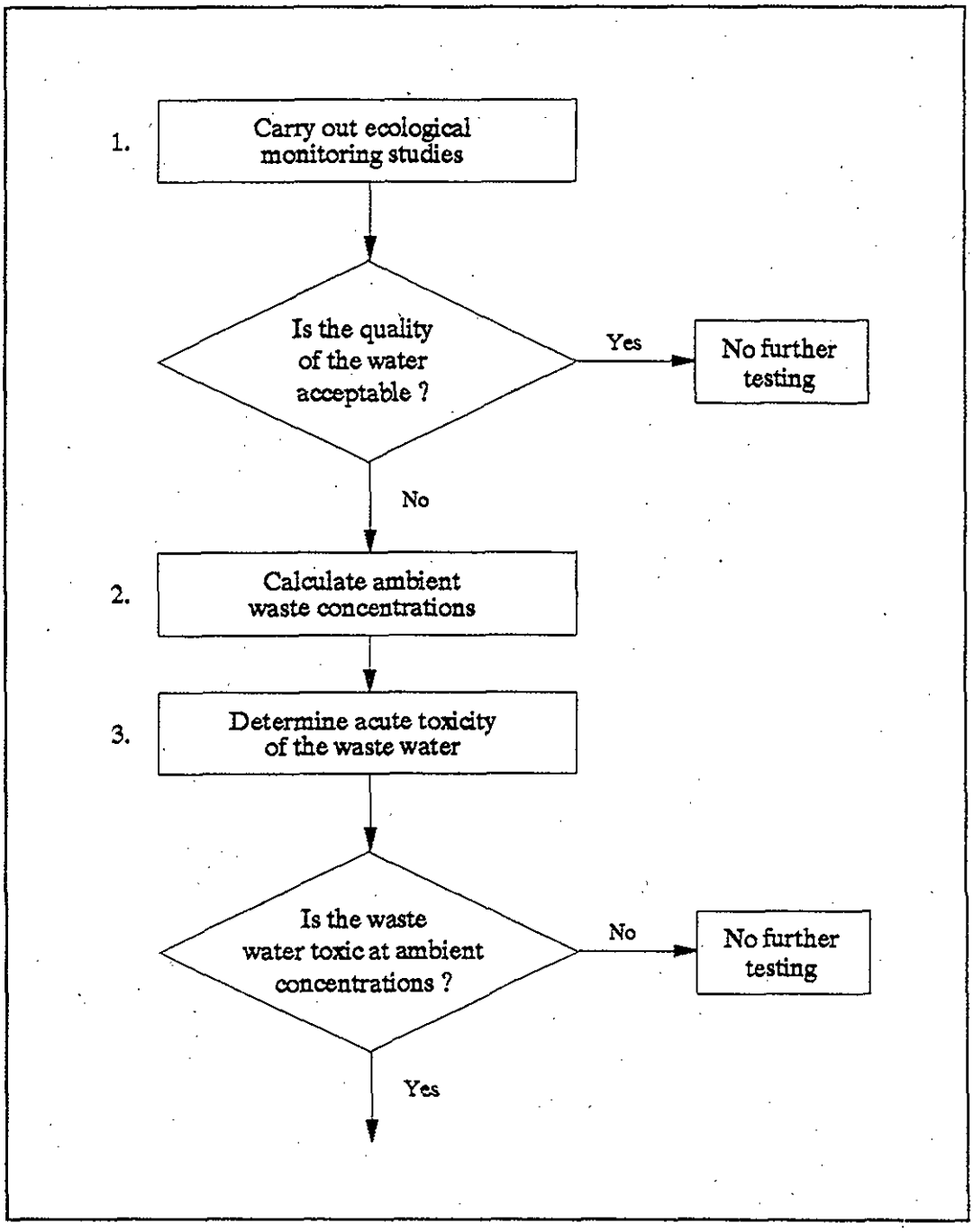

Figure 2.1.1 *A The UK proposal for monitoring and control of discharges from point sources /9/.*

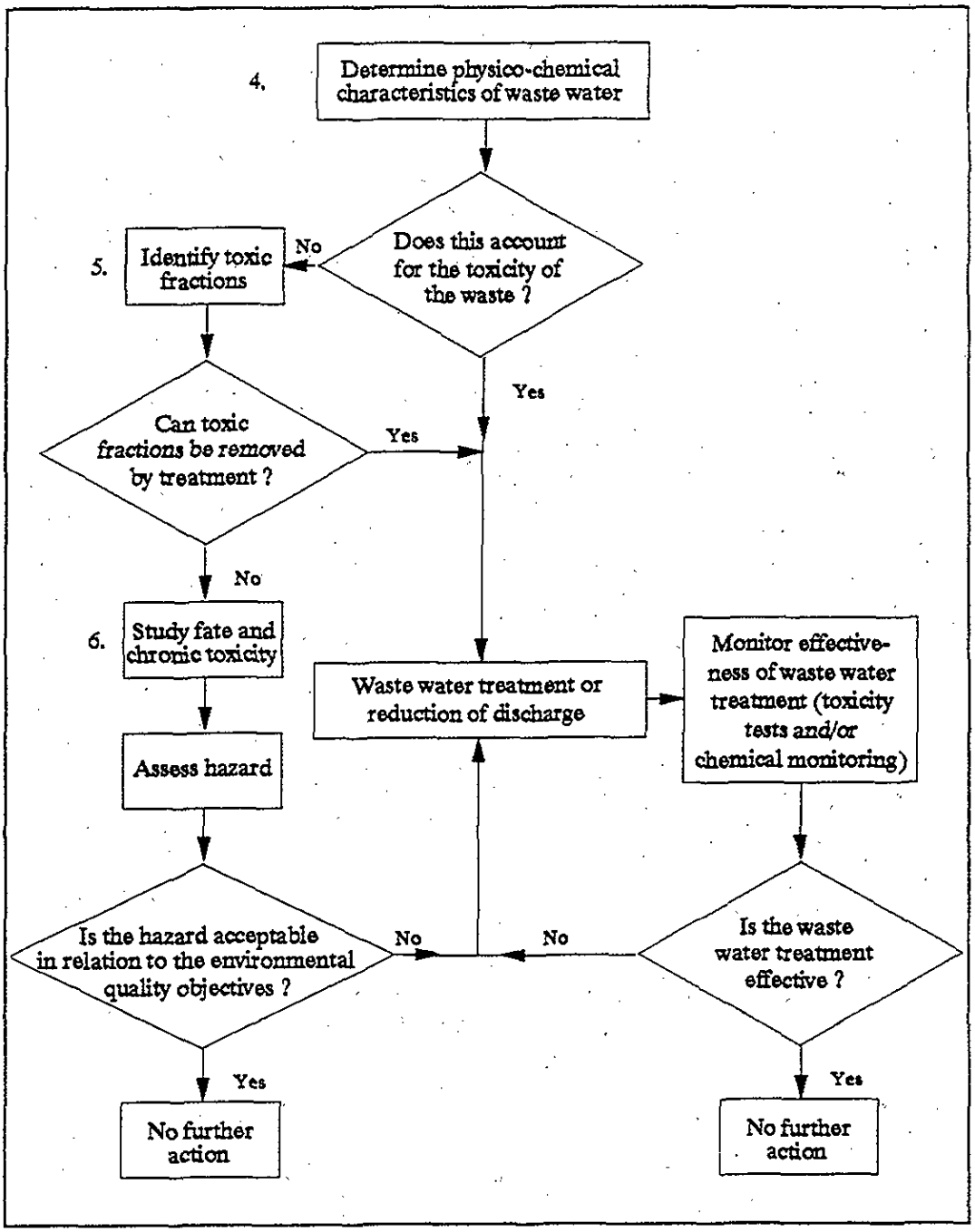

Figure 2.1.1 B The UK proposal for monitoring and control of discharges from point sources /9/.

Table 2.1.1 The Irish guidelines for development of criteria for discharge of wastewater from a point source /9/.1

Priority Group	Category	96 hours LC50	Toxic Units
A	Chemical or pharmaceutical manufacturing	4%	25
B	Mental Extractions plating or finishing	10%	10
C	Textiles, tanning, paper and glass making	20%	5
D	Agricultural and food industries, untreated municipal sewage	70%	1
E	Treated municipal sewage (secondary)	100%	1

2.1.8 Sweden

The Swedish Environmental Protection Agency has developed a strategy for "biological-chemical characterization of industrial wastewater" /10/. The strategy consists of a basic stage followed by three successive stages of investigations (see figure 2.1.2). At each stage, methods are laid down for chemical characterization of the wastewater and for evaluation of degradability, bioaccumulation and toxicity respectively. The recommended methods for each of the three stages are shown in tables 2.1.2-2.1.4.

The Swedish proposal does not contain decision criteria as such, but it does contain a number of criteria for assessment of the general environmental hazard. Thus it is recommended that discharges of persistent or poorly degradable substances should be strictly limited. Substances which in addition to being persistent are also bioaccumulative may give rise to serious environmental impacts and must therefore be minimized in the wastewater. The criterion for bioaccumulative substances is given as BCF > 1000. When evaluating toxicity, wastewater is classified as acutely toxic if the concentration after initial dilution exceeds 0.1 • EC50; if this is the case, action should be considered to reduce the discharge. If significant dispersion occurs after initial mixing, but the concentration still remains higher than 0.01 • EC50, the discharge is considered to be potentially sublethally toxic, and this would normally require additional tests after stage 2.

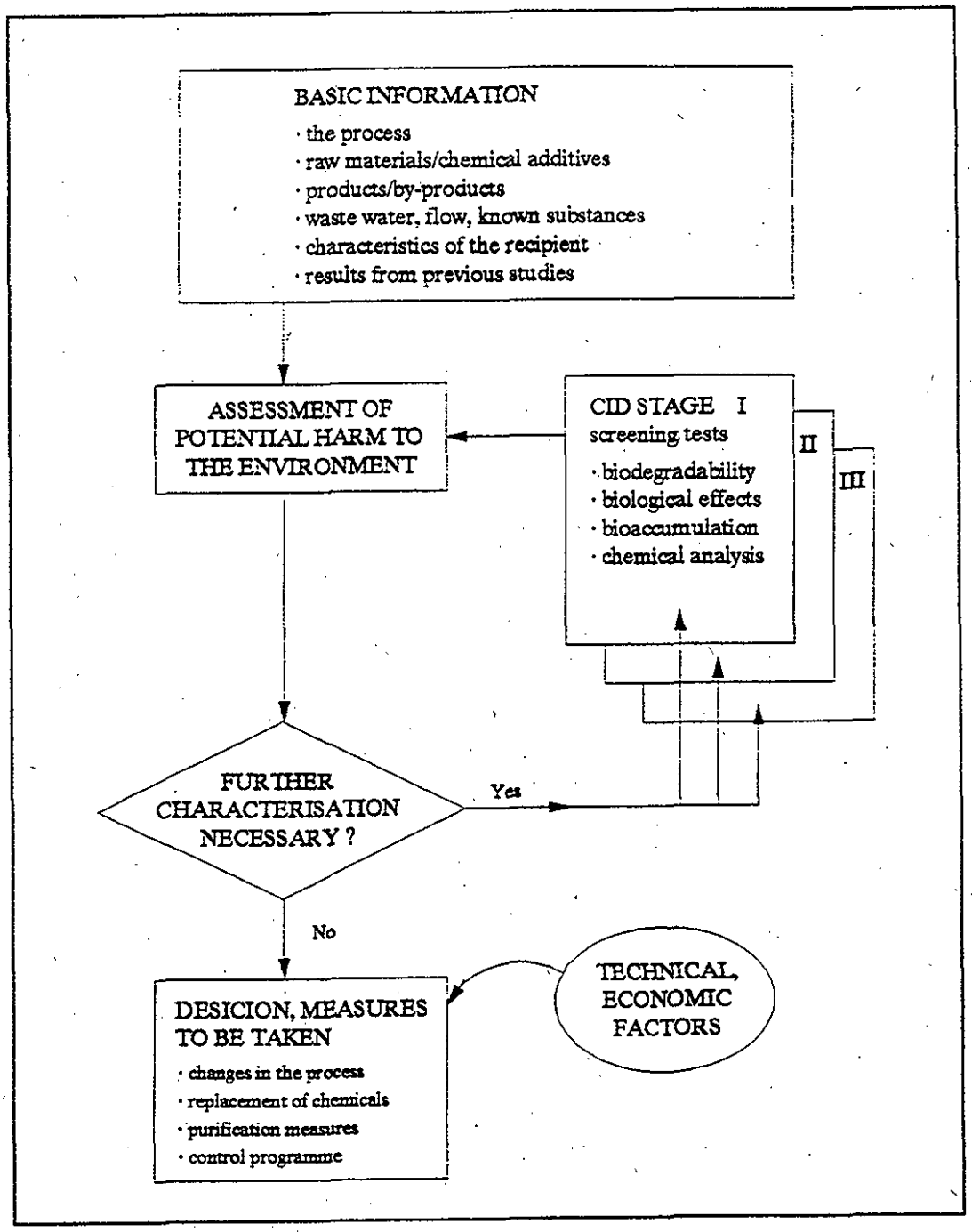

Figure 2.1.2 *Swedish proposal for investigation strategy for industrial wastewater /10/.*

Table 2.1.2 Swedish proposal for stage I tests.

TESTS	ALTERNATIVES
CHEMICAL CHARACTERIZATION	
Chemical oxygen demand COD Biological oxygen demand BOD_7 Total organic carbon TOC pH Suspended solids Conductivity Nitrogen, Phosphorus	Dissolved organic carbon DOC
	Any known or suspected substances which are throught to be toxic, persistent, or bio-accumulative
	Hydrocarbons, TOC1, EOC1, AOX
BIOACCUMULATION	
	Presence of lipophilic substances: reversed phase TLC
BIOLOGICAL CHARACTERIZATION	
Fish: 96 h. LC50	Zebra fish *(Brachydanio rerio)* Rainbow trout *(Salmo gairdneri)* Bleak *(Alburnus alburnus)* Fathead minnow *(Pimephales promelas)* Stickleback *Gasterosteus aculeatus)* Dab *(Platichthys flesus)* Cod *(Gadus morhua)*
Crustacea: 48/96 h. LC50	*Daphnia sp.* *Ceriodaphnia dubia* *Nitocra spinipes* *Cragon crangon* *Acartia tonsa*
Algae: 3 d. EC50	*Selenastrum capricornutum* *Monoraphidium griphitti* *Chlorella vulgaris* *Scenedesmus subspicatus* *Skeletonema costatum*
Higher plants: 5 d. EC50	Duckweed *(Lemna minor)* Onion *(Allium cepa)* Lentil *(Lens culinaris)*
Micro organisms:	Activated sludge Pre-screening test: Microtox

Table 2.1.3 Swedish proposal for stage II tests.

CHEMICAL CHARACTERIZATION		
Results from Stage I are followed up by wastewater analysis using more advanced techniques, for example: - capillary gas chromatography/mass spectrometry (GCMS) - "high performance" liquid chromatography (HPLC) with specific detectors (fluorescence or electrochemical) - additional specific analyses		
DEGRADABILITY		
Biological degradation followed by means of TOC or DOC analysis (Die-away) or by respirometer; only methods for readily degradable or inherently degradable organic compounds to be used. Aerobic stabilization of samples with subsequent determination of toxicity and bioaccumulation, and chemical analysis.		
BIOACCUMULATION		
TLC-analysis of lipophilic substances in stabilized samples.		
TOXICITY		
The programme builds on the results of stage I. As a rule, one or two tests are chosen from the list in an attempt to characterize the toxicity more precisely than in stage I.		
Fish:	Survival:	Egg/larvae test, zebra fish, long term exposure (14 d) using a species from stage I
	Growth:	Fry test, fathead minnow
	Physiology:	Physiological changes (Salmon/rainbow trout/ brown trout/perch)
Crustacea:	Reproduction:	*Daphnia* *Ceriodaphnia* *Nitocra*
Bivalves:	Survival:	Mussel larvae
Algae:		Algae test battery
Genotoxicity:	Ames test carried out using two Salmonella strains with/ without metabolizing system, rat liver microsomal fraction	

Table 2.1.4 Swedish proposal for stage III tests.

Stage III characterization is planned with even greater flexibility than in the preceding stages. The tests take the form of extended or more specifically aimed laboratory experiments, experiments in more realistic systems - model ecosystems or cage experiments - or, when this is found to give the best information, through investigation of organisms collected in the field from the area affected by the wastewater in question.		
CHEMICAL CHARACTERIZATION		
Additional specific analyses with advanced techniques.		
BIOACCUMULATION		
Analyses of organisms exposed to the wastewater in the laboratory, in cages in the recipient or free-living.		
	- *fish:*	*rainbow trout, perch*
	- bivalves:	common mussel, freshwater mussel
	- snails:	*Lymnea*
DEGRADABILITY		
Tests with microorganisms isolated from the receiving environment (simulation tests): specific chemical analysis of known components may be necessary.		
TOXICITY - LABORATORY EXPERIMENTS		
	- zebra fish, delayed effects - herring egg/larvae - physiological effects - polluted sediment: Tubifex, Daphnia	
TOXICITY - CAGE EXPERIMENTS		
Physiological alterations in fish: rainbow trout, perch Tainting effects in fish: rainbow trout, perch		
TOXICITY - CAPTURED FISH		
Physiological alterations: perch, flounder Skeletal alterations in garfish Morphological alterations in fish: rainbow trout, garfish		
TOXICITY - POPULATION LEVEL		
Increased tolerance in periphyte community		
MODEL ECOSYSTEM TESTS/MULTISPECIES TESTS		
Study of degradation/transformation, distribution and effects on:		
	- littoral community - soft bottom community	

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2.2 Experience with industrial wastewater examination in Denmark

The reason for summarizing Danish experience with industrial wastewater examinations is to give a baseline which can be compared with the recommendations made in this report. It is therefore important to indicate the strategy underlying the examinations carried out so far.

The strategies in the material at hand will be outlined in accordance with the structure shown in table 2.2.1.

2.2.1 The legal basis

Effluent discharge permits are issued by the authorities on the basis of the Law on the Protection of the Environment /2/. At present (1991) this law is being revised. In § 17 of the law it is stated as a general proposition that "substances which can pollute the aquatic environment may not be discharged to rivers, lakes or the sea...". Regional councils may, however, issue permits for effluent discharges to rivers, lakes and the sea (§ 18).

As a supplement to the Law on the Protection of the Environment, a set of Administrative Guidelines on Receiving Water Quality Planning have been issued /1/. The Guidelines describe a planning system which weighs up the conflicting interests and defines areas with lower water quality standards (such as effluent mixing zones) and areas with higher water quality standards. All other areas are then covered by the general water quality standard, which calls for unaffected or only slightly affected animal and plant life.

Within the zone around an effluent discharge an environmental impact is accepted, although certain minimum standards must be maintained: for example no acute toxic effects after initial mixing, and no tainting of fish. An effluent mixing zone is delimited by a "conflict boundary" bordering the areas with general water quality standards. This boundary is laid along the points where no biological effects can be observed and/or no significant changes in concentrations of natural substances can be detected.

Table 2.2.1 Main elements in the examination of industrial wastewater.

Basic information available when designing examination strategy

- chemical consumption, products, wastewater composition and properties, nature of recipient, details of processes and treatment systems

Assessment of total wastewater

- Overall strategy and stepwise toxicity testing
- Criteria for establishing no-effect-concentration and recipient concentration

Assessment of individual substances

- Chemical identification
- Ecotoxicological investigation of individual substances
- Criteria for establishing no-effect-concentration and recipient concentration
- Toxicity balance

For toxic but not xenobiotic substances (such as heavy metals, for example), discharge criteria are set so that at the conflict boundary no concentrations occur which deviate significantly from background levels. For xenobiotic substances, criteria are set taking into account the harmful properties of the substance in question, as expressed by its persistence, toxicity, and bioaccumulative tendency.

2.2.2 Experience

Danish experience with the use of ecotoxicological studies of industrial wastewater has mainly been gathered during the last 10-15 years.

The first major investigation was carried out in Køge Bay in 1977-78 by the Metropolitan Regional Council. It included ecotoxicological investigations of wastewater from three industrial enterprises, and the report was published in 1979. Since then most wastewater discharges from major Danish industrial enterprises have been investigated to a greater or lesser extent.

For most enterprises, only one or a small number of samples have been examined. Thus usually only a so-called *ecotoxicological characterization* has been carried out with any degree of thoroughness, i.e. measuring the toxicity towards a varying number of species from the ecosystem. Other enterprises are also subject to *routine ecotoxicological compliance monitoring* ranging from daily sampling, through fortnightly or monthly sampling, up to semi-annual or annual testing. Routine testing involves only a limited number of species (from one to four).

A survey of 57 of the largest Danish enterprises carried out late in 1989 /3/ gives a fairly comprehensive picture of the ecotoxicological investigations of industrial wastewater that have been carried out up to now. The 57 enterprises were distributed as shown in table 2.2.2.

Table 2.2.2 Number of Danish enterprises included in the survey of existing knowledge about industrial discharges /3/.

ISIC registration code of enterprises	Sector	Number of enterprises covered
35.119	Chemicals manufacturing	5
35.220	Pharmaceutical industry	6
34.110	Cellulose and paper ind.	6
35.300 etc.	Petrochemical industry	6
32.117	Textile dyeing	6
38.195 etc.	Iron and metals industry (inc. galvano)	16
35.12	Fertilizer industry	2
32.310	Tanning industry	3
	Other industry	7
	Total	57

The 57 enterprises were chosen on the basis of the environmental characteristics of their wastewater: toxic discharges and discharges containing heavy metals and xenobiotic substances were chosen for preference. The choice was made in consultation with the Danish Environmental Protection Agency, regional and local environmental administrations, and a number of other authorities, and all significant direct discharges of industrial wastewater in Denmark were covered.

The survey showed that 23 different industrial wastewater discharges have been investigated ecotoxicologically during the last 10 years or so, and it is thought that the investigations of these 23 discharges represent the major part of Danish experience in this area.

The scope of the investigations, and the strategies adopted for them and for any subsequent decisions on discharge requirements, differed from enterprise to enterprise. The investigations have been

initiated at different times and cover different types of discharge. In addition, the different economic and political situations relating to each discharge have resulted in differences in the investigations.

The following sections describe this material taken as a whole, using the structure shown in table 2.2.1 as a point of departure.

2.2.3 Baseline information

For many enterprises, information about the composition and characteristics of the wastewater has grown gradually. For most of the enterprises, however, a large number of the investigations have been initiated in connection with an application for approval of a discharge, and the existing information at this point can therefore be referred to as the "baseline information". By baseline information is thus meant the total mass of knowledge that is available before planning and starting additional investigations. The following section describes the general features and extent of this baseline information about Danish enterprises.

All enterprises had a knowledge of their *chemical consumption and products*, and with one or two exceptions this was available as part of the baseline information.

Initially, all enterprises had poor knowledge of their *effluent composition* including the content of individual substances. The subsequent and more thorough chemical characterizations showed that only rarely was more than a quarter of the chemical substance content known at the time of submission of the baseline information.

Similarly, the *effluent characteristics* were poorly described, and at "baseline time" most enterprises had not made any investigations into this; a few did have the results of routine toxicity tests based on a single species, and one or two had results based on several species.

The *receiving water characteristics* at the effluent discharge point were generally unknown or were described solely on the basis of a single initial benthos survey or the like. At one enterprise, routine saprobiont studies had been made and at another a very extensive recipient quality investigation had been made at an early stage.

From the outset the enterprises had knowledge about biological and physico-chemical *treatment methods*, but process optimization, substitution of process chemicals, and other approaches based on cleaner technology have only been considered - and in some cases adopted - at a later stage.

2.2.4 Evaluation of whole effluent

A total evaluation of whole effluent has formed part of the strategy at 23 of the major wastewater dischargers in Denmark. Investigations of whole effluent usually focus on toxicity. So far, there have not been many investigations of total persistence or total bioaccumulative tendency of whole effluents. Evaluation of these characteristics has been restricted to known individual substances in the discharges.

The part of the total investigation strategy dealing with whole effluent therefore deals only with toxicity.

Strategy for evaluation of whole effluent

The strategy that has been employed in most investigations has been to test the toxicity of a limited number of effluent samples on a number of species from different groups of organisms, and on this basis to take a decision about measures necessary to reduce pollution. No common approach can be discerned, and no explanations are given for the number of species included in the test programmes. Generally, 3-5 species are used, usually representing 3 groups of organisms (usually algae, crustacea and fish).

For a great number of the enterprises, an important element in the strategy has been to measure toxicity both before and after treatment of the wastewater: this might be treatment in a pilot plant or full-scale treatment.

Table 2.2.3 gives a total overview of the ecotoxicological tests used for examination of industrial wastewater in Denmark since 1978.

Table 2.2.3 Survey of ecotoxicological investigation programmes for industrial wastewater from 1978 onwards /3/.
A : acute toxicity test. Cr : Chronic toxicity test.
Ch: characterization (a few wastewater samples tested). Ru : Routine monitoring.

ECOTOXICOLOGICAL INVESTIGATION PROGRAMME - TEST ORGANISMS USED

ORGANISMS: / ENTERPRISE:	ALGAE: "Natural plankton"	ALGAE: Skeletonema costatum	ALGAE: Phaeodactylum tricorn.	ALGAE: Pavlova lutheri	ALGAE: Nitzschia palea	ALGAE: Katodinium rotundum	ALGAE: Ditylum brightwellii	ALGAE: Rhodomona baltica	PROTOZOA: Uronema marinum	PROTOZOA: Oxyrrhis marina	CRUSTACEA: Gammarus pulex	CRUSTACEA: Nitocra spinipes	CRUSTACEA: Acartia tonsa	CRUSTACEA: Daphnia magna	CRUSTACEA: Crangon crangon	CRUSTACEA: Mysidopsis bahia	CRUSTACEA: Pranus flexuosus	FISH: Guppy	FISH: Flounder	FISH: Zebrafish	FISH: Turbot	FISH: Plaice	FISH: Eel	FISH: Herring	FISH: Rainbow trout	FISH: Goby	BIVALVES: Blue mussel	BIVALVES: "Bottom fauna"	BACTERIA: Microtox	BACTERIA: Inhibition of sludge	BACTERIA: Inhibition of nitrific.	PLANTS: Eel grass	PLANTS: Cress	PLANTS: Allium	TYPE OF STUDY
Prom Kemi																				A							ACr								Ch
KVK Direct	A	Cr	ACr									ACr	Cr		A						Cr					A									Ch
KVK Hunicipal	A	Cr	ACr									ACr	Cr						A	A		ACr								Cr			Cr	Cr	Cr	Ch
Grindsted Products					Cr						A	A		A											ACr				Cr						Ch+Ru
Cheminova	A	ACr		Cr		Cr	Cr		Cr	Cr		ACr	ACr		ACr	ACr	A		A		ACr		ACr	ACr	ACr										Ch+Ru
H.Lundbeck		A	ACr						Cr			ACr												ACr					Cr						Ch+Ru
Novo-Nordisk	A		A									ACr																							Ru
Ferrosan	A	A	A										A		A				A	A						A									Ch
Junckers	A	Cr	ACr	Cr				Cr				ACr			A							ACr	Cr			A			Cr			Cr			Ch
Fredericia Cellulose	A		Cr									ACr											A				A		Cr						Ch+Ru
Maglemølle Papir					ACr																				A				Cr						Ch
Skjern Papirfabrik					ACr									A											A				Cr						Ch
Dansk Shell	A		Cr									ACr																							Ru
Statoil		A							Cr				ACr																Cr						Ch
Doras	A		Cr									ACr																							Ru
Danfoss	A	Cr										ACr	A																						Ch
NKT			Cr									A											Cr				ACr		Cr						Ch
Fjelstervang					ACr									A											A				Cr	Cr					Ch
Martensen					ACr									A											A				Cr	Cr					Ch
L.P.Hansen																													Cr	Cr					Ch
Kaj Neckelmann																														Cr	Cr				Ch
Kemira Danmark	A	A	ACr									ACr	ACr																						Ch+Ru
Faxe Kalk	A		Cr										ACr										Cr												Ch

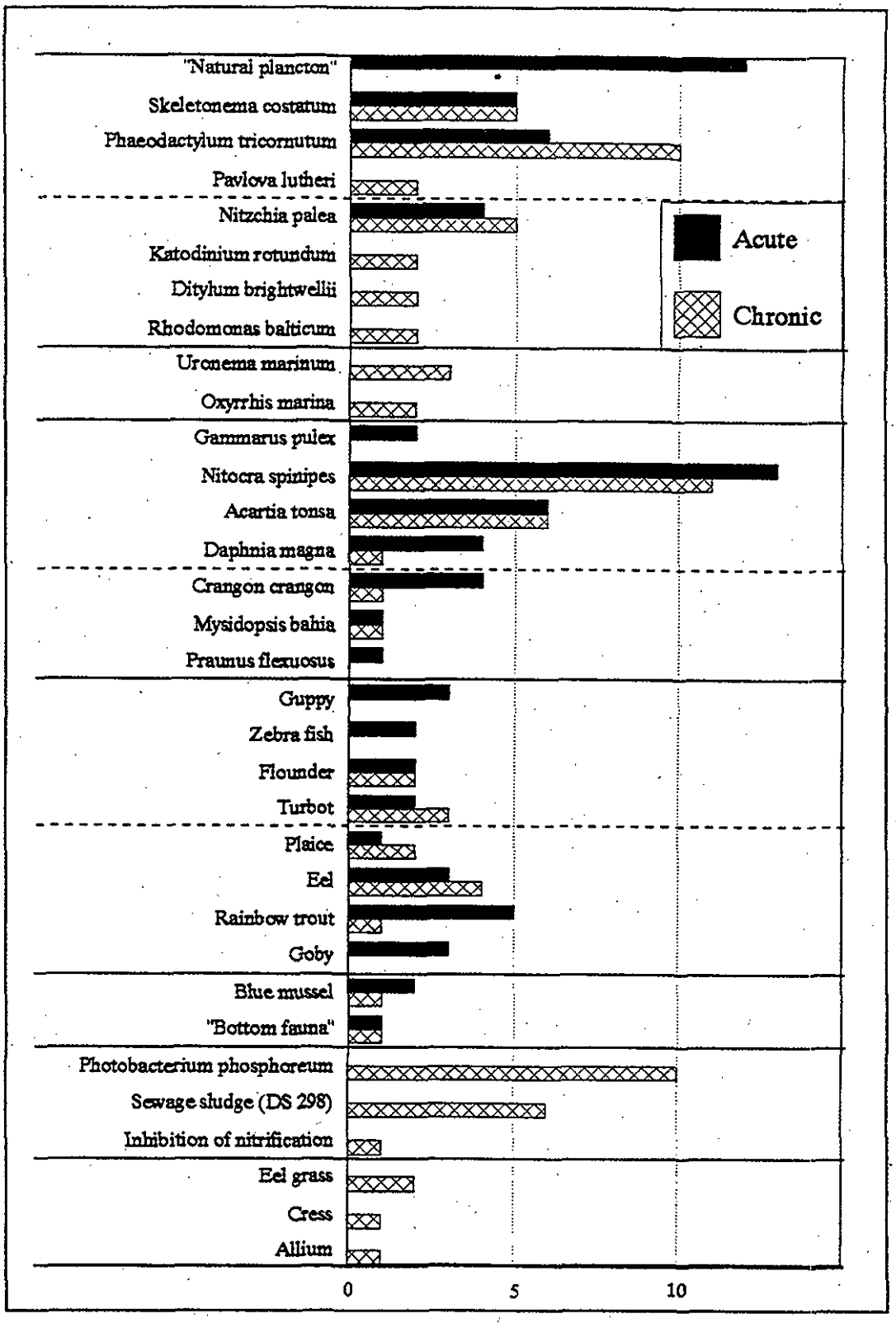

Figure 2.2.1 Frequency of use of various test species in test programmes for total wastewater from 23 enterprises /3/.

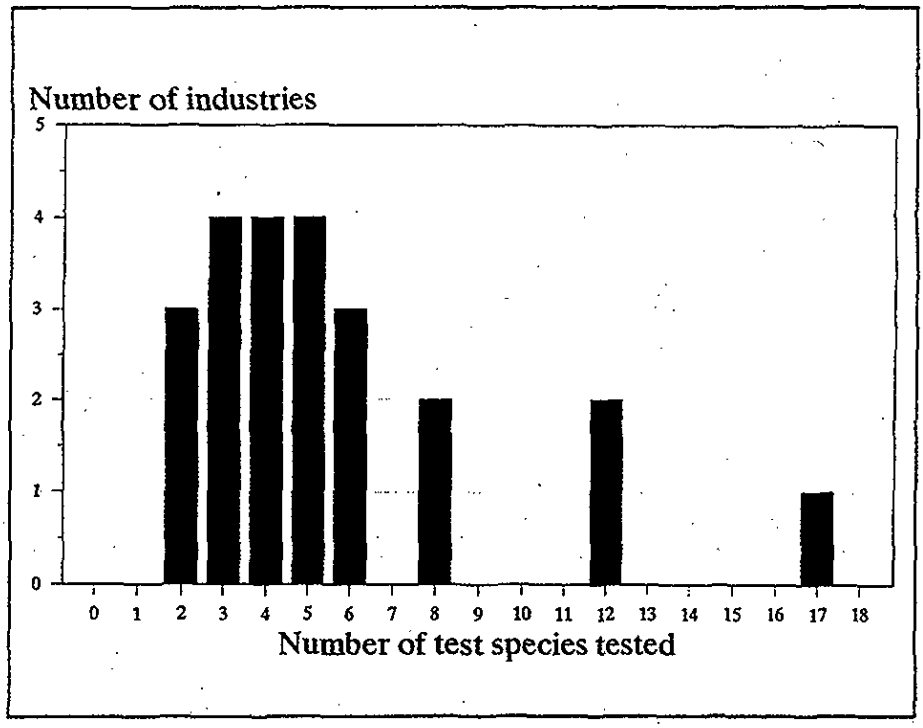

Figure 2.2.2 Distribution of test programme size, expressed as number of different species used /3/.

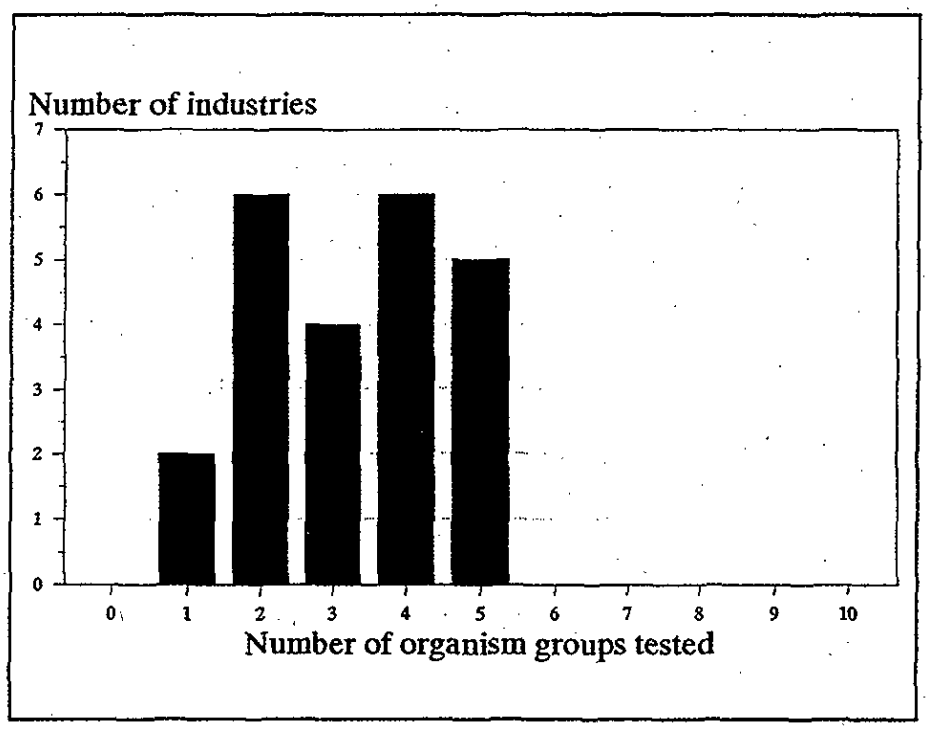

Figure 2.2.3 Distribution of test programme size, expressed as number of organism groups in the test programme /3/.

A survey of the extent of the test programmes can also be made by the use of frequency diagrams as shown in figures 2.2.1-2.2.3.

Figure 2.2.1 shows how many enterprises have used a certain species in their test programme. Figures 2.2.2 and 2.2.3 show how often a certain number of species or a certain number of organism groups have been included in the test programme of an enterprise.

As seen from these figures, a total of 33 different species have been used during the last 10 years or so in Danish ecotoxicological test programmes. This number includes tests on "natural plankton", "benthic fauna", "sludge" and "nitrification inhibitors", counting each as one species. It will be seen that *Nitocra spinipes* is the most frequently used test organism, and that a total of 13 enterprises have used it.

The test programmes have used differing numbers of species, 3 - 5 being the most usual, up to a maximum of 17 species used at one enterprise.

The species come from various organism groups: plants, algae, protozoa, crustacea, fish, "benthic organisms", and bacteria. The number of groups represented in the test programmes is evenly distributed between 1 and 5 (on average 3 organism groups).

Criteria for evaluation of potential effects

For most of the enterprises it was found that the comparison between the ecotoxicological test results and the calculated wastewater concentrations in the recipient was simply carried out by direct comparison of the lowest observed effect concentration (LOEC) and the calculated receiving water concentrations (Predicted Environmental Concentration, PEC). Conclusions about any expected toxicity problems were drawn on this basis without specifically addressing the question of how large the margin should be between LOEC and PEC for the discharge to be acceptable.

For the great majority of cases, the concentrations in the receiving water (PEC) were evaluated. In some cases, current and flow measurements were made; in other cases the evaluation was based on wind measurements and an assumed wind/current correlation. The calculations were often sufficiently comprehensive to allow the calculation of fractile diagrams showing wastewater concentrations at various distances from the discharge point. For most of the enterprises, the reference material shows that the evaluation of the toxicity effects in the receiving water was based on the highest fractiles of the wastewater concentration. For example, "median minimum water flow", the "80% fractile" or the "90% fractile" of the concentration at a certain distance from the discharge point have all been used.

An example of the overall picture of the calculated wastewater concentrations in the receiving water is given in figure 2.2.4.

It will be seen from the study of the reference material for individual enterprises that the basic procedure underlying all evaluations of the potential toxic effects in the receiving water is a comparison of PEC values with the ecotoxicological data (cf. figure 2.2.4). But there are differences in the precise criteria adopted for relating the lowest effect concentration to the PEC and for deciding on the required margin between the cumulative PEC curve on the left of figure 2.2.4 and the curve representing the ecotoxicological test

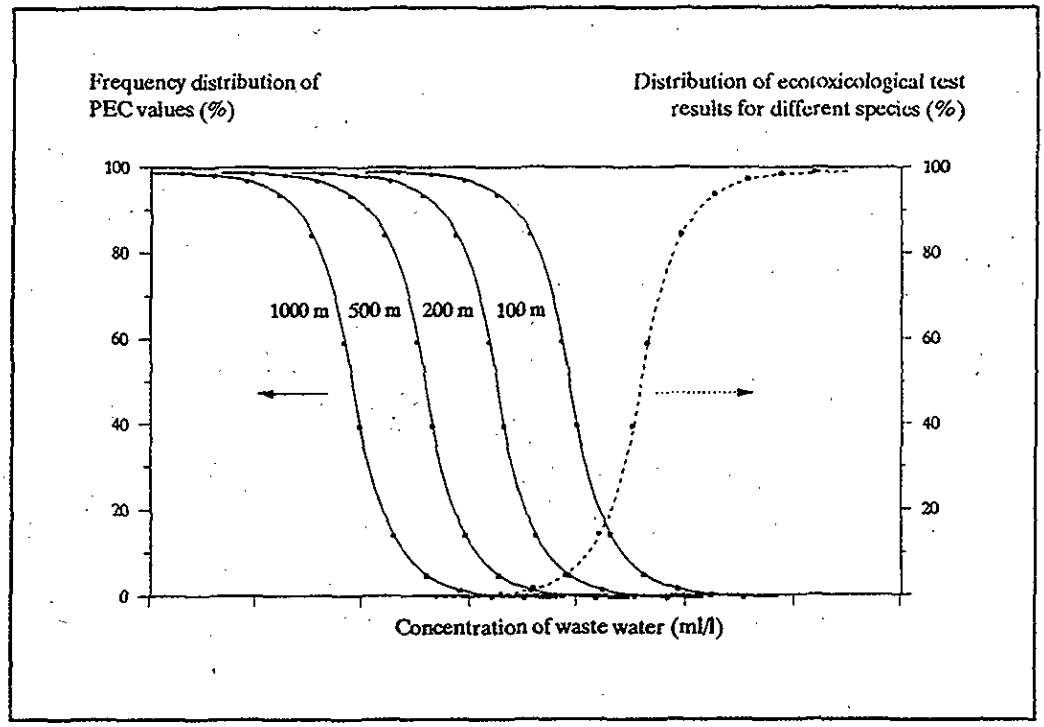

Figure 2.2.4 *Principle for comparison of ecotoxicological data with PEC values at varying distances from the discharge point.*

results on the right.

Stated in other words, there are differences in the requirements as to the value (which fractile) of the PEC that should be used, and as to which uncertainty factor should be applied when using the LOEC to derive a cutoff value for the effect concentration. This is especially relevant when estimating whether the relationship between these two is greater or less than 1.

The technical reports forming the background material for these evaluations are usually restricted to a presentation of the data, and only in a very few cases do we have detailed documentation of the evaluation carried out by the authorities before reaching their decision.

In general, the ecotoxicological test programmes contained too few investigations to permit a well-documented distribution curve to be constructed. This was in fact only done in one single case.

Furthermore, it would be theoretically correct to divide the data into distribution functions for chronic non-lethal effects (EC-values) and acute lethal effects (LC-values). This would require even more data before a meaningful picture could be obtained, and has not been done in any Danish investigations carried out so far.

Finally, it is clear that wastewater toxicity varies, so that the distribution function in fact only describes the toxicity of a single sample, and therefore the stippled curve on the right in figure 2.2.4 is therefore only one of a family of curves, where each curve in the family represents a single effluent sample. These problems are discussed in more detail in section 3.1 on effluent variability.

In the report on the strategies adopted up to now for using ecotoxicological data, it was commented that different criteria have been used for deciding how great a margin there should be between the PEC curves and the distribution function for the ecotoxicological data, or in other words, what size of safety factor should be employed when setting the lower limit for the effect concentration and the comparing this with the receiving water concentration. These criteria can be summarized as follows:

1. Quality and relevance of the data. Here attention should be paid to the number of species tested, the relevance of the tests, and any other problems affecting an extrapolation of the laboratory test data to the receiving water situation.

2. Uncertainty in the determination of the PEC.

3. Variation in toxicity of the effluent. Here attention should be paid to the fact that the effluent toxicity varies, and that this variation is usually poorly documented.

4. Duration of the effect period in the receiving water. Here attention should be paid to the fact that organisms in laboratory tests may be exposed over longer periods (applies especially to free-swimming organisms) or shorter periods (applies especially to benthic organisms) than is the case in the receiving water.

The following 7 descriptions of evaluations of industrial wastewater give different examples of the criteria that have been adopted.

Example 1

Only one enterprise has built up and analyzed a data set as shown in figure 2.2.4. As criterion this enterprise has required that the ratio between the LOEC and the 90% fractile of the PEC at a distance of 300 m from the discharge point must be greater than 10. The choice of a fractile for the PEC takes the exposure time into consideration, and the chosen uncertainty factor takes into account all the species that have been investigated. This factor also allows a margin for the uncertainty of the PEC calculation and for the variation in toxicity of the effluent.

Example 2

Here the median minimum flow in the recipient (a small river) has been used to calculate the PEC at various distances from the outfall. No attempt has been made to establish what fractile of the PEC this method arrives at. When comparing the derived PEC values with the ecotoxicological data an application factor of 2 is used relative to the LOEC value, and on this basis it is concluded that the affected zone extends a long way. 6 species from 4 organism groups have been used in the ecotoxicological monitoring programme on which the LOEC values are based.

No definite decision was taken as to which fractile of the PEC should be compared with the test data, and which value of uncer-

tainty factor should be applied to the LOEC to arrive at a value for the lowest effect concentration using the relevant data.

The variation in the wastewater toxicity is included in the data (routine ecotoxicological monitoring). The uncertainty of the PEC calculation and the effect of exposure time are not included.

Example 3

The median minimum water flow has been used, and the PEC calculated on this basis has been compared directly with the exotoxicological data. Data for 5 species from a total of 4 organism groups have been used. On this basis, the affected area has been found to be very wide. No conclusions have been reached as to the uncertainty factor that ought to be used for establishing the lowest effect concentration. In contrast to example 2, no account has been taken of the variation in effluent toxicity, since in all only 2 effluent samples have been tested.

Example 4

The initial dilution (fractiles of this were not employed) and the 80% fractile of the PEC at a distance of 100 m from the outfall were compared directly with ecotoxicological data. This included data from 4 species from 2 organism groups. By this method the affected area was found to be small, and no decisions were taken about the uncertainty factor that ought to be used for establishing a lowest effect concentration.

Example 5

The PEC was calculated from hydraulic dispersion calculations, but in contrast to the ecotoxicological data, the PEC values are given in absolute figures and not fractiles.

The ecotoxicological test programme consisted of 2 phases with acute tests on 5 species in the first phase and 8 species, mainly in chronic tests, in the second phase.

No values were established for the lowest effect concentration; the test data were used directly without applying an uncertainty factor. On this basis, effects at considerable distances from the outfall were predicted, and it was commented that other species might show greater sensitivity than those used in the test programme, so that the affected area for these species would be even larger.

Example 6

The 80% fractile of the PEC at a distance of 100 m from the outfall was compared directly with ecotoxicological data and the effect was judged to be very small. The ecotoxicological test programme was carried out in several stages and a total of 6 species from 5 organism groups were tested.

Example 7

PEC values were calculated in considerable detail, but no value was established for the lowest effect concentration. Nor has any comparison between LEC and PEC been found in the material. 3 species from 2 organism groups were tested.

2.2.5 Evaluation of individual substances

Identification of xenobiotic substances in wastewater is usually only required at the largest industrial enterprises, and even here it is usual-

ly only the enterprises with separate discharges. However, at quite a few smaller enterprises heavy metal analysis is required. The work of identifying individual substances has been carried out continuously, and one important objective in many cases has been to establish quantitative figures for the contents in and toxicity of the wastewater.

Chemical identification

About 10 of the previously mentioned 57 Danish enterprises have carried out a chemical characterization of organic compounds by means of GC-MS analysis or similar techniques which can identify chemical constituents of wastewater. Furthermore, about 10 enterprises make routine analyses for a limited number of organic substances in their effluent.

Most of the enterprises that were surveyed carry out checks for heavy metals in their effluent.

In 6-7 enterprises an attempt had been made to establish a chemical mass balance for substances in the effluent, which included comparisons of COD, TOC, total nitrogen or total phosphorus measurements with the corresponding figures for the known concentrations of substances in the effluent. In some of these enterprises the mass balance is carried out at routine intervals as a check. The other enterprises carried it out as a one-off exercise in connection with a chemical characterization of the wastewater. At 3 enterprises the mass balance showed that almost all of the quantitatively significant substances in the discharge were found again in the chemical characterization or in the routine check (70-100% recovery). At the other enterprises this was far from being the case (<20% recovery).

It should be pointed out that a high recovery percentage in the chemical mass balances does not in itself guarantee that all the environmentally hazardous substances have been identified, since many of them are often found in very low concentrations which do not show up through the uncertainty of the mass balance figures.

Ecotoxicological investigations

14 of the surveyed enterprises had carried out ecotoxicological evaluations of individual substances. Usually these were literature studies, but a few (4-5) enterprises had performed toxicity, degradation, or bioaccumulation tests on individual substances as part of the evaluation of the wastewater discharge.

Criteria for evaluation of potential effects

In general, many discharge criteria have been set for heavy metals, but in only a few cases has the toxicity of the heavy metals been compared with the expected concentrations in the receiving water. The question of the lowest effect concentration is not addressed. No criteria have been found for determining the lowest effect concentration, the PEC, or for comparing the two. Generally, the criteria for heavy metal discharges have been those in the DEPA Recommendation /1/ and other Administrative Orders.

For 7-8 enterprises, criteria have been set for discharges of organic compounds. In most of these cases, the criteria for the permitted discharge values are not apparent and no account has been taken of any lowest effect concentration. In one case, the lowest effect concentration is defined as the LOEC-values for a number of substances in the wastewater for which a large amount of data was available.

For oil refineries, criteria for oil and phenols have been set partly on the basis of international conventions, partly on the basis of what is technically achievable.

Toxicity balances

In the case of 7 enterprises, the contribution made by individual substances to the combined toxicity was evaluated quantitatively, making the assumption that the combined toxicity can be calculated additively from the individual contributions. For 2 or 3 of these enterprises, the combined wastewater toxicity can be explained on this basis. At two enterprises only half of the toxicity can be accounted for, and for the final two, only an insignificant part can be ascribed to the known substances in the discharge.

For none of the other enterprises where the toxicity of the whole effluent was examined was there any knowledge about what substances in the discharge were responsible for its toxicity.

Table 2.2.4 gives an overview of the examinations carried out for individual substances in the 23 enterprises where the toxicity of the whole effluent has been examined. The enterprises are listed in the same order as in table 2.2.3.

Table 2.2.4 *Extent of investigation programmes for individual substances in wastewater /3/. X: Fully included; (X): Partly included; - : Not included.*

	CHEMISTRY			ECOTOXICOLOGY				
ENTERPRISE:	Characterization	Routine control	Mass balance	Toxicity examination	Persistency examination	Bioaccumulativeness	List substances	Tox-balance
Prom Kemi	X	X	X	X	X	X	-	-
KVK Direct	(X)	(X)	(X)	-	(X)	X	-	X
KVK Municipal	X	X	(X)	X	X	X	X	-
Grindsted Products	X	X	X	X	-	X	-	X
Cheminova	X	X	X	X	X	X	-	X
H.Lundbeck	X	X	X	X	X	X	X	(X)
Novo-Nordisk	-	-	-	X	X	(X)	-	(X)
Ferrosan	-	-	-	-	-	-	-	-
Junckers	-	-	-	X	X	-	-	-
Fredericia Cellulose	X	X	-	-	-	-	-	-
Maglemølle Papirfabrik								
Skjern Papirfabrik	(X)	(X)	-	X	X	X	-	-
Dansk Shell	(X)	X	(X)	-	-	-	-	-
Statoil	-	X	-	-	-	-	-	-
DORAS	(X)	-	-	X	X	X	-	-
Danfoss	X	X	-	X	X	X	-	X
NKT	X	X	-	X	-	-	-	X
Fjelstervang	-	(X)	-	X	X	X	-	-
Martensen	-	(X)	-	X	X	X	-	-
L.P.Hansen	-	(X)	-	-	-	-	-	-
Kaj Neckelmann	-	(X)	-	-	-	-	-	-
Kemira Danmark	X	X	-	-	-	-	-	-
Faxe Kalk	X	X	-	X	-	X	X	-

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2.3 Setting discharge criteria and monitoring compliance

This section gives a short review of Danish and international strategies and experience with respect to setting criteria for the ecotoxicological characteristics of wastewater discharges, and to developing monitoring programmes on the basis of wastewater analyses.

2.3.1 Setting criteria and control programmes for individual substances

Danish experience

The administrative framework for setting criteria for discharge of individual substances (or mixtures of substances) in Denmark is laid down in two sets of Guidelines issued by the Danish Environmental Protection Agency in 1983 /1,2/. These guidelines regulate hazardous substances mainly by means of discharge limits.

For *naturally occurring substances* (for example, heavy metals) the guidelines lay down water quality criteria for the receiving water /2, annex 2/ based on the concept of excess concentration (immission criteria), i.e. an acceptable "excess load" is established in relation to concentrations in non-affected or only slightly affected areas (back-ground concentrations) which are comparable with the discharge area. These criteria are not based on evaluations of the NEC (No Effect Concentration) for the environment. In addition to this, discharge criteria for the heavy metals cadmium and mercury were issued in 1986 in fulfilment of EC Directives /12,13/ (see table 2.-3.1).

For *xenobiotic substances* (substances not normally found in nature), regulation today is based both on an assessment of the impact of the substances on the receiving water, and on formalized discharge concentration criteria. However, national and EC efforts have so far only led to the establishment of criteria for a very small number of substances, namely some of the so-called List I substances of the EC framework directive 76/464/EEC /3/. In 1991 List I comprised 132 substances or groups of substances, but so far EC directives and associated national regulations have only been developed for discharge of 11 xenobiotic substances /5,12,13,14/. The discharge limits are summarized in table 2.3.1 and they apply typically to enterprises producing or working with the individual substances.

Table 2.3.1 Discharge limits for List I substances according to /5, 12, 13, 14/. In addition to these, limits have also been set for discharged quantities in relation to total quantities of substances produced/treated.

	Discharge limits	
SUBSTANCE	Daily av.	Monthly av.
Cadmium (Cd)	-	0.2 mg/l
Hexachlorocyclohexane	-	2.0 mg/l
Mercury (Hg)	-	0.05 mg/l
Tetrachloromethane (CCl_4)	1-3 mg/l	1.5 mg/l
DDT	0.4 mg/l	0.2 mg/l
Pentachlorophenol (PCP)	2.0 mg/l	1.0 mg/l
Aldrin Dieldrin Endrin Isodrin	10 µg/l	2 µg/l
Hexachlorobenzene (HCB)	2-3 mg/l	1-1.5 mg/l
Hexachlorobutadiene (HCBD)	3.0 mg/l	1.5 mg/l
Chloroform ($HCCl_3$)	-	1.0 mg/l

The intention is that over the coming years, discharge limits will be established for all the substances in List I. The priorities for the substances to be included have not yet (1991) been established, but the CEC has initiated studies to define these more precisely.

In Denmark the most detailed regulation of individual substance concentrations in wastewater has been carried out by Ringkjøbing County Council in its discharge permit and monitoring programme for wastewater discharges from Cheminova A/S /7/, but other permits, for example those for Grindsted Products A/S (Ribe County Council), H.Lundbeck A/S (West Sjælland County Council) and Kemisk Værk Køge A/S (Roskilde County Council) are based on detailed regulations for a great number of individual xenobiotic substances. These permits consist of a number of criteria for acute and chronic toxicity of the whole effluent in relation to a conflict boundary in the receiving water (see section 2.3.2) *and* discharge limits for concentrations of individual substances in the effluent.

The principles used for establishing the regulations in the wastewater discharge permit for Cheminova A/S correspond to the

methods established by US-EPA for individual substances (see next page), although there are fundamental differences in method used to establish an uncertainty factor for determining the lowest effect concentration. The evaluation of the PEC takes into account some critical factors concerning the variability of the wastewater and factors affecting the dilution process.

In the Cheminova A/S discharge permit, the protection of the receiving water from acute toxic effects of individual substances is achieved by setting *maximum limits* for concentrations after initial mixing, i.e. concentration values which must not be exceeded in any sample. The values have been established on the basis of the lowest LC50/EC50 values for acute toxicity, and the lowest expected initial dilution rate (at times of current reversal). The values are expressed as discharged quantities (kg/day).

With regard to the potential chronic effects of individual substances (including bioaccumulative or poorly degradable substances) a number of *continuous average standards* have been set based on the lowest LOEC values for chronic toxicity, critical dilution in the near-discharge zone (using the 95% fractile at the conflict boundary), and the variability of the wastewater (statistical calculation of the acceptable average value and variation). As defined in the permit, continuous standards must be complied with over a certain averaging period, the control period. In other words, compliance with the permit is assessed after taking each sample, using a fixed amount of data (in the case of Cheminova A/S, for most of the parameters this is 24 samples taken during the last 6 months).

The discharge standard and the acceptable variation for it are then compared with the average value and variation in the sample data for the control period in question:

$$C = M + k_n \cdot S$$

where:
- C: the stipulated discharge standard
- M: the average value of measurements during the control period
- S: the variation of the measurements
- k_n: a constant (determined by the normal distribution function), which depends on the percentage acceptance level and number of samples (this is normally fixed at 0.95, i.e. 95% probability that the requirements will be met, if for example the 80% fractile of the discharge is lower than the stipulated value).

Experience in other countries

Earlier, the USA has regulated wastewater discharges solely on the basis of discharge limits for individual substances. In consequence of this, US-EPA has established discharge limits for a great number of substances based on extensive collections of data relating to the environmental characteristics of the substances in question /6/.

As of 1987 documented receiving water quality criteria had been established for a total of 127 substances.

To the extent that there has been sufficient data available, discharge limits have been established at the following levels of protection:

- Freshwater
  - * Acute criterion (*F*inal *A*cute *V*alue)
  - * Chronic criterion (*F*inal *C*hronic *V*alue)

- Saltwater
  - * Acute criterion (FAV)
  - * Chronic criterion (FCV)

- Protection criteria for plant life (FPV)

- Protection criteria for human load via
  - * Drinking water
  - * Consumption of fish (*F*inal *R*esidue *V*alue, recalculated to μg/l water on the basis of the bioaccumulation factor, BCF)

On the basis of these criteria, 1-hour and 4-day emission limit values are calculated which may not be exceeded more than once in a three-year period, and recommendations are made as to measures to be taken if the limits are exceeded (studies of effects on the basis of the most critical of the above-mentioned protection levels).

The one-hour requirement corresponds to 0.5·FAV-value (Maximum Acute Criterion) and the four-day requirement corresponds to the lowest of FCV, FPV, or FRV (average over a four-day period) unless other data indicate that lower values ought to be used /15/.

The two requirements can best be calculated on the basis of a dynamic dilution model, but if this is not possible, dilutions at minimum water flow may be used as shown in table 2.3.2.

Table 2.3.2 Minimum water flows recommended by US-EPA for calculation of emissions criteria on the basis of effect-based quality criteria (lowest one or seven day water flow minimum over 5 and 10 years respectively).

	Max. conc. (one hour requ.)	Mean conc. (4 day requ.)
Polluted recipients	1 Q 10	7 Q 10
Unpolluted recipients	1 Q 5	7 Q 5

Reference /6/ does not give guidelines for how to interpret "minimum dilution" for marine areas.

Traditionally in the USA the above-mentioned principles have been deployed to deal with 129 so-called "priority pollutants" when the wastewater in question has been identified as "toxic", or when there has been reasonable cause to suspect it to be so.

Since 1984 this strategy has been expanded with parallel regulation of the combined characteristics of the wastewater as described below.

2.3.2 Standard setting and control of compliance for whole effluent

Danish experience

The requirements laid down in a number of industrial wastewater discharge permits include demands for more or less regular control of the toxicity of the whole effluent by means of biotests. Table 2.3.3 gives a survey of the methods used and the frequency of sampling.

It must be pointed out that the discharge permit for a number of the enterprises in the table have subsequently been changed, and the requirement to use biotests in the control programme has been dropped. Only for a small number of the control programmes acceptance criteria are - or were - set for the results of the biotests.

The discharge limits and other aspects relating to the requirements for biotesting in the discharge permits of Grindsted Products A/S and Novo Nordisk A/S respectively are shown in table 2.3.4 - 2.3.5.

Table 2.3.3 Survey of the types of ecotoxicological test used in self-monitoring of waste water.

Enterprise	Algae		Crustacea				Bacteria
	Photosynthesis	Growth	Acute	Reproduction	Egg production	Life cycle	
Kemira (Superfos)	*Skeletonema Phaeodactylum* (combined, twice yearly)	*Phaeodactylum*	*Acartia* twice yearly		*Acartia* twice yearly		
Grindsted Products			*Nitocra* + *Daphnia* weekly				
Statoil Kalundborg (monit.programme discontinued)	*Skeletonema* 6 times/year Natural algal plankton yearly					*Acartia* twice yearly	Microtox 6 times yearly
Kalundborg municip. + Novo-Nordisk, Kalundborg (programme discontinued)	Natural algal plankton twice yearly *Phaeodactylum* monthly on stabilized + fresh sample		*Nitocra* monthly on stabilized sample	*Nitocra* monthly on stabilized sample			
Doras, Dansk Shell, Fredericia Cellulosefabrik (programme discontinued for Dora + Cellulosefabrik)	Natural algal plankton twice yearly	*Phaeodactylum* quarterly	*Nitocra* quarterly	*Nitocra* quarterly			
Lundbeck (programme discontinued)			*Nitocra* 8 times yearly				
Danfoss	*Skeletonema* yearly		*Acartia* yearly				

Table 2.3.4 Grindsted Products A/S
Requirements relating to biotests in the discharge permit (1989). The criteria refer to the necessary dilution of the wastewater at a discharge rate of 1037 m^3/d. For larger quantities of wastewater the criteria is reduced proportionally (the wastewater must be less toxic). Requirements after 01.01.93 take account of an expected improved treatment technology and closing of sulpha-drug production.

	Period when applicable			
Control of condition	01.04.89-31.12.92		01.01.93-onwards	
(x dilution)	mean	max.	mean	max.
Nitocra spinipes (96 h, LC 10)	< 40	-	< 3	< 10
Daphnia magna (48 h, EC 10)	< 6	-	< 2	< 2
Gammarus pulex (11 d, LC 20)	-	-	-	< 40
Daphnia magna (21 d, EC 20)	-	-	-	< 4
Sample type	**Weekly samples**: flow-proportional composite of daily samples			
Acceptance criteria	**Mean**: Value not to be exceeded as mean during the monitoring period (one calender year) **Max**: Not to be exceeded in any individual samples			

A number of enterprises have been required by rulings of the former land tribunals to carry out 24-hour acute toxicity tests with guppies. This requirement has been upheld in the §38 approval for some enterprises, including Cheminova A/S, as the only biotest giving a direct measurement of the total toxicity of the effluent. The enterprise is required to carry out the guppy test daily (24-hour samples). The 24-hour LC50 must not exceed a dilution of 1:15 more than once in each calendar month, and in no samples may it exceed 1:25.

Table 2.3.5 Novo-Nordisk A/S
Requirements laid down in the discharge permit for the joint discharge pipeline, dated June 1985. Monitoring is made of both raw and aerobically stabilized wastewater. (Stabilization at 15 °C to constant DOC-level; i.e. simulation of Kalundborg municipal treatment plant). A dilution factor of 50 corresponds to mean initial dilution in Jammerland Bight where the discharge takes place.

Control of condition (x dilution)		Raw wastewater	Stabilized wastewater	Control frequency per year
Nitocra spinipes	96 h, LC 10	-	≤ 50	12 months tests: flow-proportional composite of daily samples
	14 d, EC 20	-	≤ 300	do.
Phaeodactylum tricornutum	6 h, EC 20	≤ 50	≤ 25	do.
Natural algae plankton	6 h, EC 20	≤ 50	≤ 25	2 months tests in the period April-Oct.
Criteria for acceptance		Max. values, i.e. the values stated in single tests must not be exceeded.		

The permit for the wastewater discharge from Cheminova A/S /7/ regulates the *total* toxicity of the wastewater primarily by means of continuous discharge limits for the content of total-P and of a number of other individual substances. The background documentation consists of a very extensive toxicity study of the wastewater and in particular of one sample in which the toxicity contributions of the individual chemicals identified in the sample were combined additively and compared with the total acute toxicity of the sample as measured in a number of biotests. The substances that were found by means of this mass balance to be most important contributors of toxicity are phosphoric acid esters and their degradation products. It was concluded that there was a correlation within a factor of about 5 between the additive contributions of the substances and the total toxicity measured.

The biotests that are chosen for control purposes are usually those found to be the most "cost-effective" on the basis of the preliminary characterization of the wastewater using a variety of methods.

Cost-effective is here understood to include the following characteristics:

- that the wastewater is toxic to the chosen organism(s) at such a level that the distribution function for "toxicity" can be established ("low level of detection")
- that the method has a high degree of reproducibility (low "analytical uncertainty")
- that the cost of using the method is as low as possible.
  Only in relatively few preliminary characterizations of wastewater is the variation in "toxicity" reasonably well described, since usually the investigations are based on a single 24-hour or 7-day sample.

In addition to "recipient-realistic" tests, a number of wastewater characterizations have also included methods chosen solely because of their low cost (for example the Microtox test). The object here has been to examine whether the results from these tests are proportional to the results of the proper characterization methods (evaluation of lowest effect concentrations, cf. section 4.2) in order to be able to use the Microtox test (for example) as the indicator variable for the total toxicity of the wastewater.

International experience

As mentioned above, regulation of the environmental quality of wastewater discharges is carried out in most countries on the basis of a substance-specific approach. However, in recent years this approach has been supplemented in Sweden and in particular in the USA with regulations based on the whole effluent toxicity as measured in biotests.

At present there are no official guidelines for establishment of criteria and monitoring programmes in Sweden.

The strategy used in the USA has 4 main elements /10/:

- requirements based on water quality (toxicity checked by biotests)
- requirements based on clean technology principles: regulation of particularly hazardous substances (persistent, bioaccumulative, and/or highly toxic substances) by means of chemical analyses
- requirements based on technology (regulation of production, operation of treatment plant)
- ecological requirements (checked by monitoring in the field).

The use of biotest results for establishing water quality based requirements follows very much the same principles as are used for evaluating criteria for individual substances with regard to acute and chronic effect levels (cf. section 2.3.1) /11/:

- Criterion Maximum Concentration (CMC): 0,3 • TU_a (1/LC-50) (see section 4.3.1) for the most sensitive of at least three species (acute toxicity, representatives of at least three different trophic levels)

- Criterion Continuous Concentration (CCC): 1 • TU_c (1/EC-50) for the most sensitive of at least three species (as above, chronic/sub-chronic toxicity).

CMC and CCC are imission criteria, which can be converted to emission criteria using critical receiving water conditions and a knowledge of the variation in the wastewater. In some states, however, the CMC are direct emission criteria, since an initial mixing zone is not permitted. Here the CCC usually applies in relation to a maximal mixing zone defined by the authorities.

As with individual substances, the averaging time for CMC values is usually one hour, but in practice one day is used, assuming a certain amount of smoothing of peak values in treatment plant etc. The corresponding averaging time for CCC values is 4 days.

The CMC and CCC values are used to establish emission criteria in the form of maximum daily limit values (not to be exceeded by any sample) and monthly average limit values (not to be exceeded by the average of the data collected during a one month period). The two emission limit values are calculated both on the basis of critical dilution values in the receiving water, and also so as to ensure a low probability (1-5%, log-normal distribution) that variability in the effluent will lead to the limit being exceeded. Finally, attention is also paid to the load on the receiving water from other sources (waste load allocation).

US-EPA recommends that the frequency of monitoring for compliance should be related to the level of probability for compliance with the criteria (average criteria). A low monitoring frequency (minimum 4 times in the first year) thus results in stricter criteria compared with the criteria that are set when the monitoring frequency is higher.

It should be noted that US-EPA apparently does not recommend establishment of special criteria for independent monitoring of poorly degradable substances. Monitoring for these is partly covered by monitoring for possible chronic effects.

Bioaccumulative *and* poorly degradable substances are monitored independently by means of chemical analysis of the effluent or of organisms, and particularly hazardous substances (cf. EPA's 129 "priority pollutants" and the EC List I substances) are monitored by means of specific analyses.

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3 Evaluation of exposure concentrations

3.1 Characterization of wastewater and its variability

Industrial wastewater discharges can be very variable in terms of quantity and composition, and the ecotoxicological characteristics of the wastewater will vary correspondingly. In previous evaluations of industrial wastewater this variability has often been very poorly described, and there has been no specific consideration of the environmental consequences of the wastewater variation. Thus in many cases the ecotoxicological characterization of the wastewater is based on very few or even a single sample. Calculations of how the wastewater spreads in the receiving water are also usually based on constant discharge rates and there is usually no way of relating the purely hydraulic considerations to the real variation in quantity and composition of the discharge itself.

In a few cases the variation in the ecotoxicological characteristics of the wastewater is taken into consideration prior to setting the discharge criteria, but no exact rules have been laid down for doing this.

Monitoring of wastewater and wastewater variability is almost always based on grab samples. This in itself makes it difficult to give satisfactory descriptions of characteristics such as toxicity, where infrequent peak loads can cause significant problems.

The reason for evaluating the effluent variability is twofold. Firstly, it is necessary to have some idea of the variability in order to be able to set criteria for the discharge on the basis of knowledge derived from a limited number of wastewater samples. Secondly, knowledge of the variability gives a basis for planning a monitoring programme for the discharge.

3.1.1 Existing knowledge of variability in industrial wastewater toxicity

The toxicity of wastewater varies depending on its contents, and as will be seen, the variation can often be considerable. The consequence of this, as already mentioned, is that the results of the ecotoxicological test programme carried out on a wastewater sample can only be regarded as a snapshot picture. The complete picture would show a wider interval of toxicity.

The principle for comparison of the concentration of wastewater in the receiving water (PEC, Predicted Environmental Concentration) with observed effect concentrations, as shown previously in figure 2.2.4, should therefore be expanded in practice to include the variability. This can be done as illustrated in figure 3.1.1, where the upper half shows the temporal variation in the concentration of wastewater at one particular point in the receiving water together with the variation in the toxicity of the wastewater with respect to various species. The lower half of figure 3.1.1 shows the information concerning the wastewater concentration at various distances from the dis-

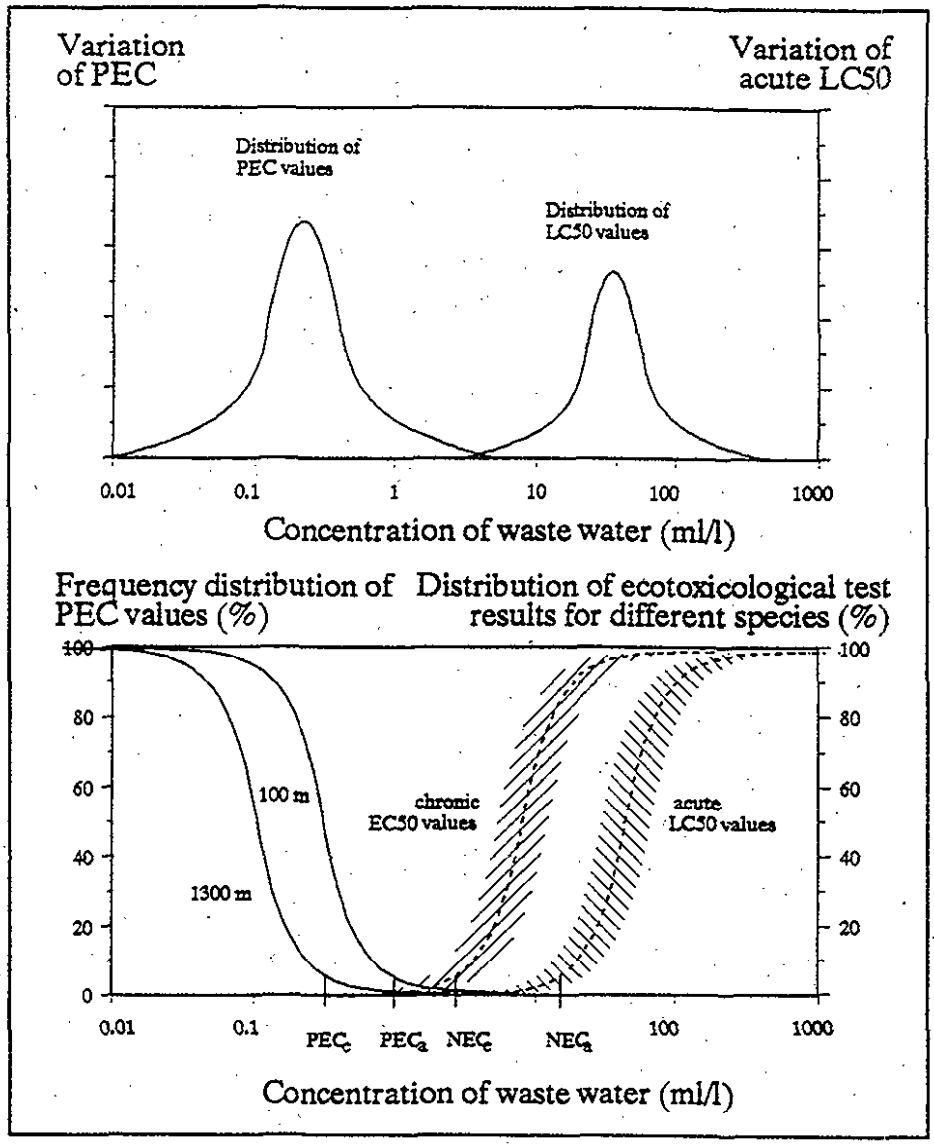


Figure 3.1.1 Principle for comparison of ecotoxicological data with PEC-values at various distances from the discharge point.
Above: the variation in the concentration at one specified distance from the discharge point, and the variation in the acute toxicity of the wastewater using tests on several species.
Below: the widths of the variations in acute and chronic toxicity values are shown (hatching) in the form of confidence bands around the distributions. The frequency distributions for wastewater concentrations at several distances from the discharge point are also shown.

charge point in the form of the frequencies at which the concentration exceeds the indicated value. In addition the figure shows the distribution function for both the acute and the chronic toxicity.

The wastewater characterizations carried out earlier have shown that it can be time-consuming and expensive to establish an adequate picture of size of the variation, since as a rule this will require investigation of a large number of wastewater samples. In

wastewater characterizations from a number of Danish enterprises an attempt was made to solve this problem by starting with a wide test programme on one or a limited number of samples.

This initial stage had as its objective firstly an evaluation of the level of toxicity in the wastewater and secondly the selection of one or more suitably sensitive test organism(s). Where necessary, this sensitive organism was then used as an indicator for toxicity in subsequent investigations of several wastewater samples in order to determine the variability in the toxicity of the wastewater.

On the basis of the observed variation a confidence interval for the range of the variation can then be estimated and plotted as shown in the figure. In other words, the range of variation can be included in the uncertainty factor when establishing the NEC on the basis of the LOEC, as described in section 5, in the same way as the other parameters that are included in the uncertainty factor.

A relevant picture of the variation in toxicity of industrial wastewater can be established by compiling the data from the routine monitoring programmes carried out at Danish industrial enterprises. A total of 8 out of the 23 wastewater discharges that have been examined ecotoxicologically have been subjected to routine ecotoxicological monitoring for a shorter or longer period /2/. 7 of these data sets give some idea of the variability in toxicity.

The best way of expressing the variation is to use the coefficient of variation, which is defined as the relationship between the variation and the mean. For the observed toxicity data, expressed as TU (Toxicity Units, equal to 1/EC or 1/LC), the standard deviation (S) and mean (M) are first calculated, and then the observed coefficient of variation is found as S/M. Table 3.1.1 gives an overview of the coefficients of variation.

It will be seen from the table that the variation in wastewater toxicity can be very large. The largest variations are seen in the figures from Grindsted Products A/S. This does not necessarily mean that the variation in this discharge really is greatest, but rather that the grab samples in the monitoring programme by chance have revealed a large variation in just those samples.

However, it should also be noted that the variation in the LC10 and EC20 values is considerably larger than the variation in the corresponding LC50 and EC50 values, and it is clear that the intrinsic variability in the test itself, and differences in the test organisms' sensitivity, can contribute to the variation in the test results. The most important reason for the difference, however, is that the experimental determination of LC50/EC50 values can be carried out with considerably greater precision than the LC10/EC20 values (cf. section 4.1), and therefore the variability in the toxicity of a wastewater discharge can be determined most accurately using LC50/EC50 values.

Table 3.1.1 Summary of observed variation coefficients for toxicity of Danish industrial wastewater.

Enterprise	Monitoring period	Number of samples	Natural plankton		Phaeodact. tricornutum		Skeletonema costatum		Nitocra spinipes						Acartia tonsa				Daphnia magna	
			EC20 6h	EC50 6h	EC20 6h	EC50 6h	EC10 6h	EC50 6h	LC10 48h	LC50 48h	LC10 96h	LC50 96h	LC10 14d	LC50 14d	LC10 48h	LC50 48h	LC10 96h	LC50 96h	EC10 48h	EC50 48h
Grindsted Products (own contr.)	1986 1987 1988	27 26 26									1.77 1.97 2.64	0.80 0.30 0.33							1.14 0.42 0.44	0.35 0.26 0.32
H. Lundbeck	1984 1986-87	6 12							1.17	1.09	0.67	0.58								
Kalundborg municip. + Novo Nordisk	1987 1988 1989	10 10 6			1.31 1.01 0.42	1.03 0.33 0.31					0.86 0.65 0.44	0.47 0.62 0.69	1.61 3.18 1.18	0.59 3.14 0.60						
Novo Nordisk (own contr.)	1987 1988 1989	11-12 8-11 3-7			0.74 0.83 0.00						0.33 0.27 0.29	0.24 0.28 0.35	0.61 0.43 1.42	0.30 1.16						
Fredericia Cellulose	1985-89	5-13	0.51	0.57	2.27	0.76					0.80	0.67	0.91	0.48						
Dansk Shell	1985-88	8-15	1.29	0.40	1.01	0.12					1.62	0.80	1.16	1.02						
DORAS	1984-86	5-9	1.25	0.76	0.63	1.24					0.88	0.65	1.38	0.71						
Kemira DK (own contr.)	1983-88	6-14			1.05	1.52	0.85	0.44			1.90	1.08	1.57	2.48	0.68	0.97	1.82	2.13		
Sodium-dichromate -reference	1989-90	3-11			0.24	0.08	0.10	0.05	0.13	0.07	0.12	0.10			0.14	0.10	0.21	0.13	0.03	0.08

For completeness, it should be added that the reproducibility of the test system is usually tested with a reference substance (often sodium dichromate), and that parallel control tests are always performed. This reduces the risk of variation creeping in because of "atypically" sensitive test organisms or other special features relating to technical aspects of the test.

As examples, the temporal variation in the toxicity of discharges from two different enterprises is shown in figures 3.1.2-3.1.5.

For wastewater discharges for which there is no information about the variability, an idea of the potential variation can be obtained from a knowledge of the variation in chemical usage or discharge of toxic substances. Examples of this are given in figures 3.1.6-3.1.9, which show the discharges of certain substances from a Danish enterprise in which usage of certain substances was monitored daily; thus the knowledge of the individual substances in the wastewater was particularly good.

For Prom Kemi A/S the relevant substances were chosen as indicators for the discharge, and they represent some of the most toxic of the known substances in the discharge. Thus the variation in the discharge of these substances also gives an indication of the variation which could be expected in the wastewater toxicity.

However, it should be emphasized that the best picture of the toxicity and its variability is obtained by ecotoxicological tests, since these take into account the internal variation between the substances themselves, any potential synergistic or antagonistic effects between them, and finally the toxicity contributed by any unknown chemical constituents in the wastewater.

At the same time it should be pointed out that the variability in toxicity of industrial wastewater varies greatly from one type of industry to another, and often from one enterprise to another. In most cases it is difficult to lay down general rules on how to handle the question of variability, and usually an individual approach will be necessary. Actual measurements, such as those supplying the data shown in the figures for individual enterprises shown in this section, can often form the basis for such an approach. Using these figures a case can be made for choosing a confidence interval for toxic effects, as shown in figure 3.1.1, and thus for deciding on the contribution made by effluent variability to the uncertainty factor when establishing the NEC.

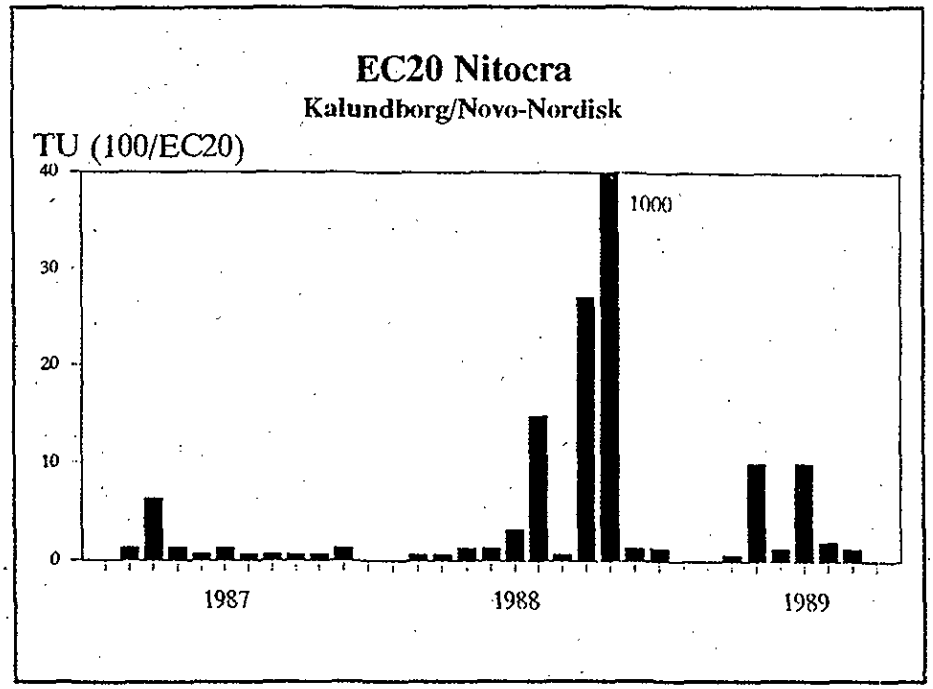


Figure 3.1.2 Time series for toxicity (EC20 Nitocra spinipes) of combined wastewater from Kalundborg and Novo-Nordisk A/S. 1987: month test; 1988/89: 24 hour test /2/.

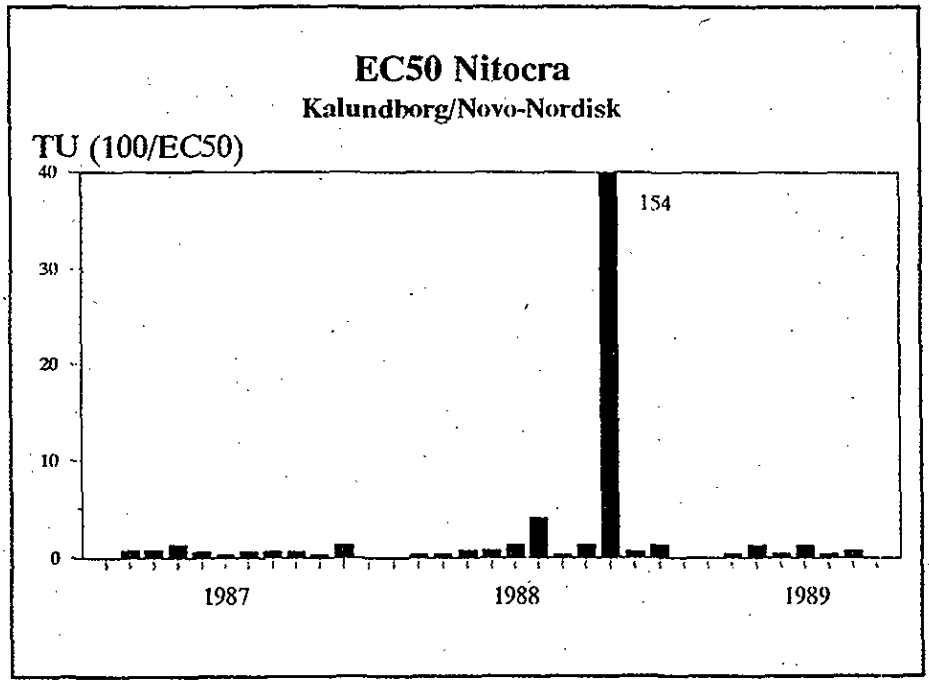


Figure 3.1.3 Time series for toxicity (EC50 Nitocra spinipes) of combined wastewater from Kalundborg and Novo-Nordisk A/S. 1987: month test; 1988/89: 24 hour test /2/.

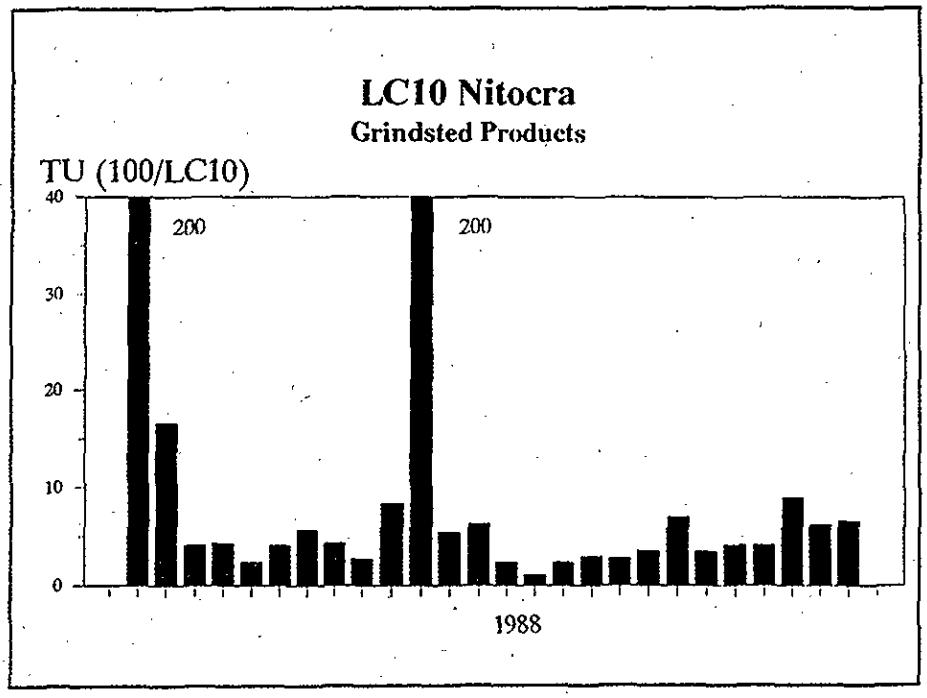


Figure 3.1.4 Time series for toxicity (LC10 Nitocra spinipes) of wastewater from Grindsted Products A/S /2/.

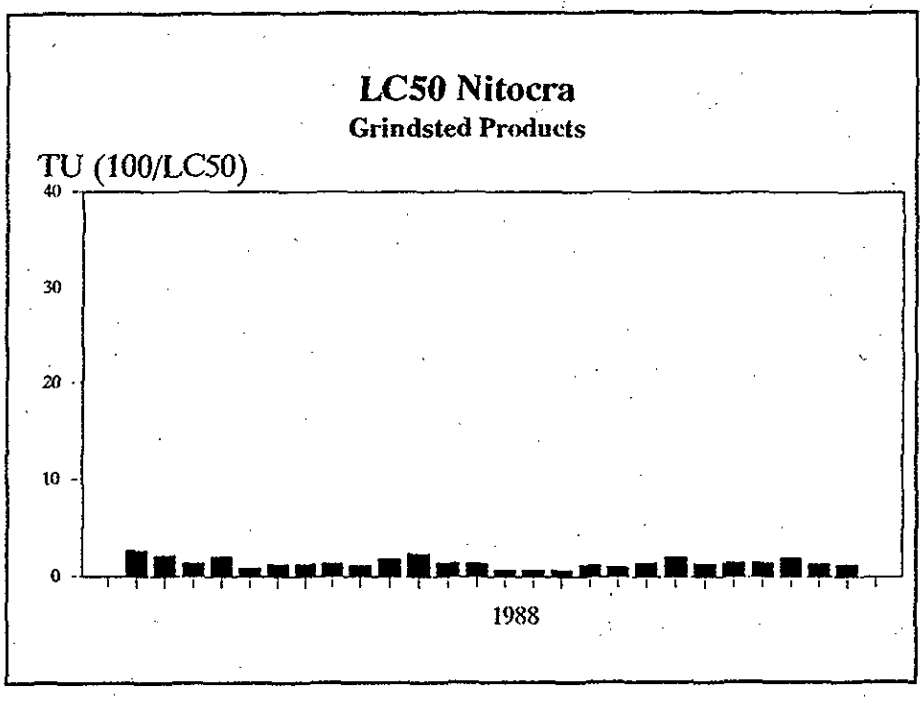


Figure 3.1.5 Time series for toxicity (LC50 Nitocra spinipes) of wastewater from Grindsted Products A/S /2/.

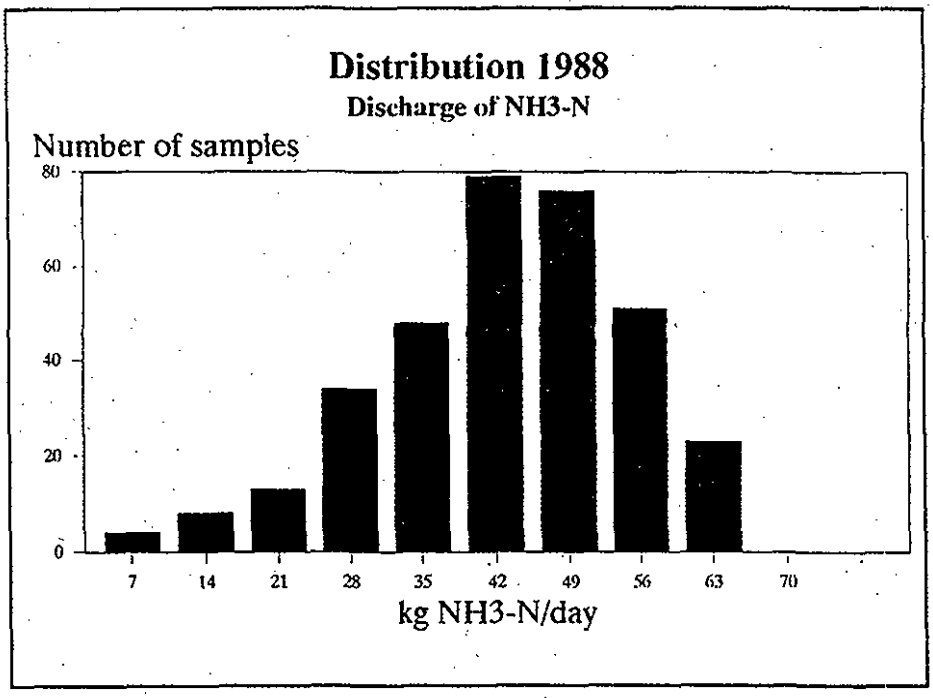


Figure 3.1.6 Distribution of discharge of ammonia/ammonium nitrogen from Prom Kemi A/S. Daily measurements /2/.

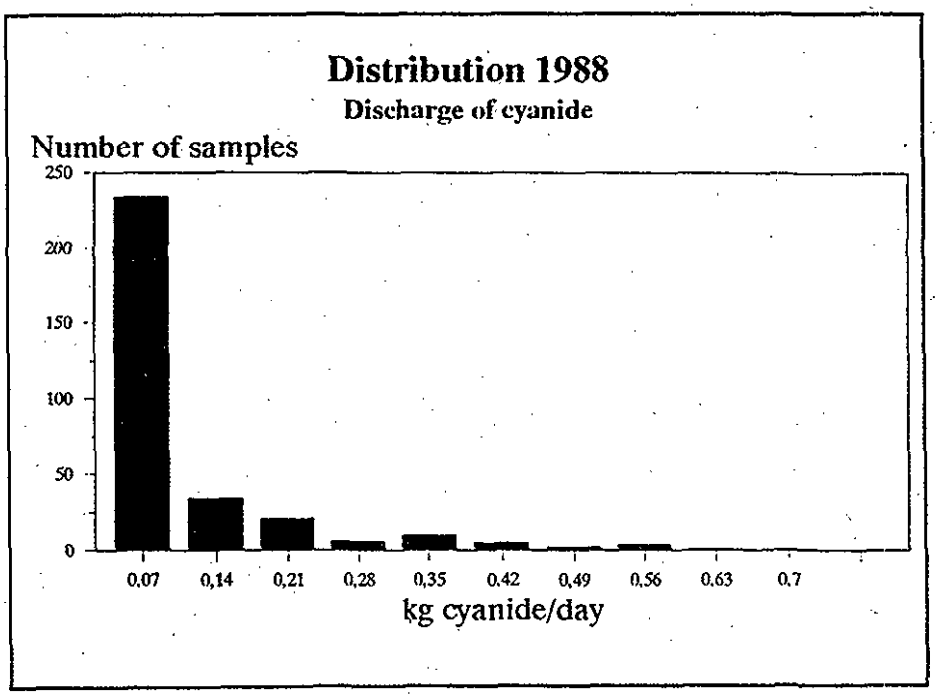


Figure 3.1.7 Distribution of discharge of cyanide from Prom Kemi A/S. Daily measurements /2/.

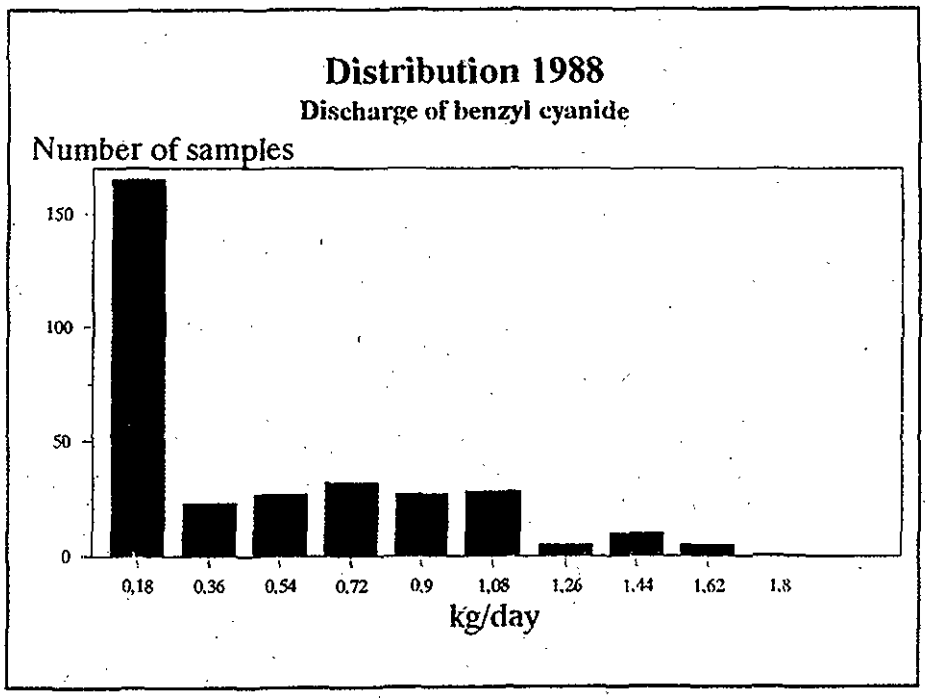


Figure 3.1.8 Distribution of discharge of benzylcyanide from Prom Kemi A/S. Daily measurements /2/.

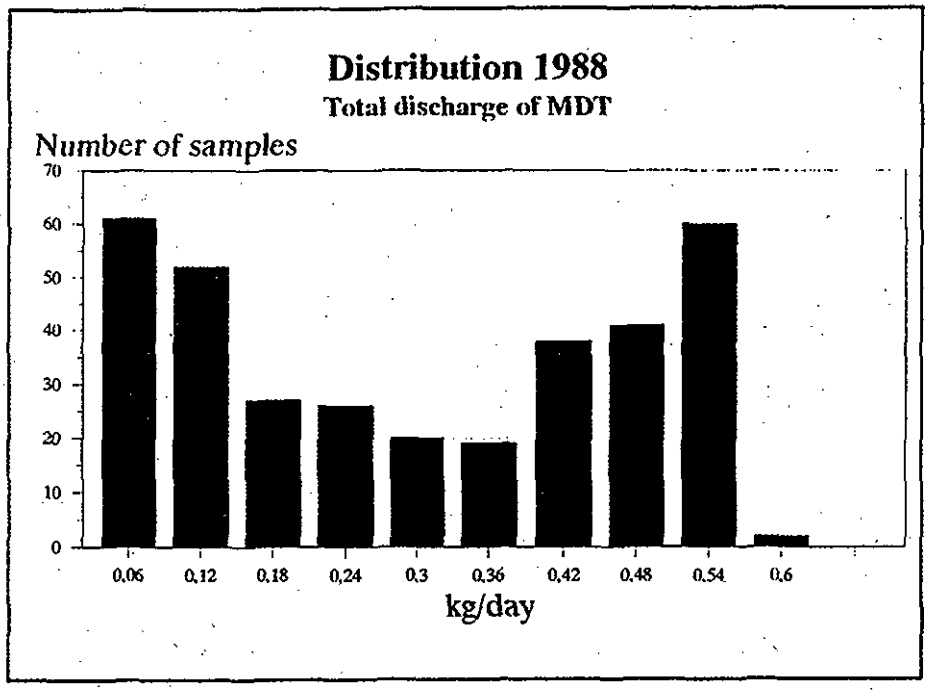


***Figure 3.1.9** Distribution of the total discharge of thiodibenzo acids from Prom Kemi A/S. Daily measurements /2/.*

3.1.2 Sampling technique and monitoring frequency

Sampling technique, and especially the averaging time used when sampling, is very important when determining wastewater toxicity.

Present experience of sampling technique

Up to now, samples for ecotoxicological investigations have usually been flow-proportional one-day composite samples.

However, examples are also known of other types of sampling principles, and 14-day or 1-month composite samples are also routine practice in some monitoring programmes.

An averaging time of 14 days is used for samples taken in the routine monitoring programme at Grindsted Products A/S /2/. The total residence time of the wastewater in retention tanks and the treatment plant is high and can amount to almost a week, but retention occurs in a series of basins so that complete mixing for the total residence time is not achieved. Mixing the wastewater from a 14-day period may therefore have smoothed out some of the variation, which in consequence may have been somewhat larger than shown in table 3.1.1.

An averaging time of 1 month was used in 1987 in the ecotoxicological monitoring programme for the joint discharge from Kalundborg municipality and Novo Nordisk A/S /2/. In 1988 the programme was altered and one-day composite samples were adopted. It is possible that the highly toxic daily samples revealed in the *Nitocra spinipes* test, cf. figure 3.1.2 and 3.1.3, would not have been detected if they had been mixed with the rest of the wastewater discharge from an entire month.

These examples are cited to show that the fundamental sampling principle is that variability and peak values in the effluent ought not to be smoothed out by the sampling. The averaging value for the sample ought not to differ significantly from the residence time of the wastewater in retention basins and treatment plants, and in estimating the residence time, attention must be given to the amount of mixing and equalisation taking place. When planning the sampling procedures and setting up the monitoring programme it is an advantage to know the overall structure in the wastewater variation.

Structure in the variation

A theoretical picture of the structure in the variation of an industrial wastewater discharge is given in figure 3.1.10.

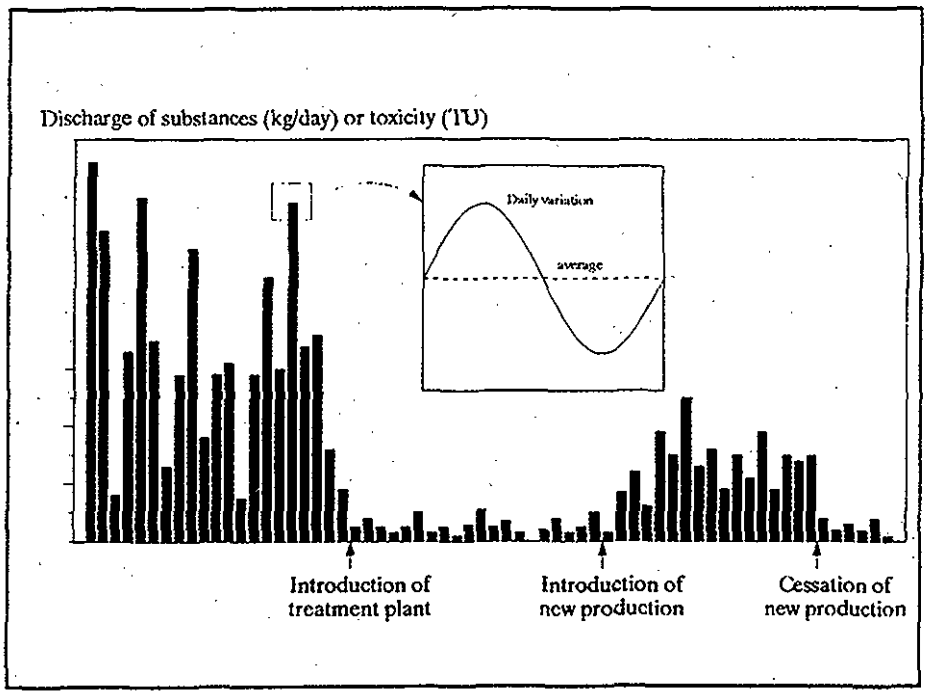
The short-term variation in the discharge is decisive for the averaging time of the sampling programme. Analysis of the short-term variation will show what averaging time ought to be used.

The long-term variation is important for planning the other aspects of the monitoring programme. The figure reveals marked changes in the discharge corresponding to changes in the production pattern.

Prior knowledge of the pattern of the variation can be an advantage when planning the monitoring and sampling programmes.

Averaging time for sampling

Precise determination of the optimum averaging time for the sampling programme will depend on a specific evaluation of the retention and mixing time in the wastewater system of the enterprise in question. If production is in batch form then the greatest wastewater load will oc-



Figur 3.1.10 Theoretical picture of the variety of an industrial wastewater discharge.

cur - after a certain delay depending on the retention in the wastewater system - after the start of each batch process. The wastewater load may also continue for a longer period than the length of the production process itself, since mixing in the wastewater system will spread the "momentary" input to the system. Even in the case of continuous production, similar situations may occur since there may be some variation in the input in the course of a working day (see figure 3.1.10).

The discharge curve for a "momentary" input to the wastewater system can be followed by using tracers, or it may be calculated from available data. An example of calculated input curves for momentary inputs via a number of fully mixed retention basins, each with the same residence time, is given in figure 3.1.11 /1/.

It will be seen from the figure that in the case of two fully mixed basins in series, each with a 24 hour residence time, the peak load will be discharged after 0.5 times the total residence time (Θ), i.e. after 24 hours. The width of the peak is also indicated in the figure. For 4 basins in series, each with a 24 hour residence time, the peak discharge will come after approx. $0.75 \cdot \Theta$, i.e. after approx. 36 hours, with a "width" of approx. 36 hours.

In practice the distribution of the residence time in a wastewater system can often be simulated as a number of fully mixed basins of different residence times combined with a number of plug-flow basins in series. The resulting distribution will be skewed to the left, and can often be approximated to a log-normal distribution. As far as is known, all Danish enterprises discharging directly to a recipient have equalization systems and a retention time of 24 hours or more. In such cases 24-hour composite samples are usually acceptable.

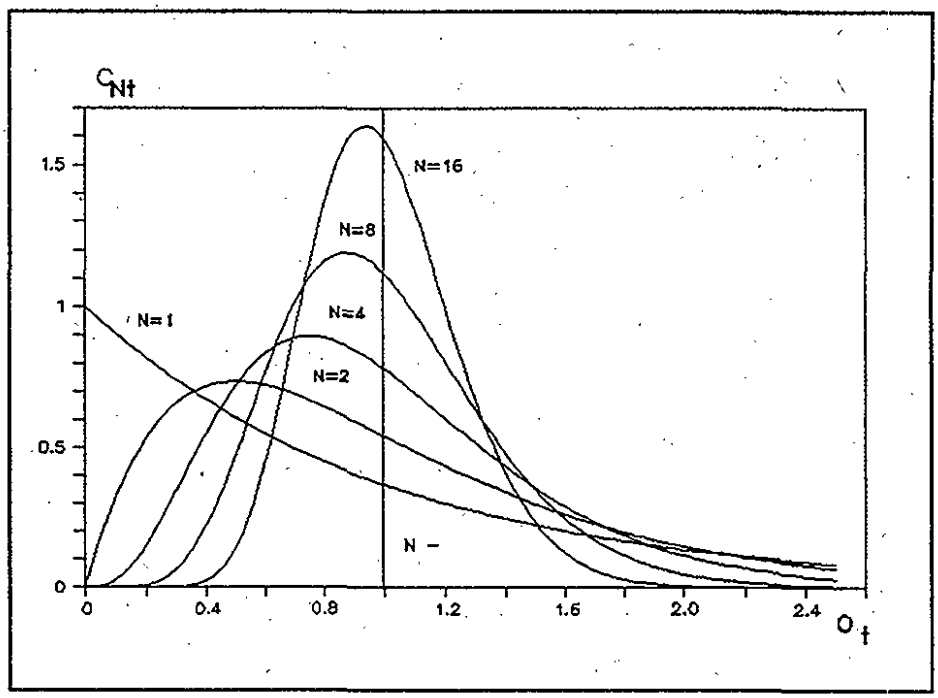


Figure 3.1.11 The normalized retention time distribution for N equal discharge basins in series /1/. $C_{Nt} = C_N/C_0$ being the normalized discharge concentration. C_N being the discharge concentration from basin no. N.

Monitoring frequency

When planning the monitoring programme, attention should be given to all the variations in the discharge which can be observed or foreseen. The monitoring programme should be dimensioned on the basis of the prevailing conditions during a period of typical variation structure, and if significant changes in the discharge occur, the programme should be revised.

However, it is clear that production on a very short timescale, e.g. batch production, can produce short-term changes in the discharge. The duration of such changes can be so short that a proper statistically-based monitoring programme can not be designed. Discharges from batch production processes must be dealt with in another way, and a monitoring programme based on random sampling is only meaningful for continuous discharges which show no significant changes over lengthy periods.

Production cycles lasting days to weeks fall typically into the "batch production" category, while production cycles measured in months to years can be regarded as "continuous".

However, unlike this simplified theoretical picture, we find that reality is considerably more complicated, since a production cycle with its corresponding wastewater discharges will often consist of a number of processes, which overlap each other and which can consist of a mixture of "batch" and "continuous" processes. The important question therefore is whether the discharge is fairly constant over a certain period, particularly with regard to toxicity.

For continuous discharges, grab samples will therefore be appropriate. The frequency of this type of monitoring will depend on the two above-mentioned factors:

- the margin between the toxicity level and environmentally problematical levels or discharge limits,

- the variability in the toxicity,

and the rules for calculating the appropriate frequency will be the same as those for statistical quality control in general.. Rules of this type are well known from the procedures for municipal wastewater discharge monitoring using standard sampling variables. The procedures have been described in a guide issued by the Danish Engineering Association /1/.

A good example of changes in a continuous discharge as a result of the adoption of new treatment techniques is shown in figures 3.1.12 -3.1.15. The figures show the sum of finished products and the sum of trialkylthiophosphoric acid esters (tri-esters) in the wastewater from Cheminova A/S in 1985 and then in 1989 after introduction of improved purification processes (including biological treatment) for part of the wastewater. These groups of substances are the critical ones with respect to the toxicity of the discharged wastewater. The different scales on the axes should be taken into account when comparing the figures. It will be seen that a knowledge of the changes is important when evaluating ecotoxicological data and when planning monitoring programmes. As shown in figure 3.1.16 and 3.1.17, the mean value of the concentration in the wastewater is reduced significantly, but the coefficient of variation (i.e. the relationship between the variation and the mean) remains more or less unchanged in the two periods. However, the relatively large variation in 1989 arises because the wastewater treatment at this time was only carried out on part of the wastewater, and the variation is more an indication of the variation in the proportion of wastewater being treated, than a sign of variation in the efficiency of the treatment process itself.

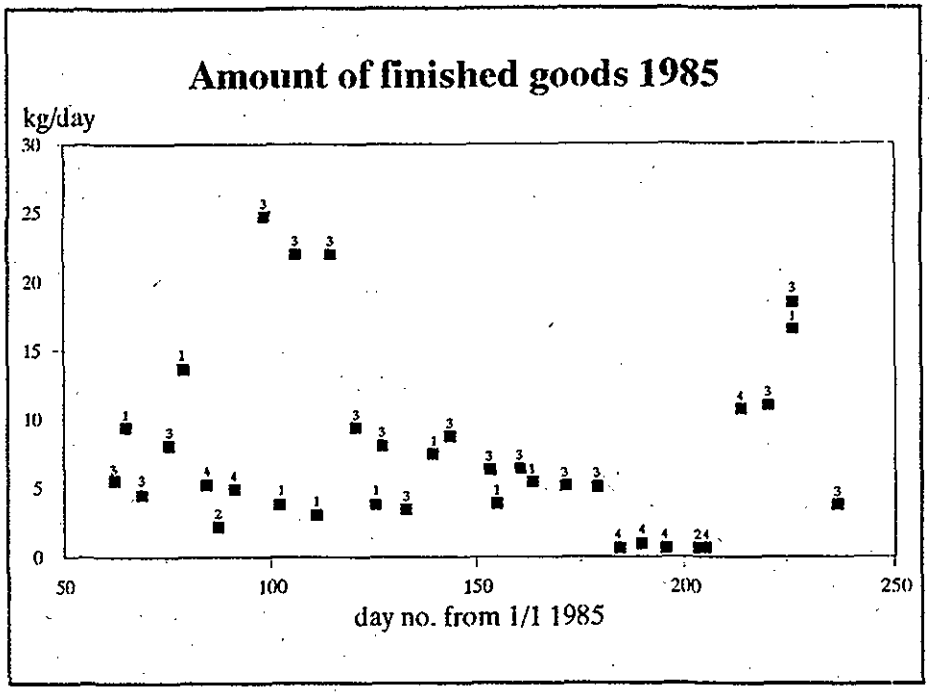


Figure 3.1.12 Discharge of finished goods in wastewater from Cheminova A/S in 1985 analyzed by the county (code 1,2) and by Cheminova (code 3,4) /2/.

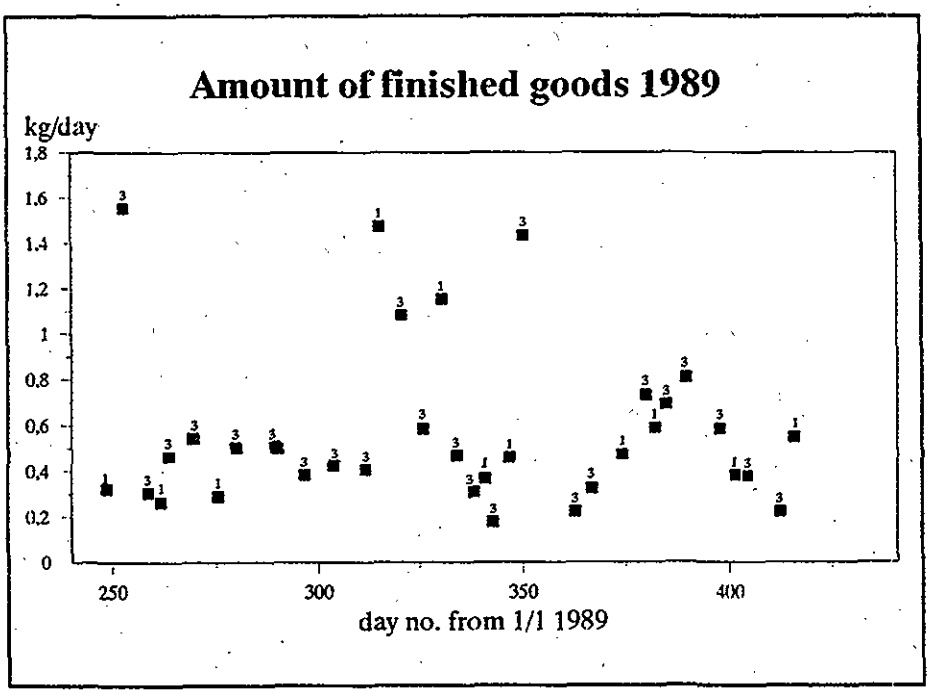


Figure 3.1.13 Discharge of finished goods in wastewater from Cheminova A/S in 1989 analyzed by the county (code 1,2) and by Cheminova (code 3,4) /2/.

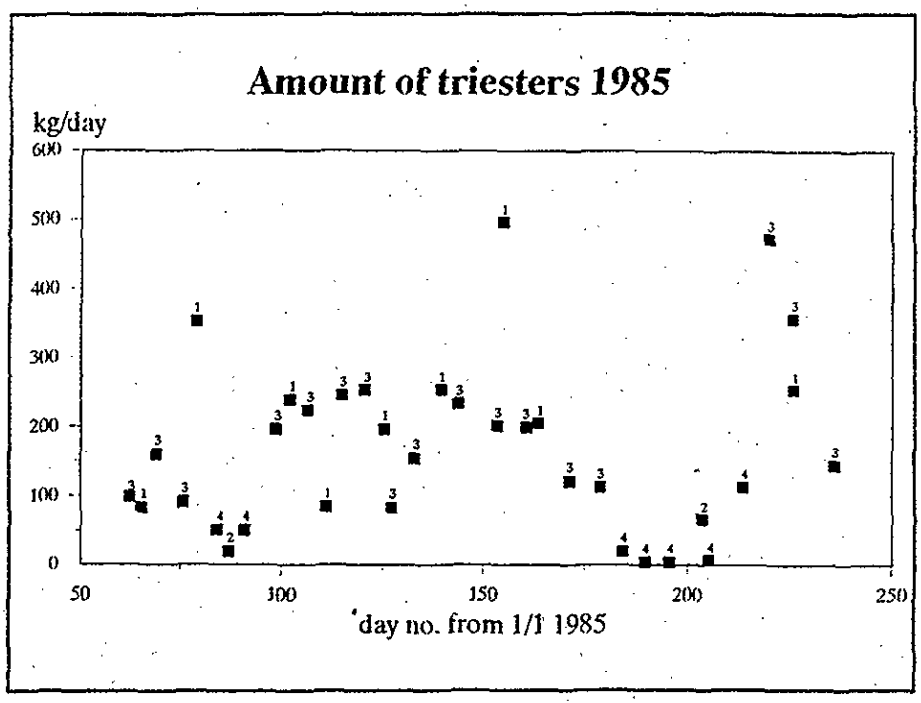


Figure 3.1.14 Discharge of triesters in wastewater from Cheminova A/S in 1985 analyzed by the county (code 1,2) and by Cheminova (code 3,4) /2/.

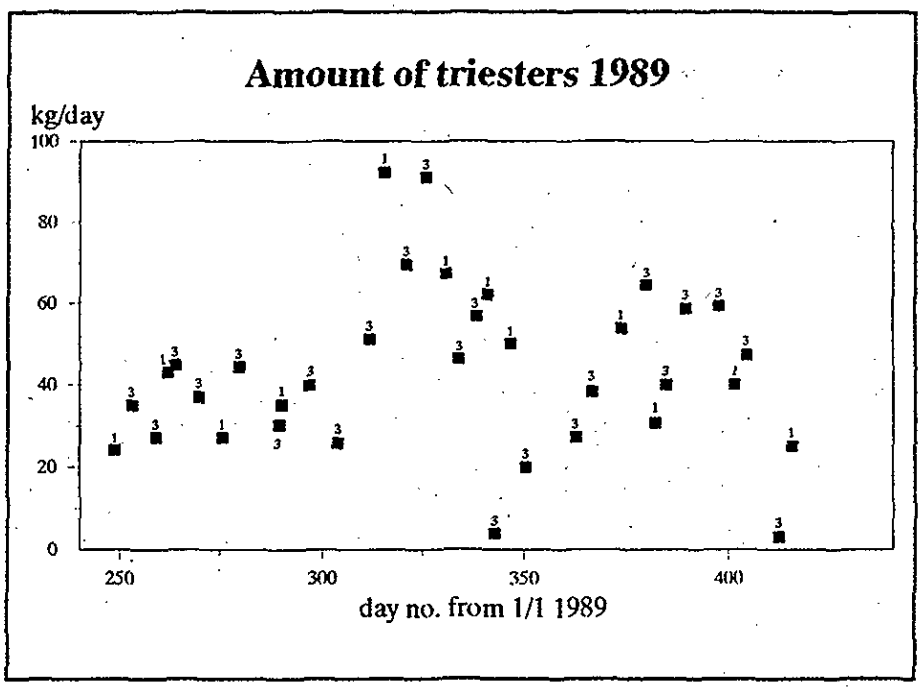


Figure 3.1.15 Discharge of triesters in wastewater from Cheminova A/S in 1985 analyzed by the county (code 1,2) and by Cheminova (code 3,4) /2/.

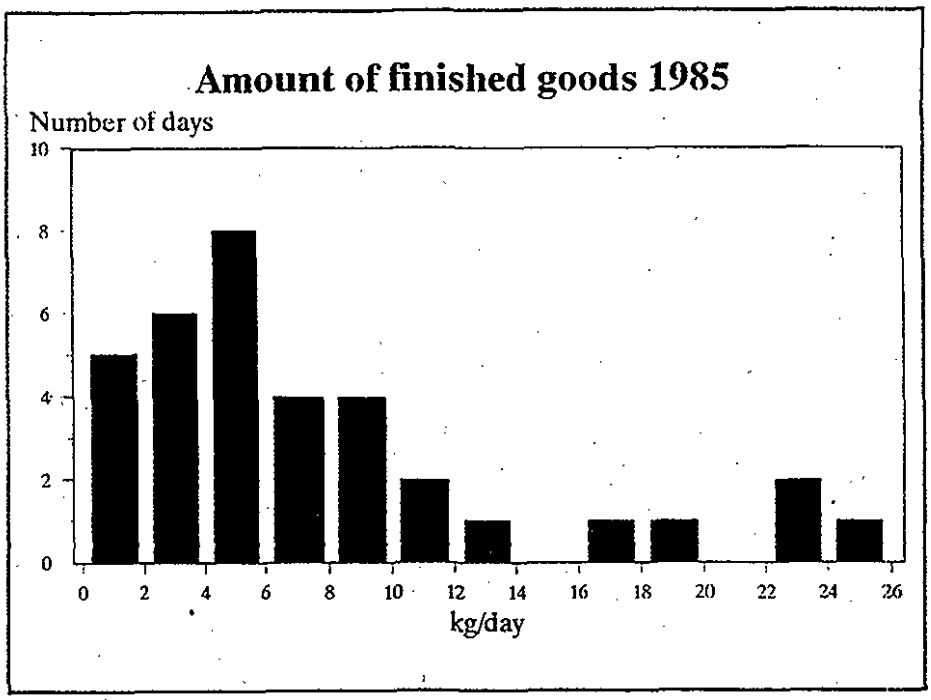


Figure 3.1.16 Distribution of discharge of finished goods in wastewater from Cheminova A/S in 1985.

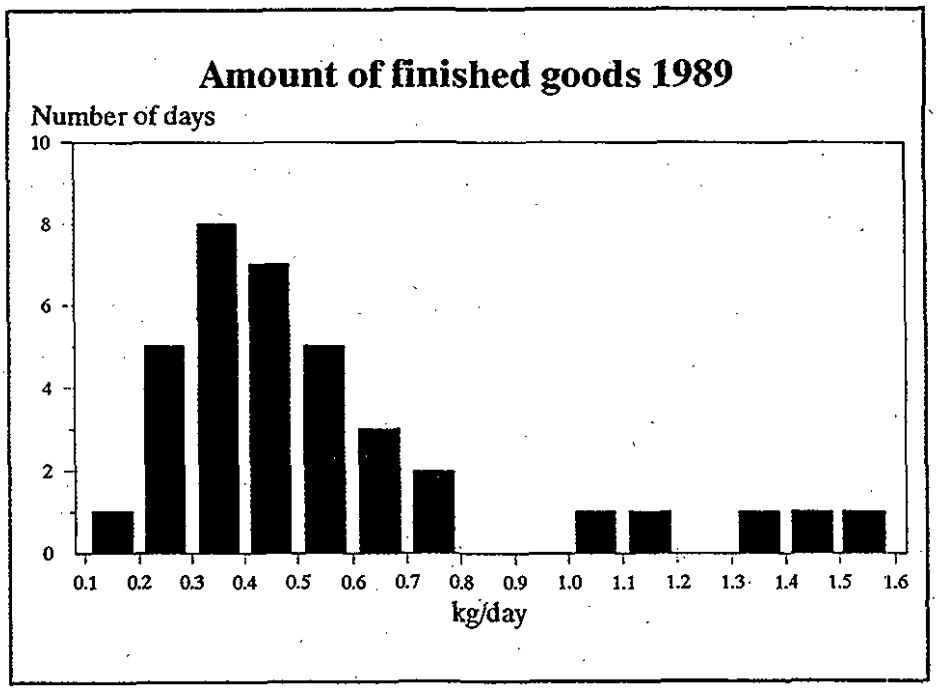


Figure 3.1.17 Distribution of discharge of finished goods in wastewater from Cheminova A/S in 1989.

If only limited data on toxicity is available it is necessary to estimate the size of the variation, and a knowledge of the variation in production processes and in discharges of individual substances will then be useful. For batchwise discharges which are not smoothed out by passing through a treatment plant, it may be rational to adopt an ad-hoc approach based on a knowledge of the production process and the potential wastewater loads, and to establish a retention system, with precautionary analysis before discharge if judged necessary. Precautionary analysis before discharge means complete retention of all wastewater in a retention basin and full characterization followed by one or several ecotoxicological tests before discharge. In Denmark this method is used by Ferrosan A/S, and it is considered that the wastewater from batch production will often be so small in quantity that total retention should be considered in such cases /2/.

References

/1/ Dansk Ingeniørforening (1981): DIFs anvisning for vandforureningskontrol. Normstyrelsens publikationer. NP-150-R. Teknisk Forlag, København, 1981.

/2/ Vandkvalitetsinstituttet, ATV (1990): Danmarks udledning af industrielt spildevand. Administration af Østersøkonventionen og Nordsødeklarationen. Miljøprojekt nr. 153, Miljøstyrelsen, 1990.

3.2 Dilution and distribution in the receiving water

When wastewater is discharged into a surface recipient it will be mixed in the receiving water and spread by currents and dispersion. Individual substances in the wastewater may also be removed from the water phase by adsorption to sediment or by precipitation if there is a significant pH or salinity difference between effluent and receiving water. Removal from the water phase by evaporation to the atmosphere is also a possibility, but in most cases volatile substances will already have been removed from the effluent before discharge.

All these processes help to reduce the toxicity of the diluted wastewater. The reduction in toxicity can be so large that toxic effects are only detected within the immediate vicinity of the outfall. The toxic effects may become concentrated, perhaps in the sediment, which may adsorb toxic substances from the wastewater. The extent of the area in which toxic effects may be expected will depend on the ecotoxicological characteristics of the effluent, the conditions under which discharge takes place, and the mixing that occurs in the receiving water as a consequence both of the movements in the water and of the density of the effluent relative to the receiving water.

The water movements will vary over time as a result of tidal activity, changes in current strengths, or seasonal changes in water flow, stratification, evaporation etc.

The temporal variation in dilution must be borne in mind when evaluating a wastewater discharge, since only those areas in the recipient where the concentration of wastewater exceeds the lower limit for effects such as chronic toxicity for significant lengths of time

can be regarded as being chronically impacted. When evaluating the dilution and spreading of the wastewater in the receiving water, the critical factors determining the minimum dilution or the greatest extent of spreading into sensitive areas must be determined. The duration of such critical periods and the frequency with which they prevail during longer periods of time should be ascertained as a basis for evaluation of the wastewater discharge.

When evaluating wastewater discharges to rivers or streams, it is customary in the USA to use the lowest average daily flow measured during the last 10 years as basis for evaluation of acute effects (1Q10) /3/. Similarly, the lowest average 7-day flow during the last 10 years (7Q10) is used for evaluation of the chronic effects. The critical factors causing acute (1 day) or chronic (7 day) effects may therefore be expected to show up with a frequency of once every ten years.

The periods of 1 and 7 days have been chosen on the basis of the special circumstances applying to rivers and streams; the essential point is that the critical factors must prevail for a continuous period of a certain length. The precise length may be determined on the basis of the nature of the recipient and the mobility of the organisms requiring protection in that water.

In marine areas, statistics are used of the meteorological conditions and wind and current speeds at the site or near to it, in order to develop a conservative but probable picture of the wastewater mixing and dilution. This could be a situation with a certain critically low level of water exchange. The length of the period during which this situation might be expected to occur must then be determined. Alternatively, an estimate could be developed of the lowest water exchange occurring during a period of a chosen length. The wind and current direction are also important in determining which and how great an area is affected around the outfall. Evaluation of wind and current directions in relation to the topography of the area are therefore also necessary elements when determining the critical factors for wastewater mixing and dilution in marine recipients.

3.2.1 Removal from the water phase

Adsorption

Individual substances may adsorb to particles in the water phase and settle out. This can reduce the toxicity of the effluent, but can also lead to toxic effects in the sediment. Adsorption occurs so rapidly that it is usually regarded as instantaneous. In real-life situations removal by adsorption will depend on the concentration of particles in the water phase, their sedimentation velocity, and the water depth (and thus the proportion of the water body in contact with the sediment). In a small volume of water there will always be equilibrium between the adsorbed concentration of a substance (C_s) and the dissolved concentration (C_w). The relationship between the two concentrations is a constant, the adsorption coefficient of the substance in question ($K_d = C_s/C_w$).

For metals and positively charged ions, adsorption to particles will take the form of complex formation. The adsorption coefficient depends on the individual substance and the nature of the particulate material (for example, the clay content). Increased salinity in the

receiving water or a fall in pH can weaken the binding of the substance to the particles, so that the concentration in the water phase increases.

For uncharged organic substances, the adsorption process is closer to true adsorption, where the hydrophobic (water-repelling) groups in the substance orientate themselves towards particles which are also hydrophobic. This process results in adsorption to the surface of the particle. The size of the adsorption coefficient depends on the hydrophobic/hydrophilic characteristics of the substance, which is normally expressed as the partition coefficient between n-octanol and water (P_{ow}). The greater the value of P_{ow}, the greater the tendency to adsorption.

A number of correlation equations have been developed for the relationship between adsorption and partition coefficients. One of the parameters used in these correlations is the content of organic carbon in the particles, which reflects their hydrophobic tendency. Higher organic carbon content in the particles gives higher adsorptive capacity.

Evaporation

Certain substances can evaporate from the water phase after discharge to the receiving water, and this may reduce the toxicity of the effluent. The tendency of a substance to evaporate depends primarily on the characteristics of the substance in question; the relationship between vapour pressure (V_p) and solubility in water (S) expressed as Henry's Constant ($H = V_p/S$). Substances with a Henry's Constant less than 10^{-3} atm · m^3/mol, however, can not be expected to evaporate under realistic conditions. The greater the value of Henry's Constant, the greater the tendency of the substance to evaporate.

The speed with which a substance evaporates depends primarily on Henry's Constant, but the situation in the receiving water, including factors such as water depth, temperature, and wind and current speeds, play an important role in determining the speed of evaporation in each particular case.

3.2.2 Dilution

Dilution of wastewater discharges occurs typically in three stages:

A) a zone in which the momentum and density of the effluent stream causes turbulent flow and therefore mixing (dilution) close to the discharge point. This is often referred to as dilution in the effluent jet.

B) a zone in which turbulent flow in the receiving water determines the transport and mixing of the effluent. This can be referred to as transport in an effluent plume.

C) a zone in which mixing is almost "complete" over a large area across the direction of recipient current flow or throughout the vertical water column. In this zone, additional dilution of the effluent takes place by advection and dispersion, which evens out the gradients arising because of variations in current flow and direction, variations in effluent flow, or

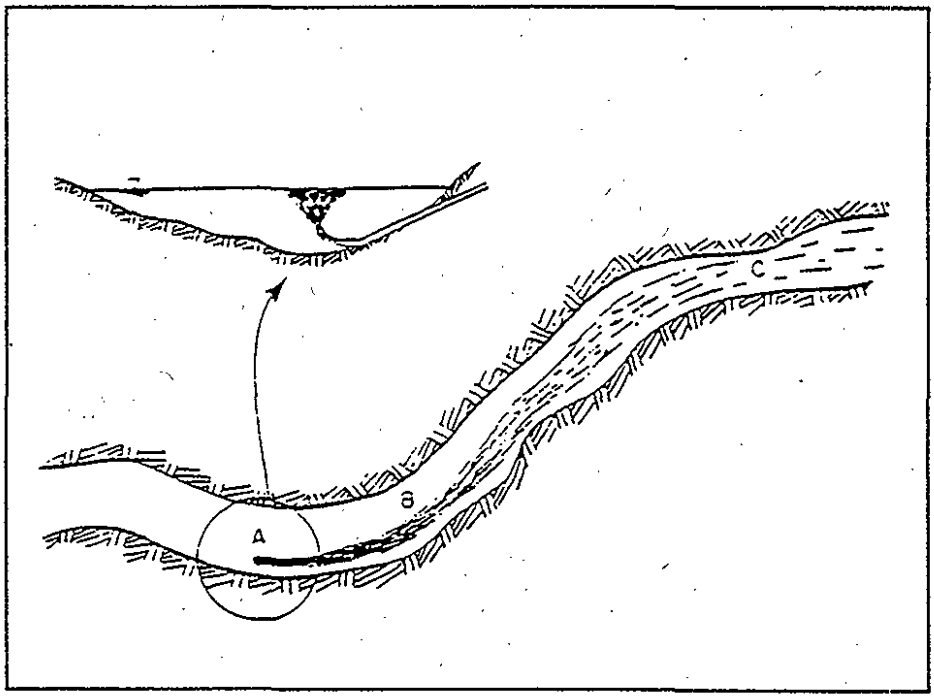


Figure 3.2.1 The dilution of the effluent in the recipient is divided up in three phases. From /4/.

variations in other discharges to the recipient in question.

The following sections discuss the factors affecting mixing in the effluent jet and plume, the factors affecting the additional dilution occurring in the recipient, and the factors affecting evaporation and adsorption.

3.2.3 Dilution in the effluent plume

Because of its surplus momentum (product of the effluent mass flux and velocity) in relation to that of the receiving water, an effluent discharge can create turbulent flow, which ensures a certain amount of initial mixing in the recipient at the point of discharge.

The dilution in the jet (and the breadth of the jet) depend on the relation between the discharged volume (Q) and the linear velocity of the jet (v). The velocity will be greatest in the centre of the jet and will decrease towards its edges (see figure 3.2.2). The further the jet moves away from the discharge point, the lower the velocity in the centre of the jet, and the greater the width of the jet.

At the limit, the effluent jet has lost so much momentum because of the resistance of the receiving water that its velocity relative to the receiving water is zero. The jet will be deflected and the diluted wastewater will spread fanwise in a horizontal direction /4/. In the great majority of cases the effluent discharge has relatively little momentum, and the extent of the jet is therefore not very significant.

As a rule, wastewater discharges have a different density from that of the recipient. The density difference can be due to temperature or salinity differences. The density difference will cause movement of the effluent (usually an upward movement, since the wastewater is usually warmer and/or less saline). The movement causes turbulent flow, and a plume will be formed.

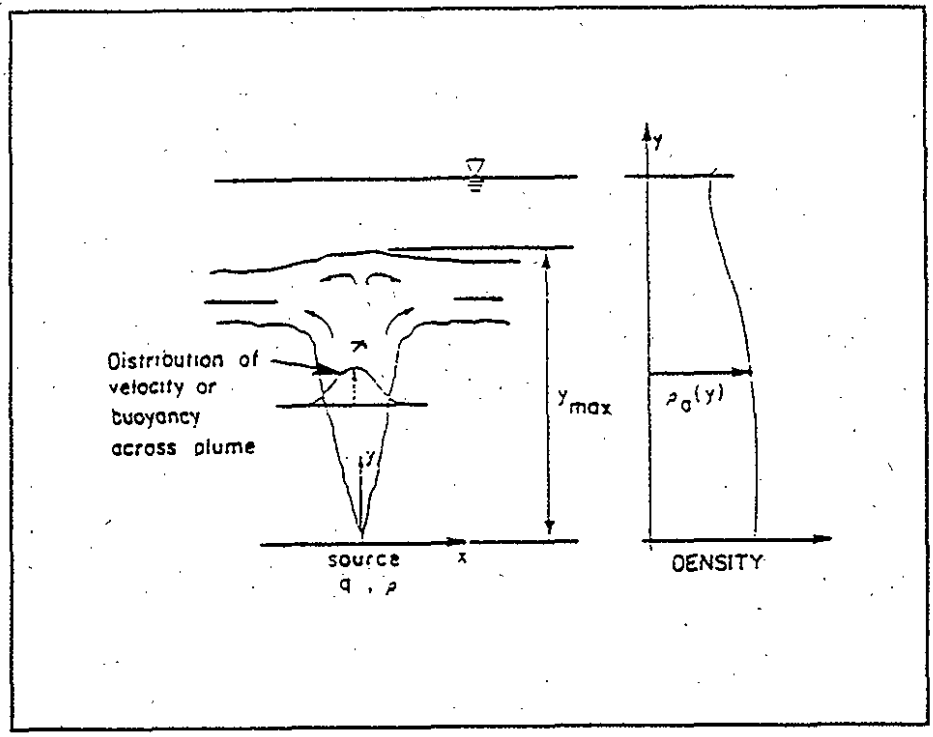


Figure 3.2.2 Flow-chart of wastewater plume

The density difference is greatest between the centre of the plume and the surrounding receiving water. The difference will decline as the distance from the discharge point increases, and the upward movement will decline and finally cease. The effluent plume will have the same structure as shown in figure 3.2.2, but it will be more "feathery" than the plume that is formed as a result of the surplus momentum of the effluent.

Density differences will generally cause more rapid spreading than the surplus momentum of the effluent, and are therefore more important for the dilution process /4/.

Discharges of wastewater having a greater density than the receiving water will result in a plume spreading along the bottom of the receiving water body. The dilution in the plume will be much less than if the effluent had moved upwards in the free water masses. Any peak loadings of toxic substances in the effluent will tend - depending on the adsorptive tendencies of the substances in question - to be evened out by adsorption to the sediment, which will reduce the availability to the water phase. The toxicity of the effluent itself is thus reduced.

The toxic effect of one or several successive peak loads will persist for a longer period of time in the pore water of the sediment, and this may give rise to acute effect concentrations during a longer period. This can lead to chronic effects in the sediment if the toxic substances are adsorbed strongly or if peak loads frequently occur.

The movement of the receiving water will have some effect on the extent of the effluent plume - especially at the "top" of the plume, where the effluent velocity has declined or where the effluent has become neutral relative to the recipient water. A cross-wise current in the recipient can "pull" the top of the plume along with it, and the plume will then spread as a layer in the water column, from

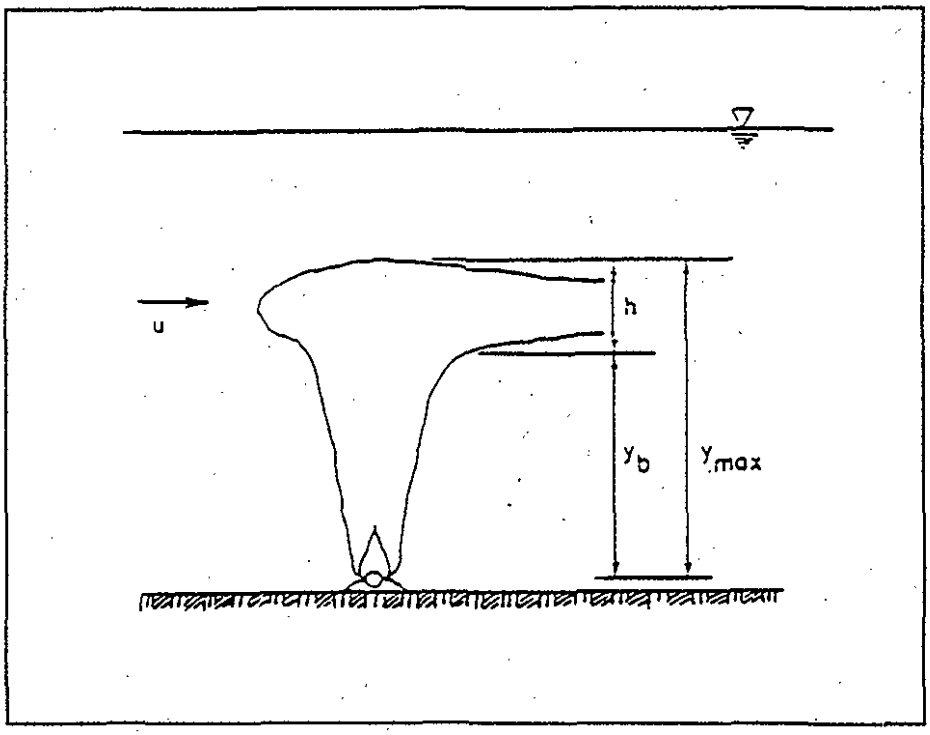


Figure 3.2.3 Spreading of an effluent in a recipient with traversing movement of the water. From /4/.

which the effluent can then spread into the rest of the water column depending on the water movements in the recipient.

A number of correlation equations have been developed which seek to describe the velocity of the effluent jet, the width of the jet and the dilution in the jet as a function of the original momentum and relative density of the effluent, the velocity of the receiving water, and the distance from the discharge point. These equations all contain an element of uncertainty, and the uncertainty increases with increasing complexity of the current pattern in the receiving water. The correlations can not usually be used to give very precise "snapshots" of the spreading of an effluent plume, but are good enough to describe the more general or typical spreading pattern of a given plume. This is often precisely what is needed for evaluating the toxicity of wastewater during the dilution process.

In connection with the calculation of the dilution in a wastewater jet one often meets the expression "initial dilution". There is no generally accepted definition for this expression, and it must therefore be described in each specific case. However, there are a number of generally used interpretations of the concept. In the case of a submerged outfall (figures 3.2.2 and 3.2.3) the initial dilution is the dilution that has been achieved in the axis of the jet when the upward movement of the jet has ceased, i.e. at the top of the jet. Depending on the characteristics of the wastewater and the recipient, this may be at the surface or at a point somewhere in the water column. If the wastewater is denser than the receiving water, two initial dilutions are often used: the first is the dilution at the top of the jet (the jet usually rises some distance above the bottom owing to the momentum of the jet), and the second is the dilution in the axis of the jet at the point where the jet or plume makes contact with the bottom.

3.2.4 *Dilution in the recipient*

After the dissolution of the wastewater jet, where the speed and density of the wastewater determine both the dilution and spreading of the wastewater, the conditions in the recipient become most important for determining the continued dilution and spreading of the wastewater plume. The speed of dilution and spreading depend on the water movements characteristic of the recipient in question.

In running water, the movements are mainly horizontal and in the same direction, but they vary in the course of the year as a result of variations in precipitation and run-off. In lakes and reservoirs the horizontal water movements are small compared with the vertical movements, which can change in direction and speed during the year. In estuaries and coastal waters the horizontal and vertical movements in the near-shore area are determined by a combination of discharges from rivers and streams, tidal movements, and meteorological factors. Further offshore the water movements become increasingly dominated by the wind.

The following sections will discuss the factors that are important for the dilution and dispersion of wastewater in the different types of receiving waters.

Running water

In running water the dilution of discharged effluent depends on the current velocity, the width of the watercourse, and the amount of effluent discharged. The maximum dilution will be achieved more quickly if the current velocity increases or if the watercourse becomes narrower.

Downstream of the outfall the effluent slowly mixes into the entire width of the watercourse. The concentration in the centre of the plume will fall while it slowly increases at the edges (see figure 3.2.4). The concentration across the entire width of the watercourse will asymptotically approach a concentration corresponding to the maximum dilution. The theoretical maximum dilution corresponds to the complete mixing of the effluent discharge in a given time interval (Q) with the entire water volume passing the outfall during the same interval of time ($v \cdot d \cdot W \cdot Q$, where v = velocity of flow of the receiving water, d = average depth of watercourse at the outfall, and W = width of watercourse at the outfall).

After this, the concentration of effluent will only change very slightly, and this concentration will pass on down the river as a slug of diluted effluent. Usually, however, the concentration will fall slowly as a result of inflow from tributaries, groundwater exfiltration, and other effluent discharges.

The distance taken before achieving maximum dilution across the entire water course will depend on the average depth in relation to the width, and the velocity of the receiving water in relation to that of the effluent plume. The shallower and narrower the watercourse, the quicker the dilution process, and the higher the receiving water velocity in relation to the effluent plume, the shorter the time before maximum dilution is achieved.

If the watercourse has sharp bends which give rise to turbulent flow, maximum dilution will be achieved faster.

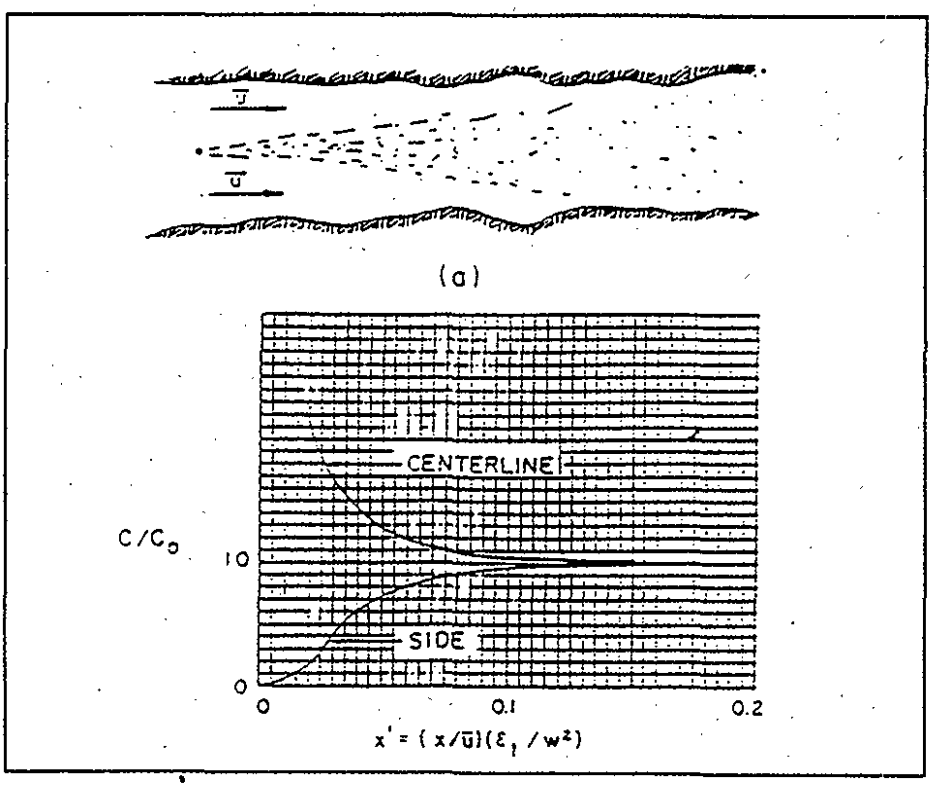


Figure 3.2.4 Concentration of effluent in the centre of the plume (centre line) and at the sides of the watercourse as a function of the distance from the point of discharge. From /4/.

In water courses the critical factors for acute toxicity effects will be the lowest average flow during short periods of time (1 day or thereabouts), while the critical factor for chronic toxicity will be the lowest average water flow over longer periods (7 days or more). The frequency of occurrence of these situations can be determined by checking the lowest water flow figures for a period of 10 years or so.

In the USA the authorities recommend using the lowest average 1-day and 7-day flows measured during the last ten years (1Q10 and 7Q10 respectively) for calculating the extent of the areas around the outfall where acute or chronic effects might be expected /3/. However it should be noted that usually the rule applies that unless a "high rate diffusor" (achieving a diffusion rate of above 3,0 m/s) is fitted to the outfall, no acute effects are permitted in the undiluted effluent. The general requirement to use 1Q10 and 7Q10 also applies for discharges to reservoirs with retention times of less than 20 days.

Lakes and estuaries

In lakes and estuaries the horizontal water movement is often very limited, but dilution of the lake water will still take place because of meteorological variations in the course of the year.

In the autumn the surface water cools and becomes heavier than the deeper water. The surface water sinks towards the bottom and this gives rise to mixing. Strong winds can also increase the mixing process.

In the spring and early summer the surface water is warmed by the sun and becomes lighter than the deeper layers. This stops the vertical water movement caused by the density differences. However,

the wind can still create waves on the surface, but the mixing arising from these movements only affects the upper layers in the water, which in deep lakes are sharply separated from the deeper, cooler layers. The marked boundary between the layers can be detected by the pronounced change in temperature (thermocline). (see figure 3.2.5). Similar situations can arise because of salinity differences (halocline).

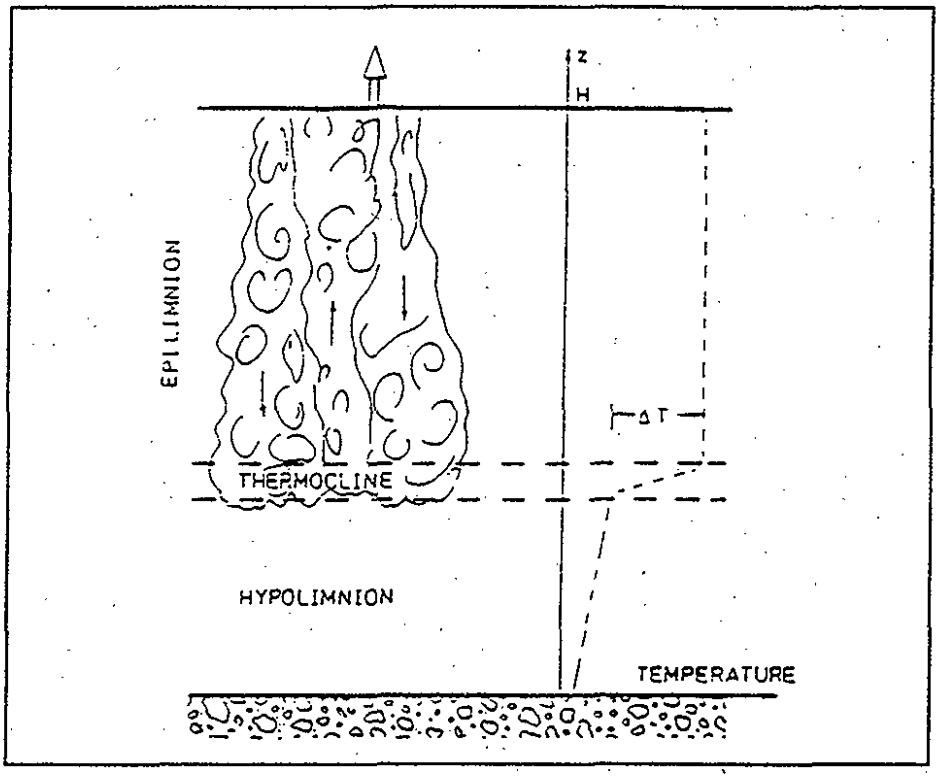


Figure 3.2.5 Temperatures and water movements in a stratified lake. From /4/.

In the summer period solar radiation can give rise to evaporation from the water surface. This can increase the density of the surface water, but at the same time the heating of the water leads to a fall in density, and the vertical water movement induced by evaporation will therefore be counteracted.

Discharges into lakes from streams and rivers, and the lake outflows themselves, can cause water movements in the lake which increase mixing. If the inflowing water is colder than the lake water, or if for other reasons it has a higher density, the inflowing water will tend to follow the bottom and will not mix so much with the upper layers.

Because of the more complex and seasonally variable water movements in a lake, calculation of the mixing of an effluent stream in the lake will be more complicated than in a river. A start can be made by calculating the extent and velocity of the wastewater plume and the concentrations at various parts of it. The next step, calculating how the plume mixes into the lake water, requires the use of mixing models which use the measured seasonal water movements in the lake itself as input together with information about the wastewater plume (see section 3.2.1).

The critical factors in lakes can be identified by analyzing the seasonal variation in water depth, wind speeds and directions, and solar radiation. The density of the wastewater in relation to the lake water may vary through the year, and therefore no particular season can be picked out in advance as the most critical. Under all circumstances, the velocity of the lake water can be set at zero unless it can be documented that there is a permanent current through the lake.

"Closed" estuaries can be treated in very much the same way. Estuaries are parts of the sea where freshwater from rivers meets the salt water of the sea and mixes with it. Estuaries can be divided into:

1) fjords, which are narrow, long and deep, with strong stratification between salt and fresh water,
2) shallow coastal areas where river water gradually mixes with salt water from further offshore,
3) "closed" marine areas, separated from the open sea by spits, bars or the like, which reduce the water exchange.

The inflowing freshwater will have a lower density than the salt water, and will therefore lie on the surface as a separate layer, on top of a layer of salt water.

This type of situation will often exist in a fjord. Wind action on the surface with wave formation will cause mixing between the fresh and salt water layers, thus reducing the salinity gradient up through the water column. This phenomenon is often seen in shallow coastal areas. If the estuary is "isolated" from the sea by sandbanks etc., the inflow of sea water to the area will be reduced and the mixing in the closed estuary will be greater.

Estuaries are also influenced by tidal movements; during ebb tide there is a relatively greater inflow of fresh water, while during flood the inflow of salt water is greater. In fjords the salt water will mainly enter along the bottom because of the density difference. The tidal movements will cause movements in the layered water column which will tend to cause mixing between the layers.

Marine areas

In open coastal areas the tidal movements are of less importance, and a large amount of the mixing will be caused by wind action at the surface and by currents in the deeper water masses.

Calculations of mixing and dilution in marine recipients requires modelling of the water movements on the basis of calculations of the extent and distribution of concentrations in the plume (see section 3.2.1).

In coastal areas the critical factors can be identified by analysing the various parameters (tides, discharges from rivers, wind strength and direction, and stratification). In unstratified areas the critical situation will be a combination of minimum water exchange and minimum freshwater discharge. If stratification occurs, it is necessary to consider both situations: stratification and full mixing. In addition to these critical design situations, analyses should also be made for other situations in which mixing may be better, but where the extent of the plume is greater.

Marine areas require a far better knowledge of the discharge characteristics, the tidal and oceanographic circumstances (spring and neap tides etc.) and topography, than do lakes or rivers.

3.2.5 Simulation models

Today there are a great number of simple and more advanced computer models for simulation of water movements, effluent mixing, and even the fate of individual components in the effluent /1,5/.

Simulation models which describe the spreading of the effluent may be models which only calculate the spread of the plume, or models which also calculate the additional dilution of the effluent when it has mixed into most of the water column. Models of the latter type may also be able to calculate the fate of individual substances. A number of models of this type which are relevant for various types of receiving waters are described below.

Spreading in the effluent plume

Spreading of the effluent plume in marine recipients can be calculated by analytical methods as described above, but more accurate calculations can be made using numerical models (e.g. NEWJET /1/). Numerical plume models can calculate the horizontal extent of the plume, either as a surface plume or a heavy underlying plume. The latter type of calculation will be an approximation, since the model regards the bottom plume as an "inverted" surface plume. This assumes that the bottom is plane and horizontal. In cases where the plume lies at an intermediate level in the water column, it is not possible to make direct calculations of the horizontal extent of the plume, and an analysis of the situation must be confined to the initial dilution and the dilution after mixing into all or most of the water column.

Under all circumstances calculations can be made for various specified current velocities in the receiving water, which permits critical or more representative situations to be studied.

The input to the numerical models consists of the results of analytical calculations of dilution in the effluent jet (initial dilution, width of the jet, etc.)

Spreading in rivers, lakes and similar recipients

Spreading of effluent in rivers, lakes, and other recipients where currents can be treated as one-dimensional along the extended axis can be simulated using numerical models (such as MIKE 11 or MIKE 12 /1/). The simplest of these models (such as MIKE 11) are used for simulation of well-mixed receiving waters, while the more advanced models such as MIKE 12 can be used for stratified recipients. These types of model can be used to simulate spreading in lakes or rivers and in closed marine recipients such as small fjords or harbours.

Both types of model calculate the concentration of the discharged effluent in an area defined by the user. The models take topographical information into account and solve the hydrodynamic and transport/dispersion equations dynamically: in other words the concentration varies with time depending on variations in flow and current, effluent discharge, meteorological factors, etc.

The input to these models consists partly of the topography of the area in question (shape, depth etc.) and partly of figures describing a number of parameters at the limits of the chosen area

(water flow, water depth, effluent concentration or concentration of individual substances, etc.). Since the input section of the model is fully menu-driven, it is far easier to use than similar models have been in the past. PC-based versions are available which have been designed for use by non-specialists.

The results of the dispersion calculations can be displayed graphically, giving presentations of variation with time at chosen points, or variation throughout a chosen area at a given time.

The models can calculate the dispersion of complex effluents, and it is also possible to calculate dispersion of individual substances, taking transformation or breakdown into account.

Spreading in marine areas

In marine areas it can rarely be assumed that current flow is one-dimensional. Usually it is two-dimensional and may be stratified in two layers. The effluent will therefore also spread in two horizontal dimensions, and if stratification exists there may be transfer between the two layers. If the receiving water is well mixed the effluent will mix into the entire water column, except in the immediate vicinity of the outfall where the effluent plume can be detected.

More complicated models such as MIKE 21 /1/ have been developed to calculate dispersion of wastewater in well-mixed marine recipients. MIKE 21 is parallel to the above-mentioned models but describes the situation in two dimensions. The computing capacity requirements are therefore larger, and the model does not run on a PC but on a more powerful Workstation.

When using the model the area to be investigated is divided into a rectangular network of desired grid size. The finer the grid, the more detailed the description of the situation, but the longer the calculations will take. The topography, particularly the water depth, is described in as much detail as the chosen grid size permits. The output from the model consists of the figures for the effluent concentrations at each grid point at the specified times in the specified period. The results can be presented graphically as time series at chosen points, as isoline plots at a chosen time, or as the means for a chosen period.

Corresponding models (such as SYSTEM 22 /1/) for stratified waters are also available.

The input to the two-dimensional models is similar in character to the input to the one-dimensional models described above.

The two-dimensional models can also simulate the dispersion of effluent or dispersion and transformation of individual substances.

Other types of models

A number of other models which can simulate the dispersion in the receiving water of individual substances in a wastewater discharge are described in /6/. Some of the models are simple ones which describe the dilution or fate of individual chemicals. Others calculate the dilution within a number of compartments into which the water system is subdivided. Only a small number of the models can be used for calculations in marine recipients, since only a few of them take tidal movements into consideration.

Table 3.2.1 Outline of model types for simulation of water movements and dilution in recipients.

Surface water	Level	Type	Method
Freshwater	Simple models	Dilution model	RDM DYNTOX PDM3
		Analytical fate model	Reachscan SARAH MEXWA
		Compartment model	QWASI EXWAT
	Advanced models	Hydrodynamic model	EXAMS WASP4 WAQUA XTEM TDTIM
Seawater	Advanced models	Hydrodynamic model	WAQUA XTEM TDTIM

Only rather advanced hydrodynamic models can be used for simulating aquatic systems that are more complex than rivers.

RDM (River Dilution Model), DYNTOX (Dynamic Toxicity Model) and PDM3 (Probabilistic Dilution Model) are simple dilution models for flow in rivers. RDM cannot allow for seasonal variations in current patterns or for daily/weekly variations in the wastewater discharge. In addition, this model does not include removal or breakdown processes in the recipient. When used as a model for specific environments, DYNTOX makes a number of assumptions about the nature of the receiving water which will not always be appropriate. PDM3 assumes that the wastewater discharge is log-normally distributed over the year. This means that batch productions which only operate at certain times of year can not be modelled. The flow in the receiving water (river or stream) is likewise assumed to be log-normally distributed, and this will not be true in larger rivers where there may be large seasonal variations. For making preliminary estimates of wastewater concentrations in watercourses, DYNTOX is probably the most suitable model.

Reachscan, SARAH (Surface Water Assessment Model) and MEXWA (Model of Exposure Assessment in Water) are simple mixing models which include a description of the fate of chemical substances. Reachscan combines a database, in which the river system is described, with a simple model for the mixing in the recipient. Downstream concentrations can be calculated in a dilution model or

in a fate model which includes adsorption, evaporation and degradation. The database describing the river system consists of average figures, and a highly simplified fate model is used, so that the model as a whole should only be regarded as a tool for simple screening of the fate of chemical substances in watercourses. SARAH is a steady-state model which calculates dilution, diffusion, adsorption, evaporation, hydrolysis, and bioaccumulation in fish. This model has been developed to permit evaluation of human exposure via foodstuffs (fish) and drinking water (surface water). The method does not include biodegradation and can not model accumulation/concentration processes in sediment and bottom water. MEXWA subdivides the dilution calculations into a mixing process close to the outlet, where both horizontal and vertical dispersion are included, and mixing in the receiving water, where the extent and horizontal dispersion of the wastewater plume are described. The second phase also includes evaporation, adsorption, and degradation. In a third phase, the model calculates dilution, evaporation, adsorption and degradation in the far field. The model is theoretical and assumes that there are no bends in the river and that the current velocity is constant. For later and more detailed studies of wastewater concentrations MEXWA appears to be the preferred model, since it includes both horizontal and vertical dispersion and adsorption to the sediment.

The modified QWASI Model and EXWAT (Exposure of Surface Water Bodies) are compartment models which subdivide the receiving water system into a number of boxes. QWASI divides it into water, bottom sediment, suspended matter, and biota. Because of the general lack of information about distribution and diffusion coefficients, these are assumed to be the same for all substances. Another weakness is that the model assumes that the concentration of each substance is homogenously distributed within each compartment. EXWAT is a further development of QWASI and consists of a number of boxes consisting of water with suspended matter and biota, and sediment with pore water. The model includes sedimentation processes and resuspension of suspended matter, and calculates the distribution between water/suspended matter and sediment/pore water, and the exchange between pore water and water. The model includes evaporation, degradation and bioaccumulation. It can only calculate steady-state situations and cannot calculate dilution in stratified water bodies. The possible influence of temperature is not taken into account. If an analysis of the dispersion of individual substances in a recipient is needed, EXWAT is the best choice of model, since this model can estimate concentrations in specific compartments (e.g. bottom water or sediment) on the basis of relatively simple input.

EXAMS (Exposure Analysis Modelling System) and WASP4 (Water Analysis Simulation Programmes) are refined hydrodynamic models which can be used to simulate fresh water systems. Both are compartment models which describe the receiving water system in terms of boxes, with an exchange of substances between the boxes as a result of water transport. Within each box the adsorption and sedimentation, evaporation, bioaccumulation, abiotic transformations (photolysis and hydrolysis) and biodegradation are calculated. EXAMS cannot be used to simulate short-term discharges at high concentra-

tions. The model can only simulate low concentrations of individual substances (concentrations lower than 50% of their solubility). EXAMS also assumes that the discharge is so small that the substances in the effluent do not affect the general characteristics of the receiving water system (pH, dissolved oxygen, biomass etc.). The same restrictions apply to WASP4, which in addition is unable to simulate dispersion of a wastewater discharge with a density different from that of the receiving water. Both WASP4 and EXAMS are highly developed models which require detailed information about the receiving water and the discharge before they can calculate the dispersion of the wastewater.

WAQUA (Water Quality Model), XTEM (Xenobiotics Transport and Effect Model) and TDTIM (Transport and Influence Model) are refined hydrodynamic models which can be used to simulate both fresh water and marine areas. The models use a two-dimensional grid in which the concentrations at each node are recalculated with each iteration of the model. All these models take removal and breakdown of chemical substances into account. WAQUA can only be used for simulation of water bodies with low degrees of bottom inclination and without stratification. Mixing is assumed to occur mainly by turbulent diffusion. All breakdown reactions are assumed to be linear. XTEM and TDTIM calculate the water movements on the basis of information about wind speeds and directions, water inflow and outflow, topography, and density differences. In addition, the models take into account the possible inhibition of microbial activity as a result of substance concentrations, and the effects of temperature on degradation processes. XTEM can only simulate one water layer, while TDTIM can simulate several layers simultaneously. For each simulation in XTEM only one removal process can vary in speed throughout the simulated area; in addition, all reaction parameters are assumed to be constant with the exception of the temperature effect. TDTIM does not have this limitation, but on the other hand this model is expensive to use, both at runtime and beforehand when collecting the necessary information. Thus the three models WAQUA, XTEM and TDTIM represent an increasing level of complexity. This means that the models should be used in that order, depending on the level of detail desired.

Conclusion

For calculating wastewater dilution, one can begin with relatively simple calculations of the initial dilution - and the dilution in the near field in freshwater recipients - using the relationship between the discharge velocity and the current velocity in the recipient. In marine recipients with tidal movements, the water movements are more complicated, and therefore the simple relationships (mass balances) cannot be used. For more precise estimates of dilution and dispersion, relatively simple computer models can be used as a start, and later (if necessary) more complicated models can be brought in, together with the necessary expert assistance for developing the model scenario.

If the concentrations of individual substances are expected to be important for the ecotoxicological load on the receiving waters, computer models may be used which can simulate the dispersion and dilution of individual substances. These models can include some or

all of the following parameters: biological/chemical/photochemical degradation, and removal by adsorption or evaporation.

3.2.6 Examples of PEC calculations

Rivers

Before issuing a discharge permit to Grindsted Products A/S, Ribe County carried out an evaluation of the dilution in the receiving water (Grindsted Stream). The evaluation was carried out on the basis of water transport measurements in Grindsted Stream measured in 1987 at Eg Bridge (10 km downstream of the discharge point); these measurements were recalculated to give the water flow at Tingvejen just upstream of the discharge /7/. The median minimum daily flow at Tingvejen was found to be 976 l/s.

Since Grindsted Products had applied for a permit to discharge 20 l/s, the dilution after complete mixing (used here as "initial dilution") could be calculated to be an average of 48.8 times (= 976/20). In the discharge permit it is noted that the mixing does not occur instantaneously, but that investigations of pH profiles in the river had indicated a mixing zone extending some few hundred meters downstream of the discharge point.

The calculated dilution factor and the knowledge that the mixing was not instantaneous were also used when setting the discharge criteria for the wastewater toxicity.

Estuaries

Discharge of wastewater from Junckers Industrier A/S in Køge Bay is an example of a discharge to a recipient where tidal movements are not very important for the dilution /9/. The wastewater is discharged through a diffusor 5.3 km from land at a depth of 10 m. In 1987 the discharge rate was approx. 58 l/s. The discharged wastewater is warmer than the recipient water, but owing to a large content of cellulose fibre the density is greater than that of the recipient seawater.

For 25% of the time, however, the recipient water is assumed to be stratified, so that the wastewater is less dense than the bottom water around the discharge point, and an upwardly moving effluent plume will form, with a dilution along the central axis of between 3 and 15 times. However, the stratification in Køge Bay is not thought to be so pronounced as to cause the plume to reach the surface.

For the remaining 75% of the time, it is assumed that the wastewater will be denser than the bottom water, so that the effluent plume will spread along the sea bed with an initial dilution factor of about 3. Model calculations carried out by the Danish Hydraulic Institute show that for 50% of the time, the effluent plume will spread to the south-east. 1 km from the discharge point the dilution at the center of the plume is approx. 100 times - more or less regardless of the current speed and direction. At 5 km the current is more important and the dilution at this distance from the discharge point will vary between 300 and 1000 times.

Marine areas

The wastewater discharge from Cheminova A/S into the North Sea is an example of wastewater discharge to a coastal recipient /8/. The discharge point is approx. 500 m from the shoreline at a depth of about 8 m. The discharge permit states that if the wastewater has the

same temperature as the receiving water, it will be denser 20-30% of the time, and will therefore spread along the seabed. Therefore the permit stipulates that unless it can be proved that the wastewater is warmer than the seawater and therefore will rise towards the surface, measures must be taken to ensure that the discharge mixes into the entire water column above the discharge point. This is expected to give an initial dilution of at least 20 times.

The total picture of the dilution occurring in the near zone is calculated as a series of distribution functions for the dilution at different distances from the discharge point. The frequencies are determined from model simulations based on current measurements. Thus it is stated that the dilution factor 200 m north of the discharge point will be greater than 525 for 90% of the time, and greater than 360 for 95% of the time. 200 m south of the discharge point, the dilution will be greater than 1000 for 90% of the time and greater than 555 for 95% of the time.

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3.3 Degradability

An assessment of the degradability of chemical substances has as its ultimate aim the determination of the speed of transformation of the substance in the environment. This information, together with information on the volatility, adsorptive ability, and solubility of the substance, may be used to evaluate or even to calculate the concentration of the substance in the recipient. The result can then be compared with the effect levels for the substance.

When evaluating the degradability of chemical substances, a great number of factors can influence the degradation of one particular substance. The general biodegradation route for organic substances under aerobic conditions is the full degradation of the organic material to carbon dioxide and water. This process releases energy and organic carbon which the microorganisms responsible for the biodegradation utilize for growth and maintenance of metabolic processes.

Degradation of a specific substance within this general process depends especially on:

- the organic substance
- the environment in which the substance occurs
- the species and populations of microorganisms present.

When individual substances in a mixture are biodegraded, the other substances may affect the degradation process compared with the situation where the substance in question is the only energy and carbon source. The differences may be due to alterations in the characteristics of the substance in question or to changes in the way in which the microorganisms exploit the substance.

3.3.1 Intrinsic properties

The intrinsic properties of the organic substance which influence its biodegradability are primarily related to its chemical structure:

- molecular weight and molecular size
- degree of polymerization
- aromatic or non-aromatic character
- degree of substitution
- amount of branching in the molecule

Biodegradability generally declines with increasing molecular weight and size, with increasing degree of polymerization, increasing number of aromatic rings, degree of substitution, and branching of the molecule. In addition, factors such as solubility in water and toxicity towards microorganisms play a decisive part, with increased solubility increasing biodegradability and increased toxicity generally reducing biodegradability.

The relationship between biodegradability and *molecular weight and size* arises because molecules with molecular weight above 500 - 800 have difficulty in passing through the microorganisms' cell membrane. Degradation of high molecular weight substances therefore

requires the presence of exoenzymes (enzymes released from the microorganisms into the surrounding environment).

The relationship between biodegradability and *aromatic or non-aromatic character* is the empirical observation that substances with three or more aromatic rings are degraded with great difficulty. The explanation lies partly in the high molecular weight of such substances, and partly in the lower solubility of substances with several aromatic rings. In addition, the breakdown of aromatic rings requires the presence of oxygenases to hydroxylate and break open the rings.

The relationship between biodegradability and *degree of substitution* of aromatic rings or branches in the molecule for example, is more complex. Biodegradability increases with an increasing number of substituents, but the degree of biodegradability also depends on the chemical characteristics of the substance and the position of the substituents in the aromatic ring /15/.

This connection between biodegradability and chemical structure has been developed into a number of "rules of thumb", which can predict which chemical structures may be expected to be biodegradable /15/. During the last decade attempts have been made to identify more precise correlations between biodegradability and chemical structure, but progress has been slow, and no general models seem to have reached a level where they are suitable for practical administrative use. One difficulty in developing empirical correlations is that the data for biodegradability are rarely quantitatively comparable /7,29/.

One of the greatest difficulties in developing structure-biodegradability relationships is that tests carried out on the same substance, but using different populations of microorganisms, may give different results. This has been demonstrated, for example, with 4-nitrophenol, which in some tests turned out to be fully biodegradable and in other tests was not degradable at all /15,18/. This variation in test results is due to differences in the microorganism species and the population present in the test system when the test was carried out, and this in turn depends on the inoculum used to set up the system.

The solubility of a substance can often set limits for its biodegradability. The solubility in water may be increased if it is mixed with other substances such as solvents, surfactants, or emulsifiers. These increase the availability of the substance to the microorganisms, and may thus increase the biodegradability. These effects are exploited in order to be able to test the biodegradability of poorly soluble substances /22/.

Other types of substance may reduce the solubility of the test substance by forming complexes or adsorbing it. Thus the biodegradability of 2,4-D-esters and substituted phenols is reduced in the presence of humus /25,30,31/.

Increased solubility does not necessarily lead to increased biodegradability of individual substances, however, since the increased solubility may also lead to available concentrations so high that they inhibit the microorganisms.

The presence of several individual substances, which on their own do not have an inhibiting effect at the relevant concentration,

may lead to inhibition as a result of additive effects. This has been observed with mixtures of chlorophenols.

3.3.2 Biomass

It has been shown that the origin of an inoculum is very important for the biodegradability of poorly degradable substances. 4-nitrophenol was found to be broken down in 3 days by microorganisms from fresh water, but in 70 days by microorganisms from sea water /14/. In a test system using only one substance as carbon and energy source a rapid selective growth will take place of those microorganism species which are best able to exploit that substance. This leads to a change in the composition of the biomass in the direction of a few dominating species.

If initially there are no microorganisms present which can utilize the carbon and energy source, the substance in question will not be broken down immediately. Biodegradation may however begin after a shorter or longer period which is termed the *lag phase*. During this period some of the microorganisms present develop enzyme systems which are able to exploit the test substance. The formation of new enzyme systems can take place through expression of inactive genes, through modification of existing enzyme systems, or through transfer of plasmids from one species to another, enabling them to exploit substances both as energy and carbon source.

In addition, an apparent lag phase may be observed because the microorganisms which are able to break down the test substance are only present in small numbers and only multiply slowly on the substrate. This increase in numbers may not take place before other more accessible carbon sources in the test system have been used up.

The reason for an apparent lag phase is poorly understood and can vary from substance to substance, and from microorganism population to microorganism population. This also makes it possible that a similar adaptation of microorganisms towards degradation of a specific substance may not always occur in natural environments, where there will always be alternative supplies of carbon and energy available.

Biodegradation of individual substances in a mixture may be affected by:

- changes in degradation mechanisms
- competition between microorganisms for energy and carbon sources
- changes in production or activity of enzyme systems.

The result of a test for biodegradability of an individual substance as sole energy and carbon source can be:

- mineralization
- transformation without growth
- transformation with growth
- no transformation

Mineralization means that complete biodegradation occurs to the end products carbon dioxide and water. Transformation means that biodegradation ceases at a certain stage where a stable metabolite is formed. Transformation can occur both with and without growth, depending on whether the part of the molecule that has been removed can be broken down to carbon dioxide and water and thus supply carbon and energy for growth. These four mehanisms for breakdown of individual substances are shown in figure 3.3.1. Below the stippled line in figure 3.3.1 are shown the four possible results for an individual substance in a biodegradability test for a mixture of substances:

- no transformation owing to diauxy
- mineralization
- transformation
- no transformation

These types of biodegradation are grouped under the term co-metabolism /17/.

Mineralization

Substances which are mineralized when used as the sole carbon and energy source, are broken down either by enzyme systems which are always present at a certain concentration and activity (*constitutive enzymes*), or by enzyme systems which first must be induced, developed/modified, or transmitted by plasmids (*adaptive phase*).

Xenobiotic substances which can be biodegraded by constitutive enzymes will usually be broken down more rapidly in the presence of other carbon sources. This is due to the fact that the biomass in the system will be further increased because of the greater supply of energy and carbon /16,19/.

The adaptation process can be affected by the presence of other organic substances. If the breakdown of some of the other organic substances involves the same enzyme systems as for the substance in question, the adaptation process may be shortened because of the increased induction of the enzyme systems (*analogous induction*) /11/. The growth in biomass on the other organic substances is less impor-tant for the length of the adaptation period /15/.

The presence of readily degradable organic substances, which can increase the biomass in the system, can at the same time inhibit the biodegradation of other substances /15,24/. The reason for this phenomenon has not been determined in all situations, but in the case of carbohydrates it has been shown that the inhibition is due to *catabolite-repression*, either by reducing the activity of the enzymes which do not take part in the carbohydrate degradation, or by reducing the production of these "superfluous" enzymes. This phenomenon is known as "*diauxy*".

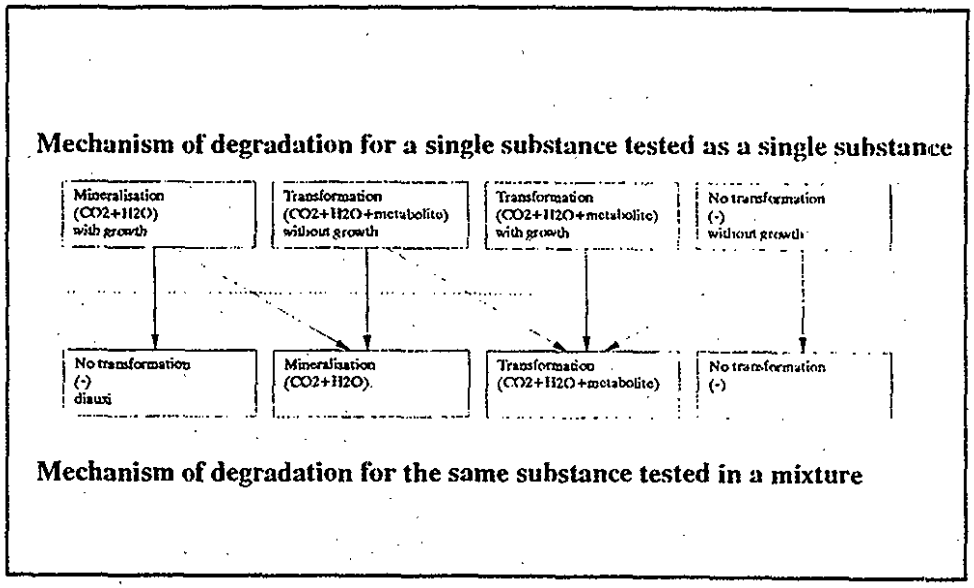

Figure 3.3.1 *Possible degradation results for a single substance tested in a single-substance test and corresponding results when tested in a mixture of substances.*

Transformation with growth

Transformation with growth occurs when the substance is split into two products, one of which can be degraded and support growth. The biodegradability of the less degradable metabolite will normally be unaffected by the addition of other readily biodegradable carbon sources /22/. The biodegradability of the substance in question will only be increased in those cases where analogous substances are present amongst the added substances which - unlike the substance in question - can induce active enzymes.

Transformation without growth

Substances which when supplied as the only carbon and energy source are transformed without causing growth in biomass are not a suitable carbon and energy source for the growth of the microorganisms, and they are transformed into stable metabolites. When other organic substances are added which can act as carbon and energy source, the stable metabolites may be broken down, if the appropriate enzyme systems are induced by the other organic substances.

This phenomenon is today known as *co-metabolism*. Previously it was called co-oxidation, because it had only been observed in connection with oxidative processes /15/. Co-metabolic ability is related both to the substance and to the environment in which it is biodegraded.

Co-metabolism is often used as a term for biodegradation mechanisms for xenobiotic substances at low concentrations, since in such circumstances significant biomass growth can not be measured. At these low concentrations of xenobiotic substances the expected growth will often be small compared with the growth arising from the exploitation of other organic substances that are present, and the growth due to the xenobiotic substance can therefore not be measured. It is therefore possible that the term is used incorrectly.

No transformation

Substances which are not transformed when present as the sole carbon and energy source are usually poorly degradable because of the chemical structure of the molecule, which cannot be transformed by enzymatic processes. Such structures include for example ether bonds, quaternary carbon atoms, branched carbon chains, and halogenated groups. It is therefore not to be expected that the addition of other organic substances will affect the biodegradability.

Other mechanisms

The presence of other carbon sources in the complex mixture may also influence the degradation of the substance in question in other ways than by direct activation or inhibition of the active degradation enzymes.

Transport across the cell membrane may be increased by the induction of transport enzymes, which increases the transport of the substance and thus increases the rate of its biodegradation.

A complex mixture of energy and carbon sources can ensure a high diversity in the biomass. This can result in increased biodegradation of the individual substance, since a number of substances - especially xenobiotic substances - are transformed by one type of microorganism, whereas their metabolites are mineralized by other types.

3.3.3 Environmental factors

The most important environmental factors influencing the biodegradability of a substance under aerobic conditions are the following:

- oxygen concentration
- concentration of the test substance
- nutrients, trace elements and vitamins
- temperature
- pH
- particles and available surfaces
- other organic substances.

The minimum *oxygen concentration* required for biodegradation of organic substances under aerobic conditions is about 1 mg/l for most microorganisms. At lower oxygen concentrations biodegradation may take place using other electron acceptors instead of oxygen /3/. Under anoxic conditions, nitrate is used and reduced to nitric oxide; under anaerobic conditions, sulphate is reduced to hydrogen sulphide, and under strictly anaerobic (methanogenic) conditions, organic substances such as formiate, acetate, are used as electron acceptors. In the following we shall only consider aerobic conditions.

The rate of biodegradation for organic substances is generally highest at *pH values* of 6-8. In addition, the rate increases with increasing *temperature*. For each bacterial species there is one optimum value for pH and temperature at which maximum growth occurs. A change in pH and/or temperature can thus influence the composition of the bacterial population. If the test substance can only be biodegraded by one or a few species, a change in conditions towards the optimal values for these species will ensure a higher rate of degradation.

Particles and surfaces influence the rate of biodegradation, because substances with low solubility (high partition coefficient n-octanol/water, log P_{ow}) have a tendency to adsorb to particles and surfaces. This will reduce the availability of the substance for the microorganisms.

In test systems with a high biomass, adsorption will be significant and the rate of biodegradation low, since the concentration in the water phase will remain in constant equilibrium with the adsorbed concentration. Adsorption is a rapid process (timescale on the order of hours) and as a rule can therefore be distinguished from biodegradation by measuring the dissolved concentration a few hours after adding the test substance.

The *concentration of test substance* also influences the rate of biodegradation. High concentrations may be toxic and may inhibit the breakdown process - or even stop it completely. Certain types of xenobiotic organic substances seem not to be broken down at very low concentrations /32/. This phenomenon may arise because the substances are found in so low concentrations that adaptation of potentially biodegrading microorganisms does not occur. The reason may be that the very low concentrations only give a very short contact time between organic substance and microorganism, or that the energy consumption during the adaptation process is greater than the energy obtained from the biodegradation of the small amount of substance that is available, which hinders the growth of actively biodegrading microorganisms.

In addition to energy and carbon sources, microorganisms require *nutrients* at relatively high concentrations and *trace elements* at low concentrations to enable them to form new cells and to obtain energy through metabolic processes. The nutrients comprise nitrogen (nitrate or ammonium), phosphate, sulphate, calcium and magnesium. The trace elements include iron, copper, manganese, and zinc. In addition, certain microorganisms require *vitamins* of the B-group or substances which can be transformed into B-vitamins.

Other organic substances can play a role in the biodegradation of xenobiotic substances, since some of these can only be broken down in the presence of other organic substances. The reason for this phenomenon, which is known as co-metabolism or simultaneous degradation, is that the substance is biodegraded by the enzyme systems of the microorganisms without contributing energy to support their growth. This energy must therefore be obtained by metabolizing another organic substance /8/. This phenomenon manifests itself as a linear (non-exponential) biodegradation kinetic.

3.3.4 Methods for studying biodegradability

Individual substances

With the aim of harmonizing international test methods for use when evaluating chemicals, OECD has standardized a number of test methods for the study of biodegradability, and has outlined a strategy for testing /19,20,21/. This strategy and the standardized methods have by and large been adopted by the EC /6/.

The test methods aim at identifying the readily biodegradable chemicals using a relatively simple procedure (*screening*). The biodegradability is defined on the basis of the extent of degradation of the

substance after a predetermined period of time, taking into account the favourability of the conditions in the chosen test system towards biodegradability.

The term "*readily degradable*" is applied to substances which are degraded "significantly" using test methods which are relatively unfavourable to biodegradation. "Significantly" is here taken to mean removal of 70% of dissolved organic carbon (DOC), 60% of the theoretical oxygen demand (ThOD), or 60% formation of the theoretical carbon dioxide quantity ($ThCO_2$), depending on the parameter measured in the test method. The degradation must have started within 28 days, and must reach one of the above-mentioned figures within 10 days after the start ("time window"): start of degradation is defined as the time at which degradation exceeds 10%. The requirement of a 10-day time window ensures that degradation proceeds rapidly.

The unfavourable conditions for biological degradation are reflected in the low biomass (10^2-10^6 cells/ml), in the relatively low ratio between biomass and test substance concentration (2-100 mg/l), and in the short test period. Regardless of the fact that the biomass is low in the test methods, there is great variation in the size of the biomass in the different test methods, however, which leads to differences in the degradative capacity /2/.

OECD has attempted since 1988 to achieve greater harmonization of the methods with regard to the size of the biomass. At the present time this has resulted in a proposal to standardize the methods into two groups, one with 10^6-10^7 cells/ml (30 mg SS/l, activated sludge) and one with 10^2-10^3 cells/ml (15 ml/l, secondary settled wastewater).

The test methods in the first group are:

- Modified AFNOR Test (OECD 301A)
- Modified Sturm Test (OECD 301B)
- DOC Die Away Test (ISO standard)

In addition the Modified MITI(I) Test (OECD 301C) may be used since this test contains the same amount of biomass, although it uses a different test medium.

The test methods in the second group are:

- Closed Bottle Test (OECD 301D)
- Modified OECD Screening Test (OECD 301 E).

All the test methods have the common feature that the test substance is added as the sole carbon and energy source. In addition, nutrients are added to the test medium at a concentration sufficient for growth during the breakdown of the carbon source. In the MITI(I) test a different nutrient composition is used from the other tests.

The term "*inherently degradable*" or potentially degradable is applied to substances which are biodegraded in test systems favourable to biodegradation. In these tests the substances must be broken down by over 70%, measured as DOC, within 28 days.

The favourable conditions for biodegradation are ensured by the presence of a high biomass (10^6-10^7 cells/ml) and a high con-

centration of the test substance (200-1000 mg/l). In addition, the tests may be performed over a longer exposure time which gives better possibilities for adaptation. The high biomass is obtained by using activated sludge as inoculum (seeding material).

The test methods that may be used are:

- Modified Zahn-Wellens Test (302A)
- Modified SCAS Test (302B)

As with the test methods for ready degradability, adequate quantities of nutrients are added to ensure degradation. A buffer is also used to ensure a pH value within the range 6-8.

Substances which are degraded by less than 20% in test methods for inherent degradability are considered to be *poorly degradable*, and may also be referred to as *persistent*. Substances which are degraded by 20-70% are normally considered to be degradable, but it is possible that this degradation only goes to the stage of stable metabolites.

The OECD strategy for testing of chemicals for ready or inherent degradability is shown in figure 3.3.2.

Substances which are found to be readily degradable in the OECD test strategy are expected to be degraded rapidly in natural aquatic environments (for example in receiving waters), and it is thought that they will not occur in significant concentrations. If the discharge concentration is high, however, areas with significant concentrations may occur in the region around the discharge point. In these cases it may be necessary to carry out simulation tests to determine the rate of biodegradation and, from this, the concentration gradients around the discharge point.

Substances which are not readily degradable but are inherently degradable are not necessarily degradable at all in natural environments such as fresh water. In such environments the degradability and rapidity of degradation must be determined using tests which simulate the environment into which the substances are discharged.

The principle behind *simulation tests* is that they use water, sediment, and/or activated sludge from the environment into which the substance is to be discharged, as the inoculum for the degradation test. In addition, the test substance is added in realistic (low) concentrations, possibly using ^{14}C-marked preparations, and the temperature of the system is also kept at the level found in the receiving water. Simulations tests give an absolute measure of the rate of degradation.

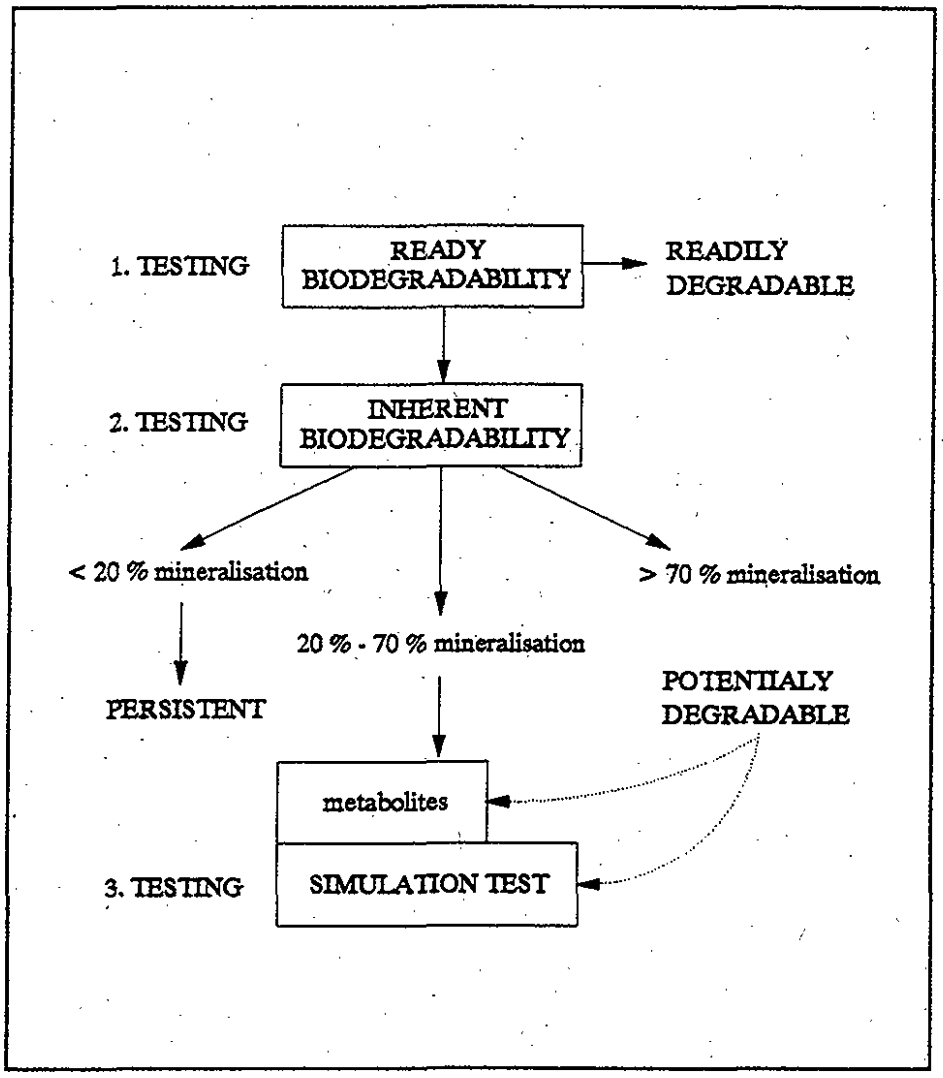

Figure 3.3.2 OECD's strategy for investigations of biodegradability of chemicals.

OECD has subdivided simulation tests into the following categories:

- biological treatment plant (aerobic)
- biological treatment plant (anaerobic)
- river
- lake
- estuary
- marine
- soil

So far, however, standardized specifications have only been developed for simulation of aerobic treatment plants. Work has gone on for some time to standardize the methods for surface waters /27/, but standardization of methods for simulating these environments is difficult, because the environments are so complex, and according to OECD's principles the results of the tests must be valid for all situations where the simulated environment might be encountered. In Denmark, simulation tests have been carried out for degradation of

organic substances in seawater and in seawater/sediment, in order to evaluate discharges of wastewater /34,35,36/.

Mixtures of substances

The purpose of investigating the degradability of mixtures of substances is to investigate the effect of the presence of other organic substances which can serve as carbon and energy source. Investigations of this type can either focus on known substances in the mixture, on the total quantity of organic substances, or on the characteristics of the mixture.

The method used so far for investigating the degradation of mixtures has been the analysis of oxygen consumption over a 5 day period (BOD_5). This sort of analysis is only relevant if the theoretical oxygen demand of the mixture is known, for example in terms of the chemical oxygen demand, COD. Poorly degradable substances at low concentrations will not be measured by the test because of the low oxygen consumption. Because of this uncertainty, the method is not appropriate for investigating the potential content of persistent compounds in a mixture /5/.

Lindén *at al.* /13/ have suggested the use of one of the screening methods for ready degradability of individual substances (Modified OECD Screening Test) for investigating the degradability of mixtures. The criterion for ready degradability is proposed to be 70% removal of DOC in 28 days. However, this says nothing about the presence or absence of persistent substances in the remaining 30%. If 30% of the organic material is still undegraded after 28 days it would seem more reasonable to conclude that the remaining material is persistent, and it would therefore be reasonable to proceed to an evaluation of the remaining material with regard to fate, bioaccumulative tendency, and toxicity.

de Kreuk & Hanstveit /4/ have concluded that neither DOC nor specific analyses are sufficient to evaluate the degradability of mixtures, since it is impossible to analyse all the individual substances in the mixture. Instead, they propose using a combination of sum parameters such as DOC, AOX (adsorbable organic halogens) and finger-print analyses, supplemented with functional analyses such as toxicity tests. Finger-print analyses consist of an incomplete chemical characterization, and degradability is evaluated solely on the basis of whether the observed response disappears. If all responses do not disappear completely, a further characterization of the remaining organic material must be performed with regard to bioaccumulation and toxicity.

A further development of the above-mentioned strategies for investigation of complex mixtures has been utilized in Denmark in recent years /15,37,38/. The strategy is described below.

Readily degradable substances in mixtures may be investigated using a test method corresponding to the Modified OECD Screening Test, adding the mixture at a concentration corresponding to about 5-40 mg DOC/l. Nutrients and inoculum are added corresponding to about 1-5 ml secondary wastewater or surface water per liter. Degradation is followed by means of DOC analyses, and the amount of organic substance removed after 28 days is considered to

be the readily degradable fraction. The remainder may be inherently degradable or degradable in the environment.

Inherently degradable substances may be investigated in mixtures by means of a test method corresponding to the Modified Zahn-Wellens Test. The mixture is added at relatively high concentrations (100-500 mg DOC/l), with nutrients at the same concentration as in the original Modified Zahn-Wellens Test. The medium is inoculated with receiving water, possibly with added sediment, in order to achieve a high biomass of 10^5-10^7 cells per ml. If sediment is added, problems may arise - as in the Modified Zahn-Wellens Test - with adsorption of certain substances. During degradation the medium must be aerated (ordinary air) to ensure a high partial pressure of oxygen, and the emergent air must be refluxed through a cooler to reduce evaporation of water and test substances. The test apparatus is shown in figure 3.3.3.

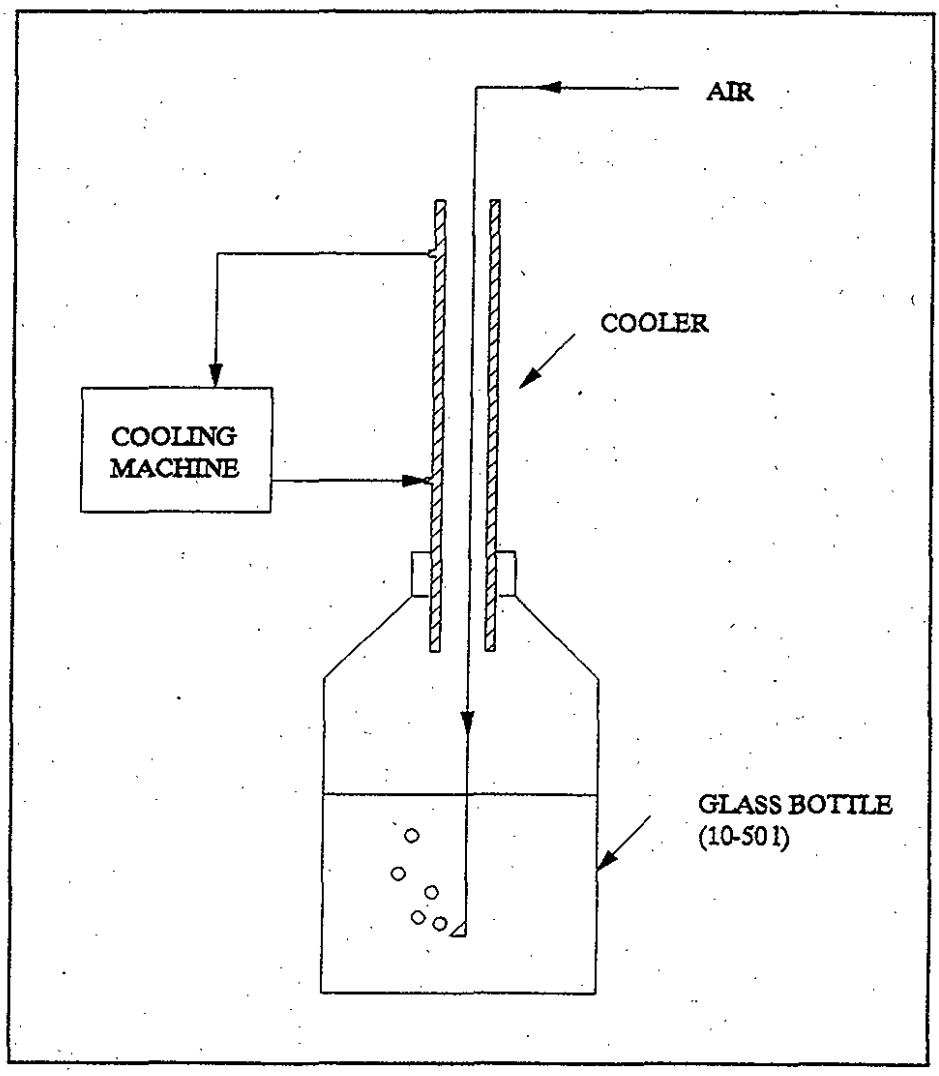

Figure 3.3.3 Test apparatus for degradation test for mixtures.

During the test the degradation is followed using sum parameters such as DOC and AOX and, if required, by screening for toxicity (for example, the Microtox test). At start and finish samples are removed for tests of acute and chronic toxicity on various organism

groups (such as bacteria, algae, crustacea and fish), chemical analysis of the individual components, and screening for potentially bioaccumulative substances. Typical results from such a degradation test on a mixture are shown in figure 3.3.4.

Individual substances which are broken down by more than 20% during the test period may be characterized as inherently degradable, whereas substances which are degraded by less than 20% must be regarded as persistent.

The fraction of the mixture which is not readily degradable, but which may be inherently degradable, is not necessarily degradable in the receiving water. This can be tested for in a *simulation test*, which can be carried out as a batch or continuous flow process. Simulation tests employ /1,16/:

- natural receiving water both as dilutant and as inoculum. For simulations of shallow receiving waters, sediment may also be added at concentrations of 1-10 g SS/l /26/.

- low concentrations of the mixture, typically corresponding to the initial dilution of the wastewater in the recipient. In some cases, one ^{14}C-marked substance is added in small quantities so that the degradation of this marker substance can easily be followed /28/.

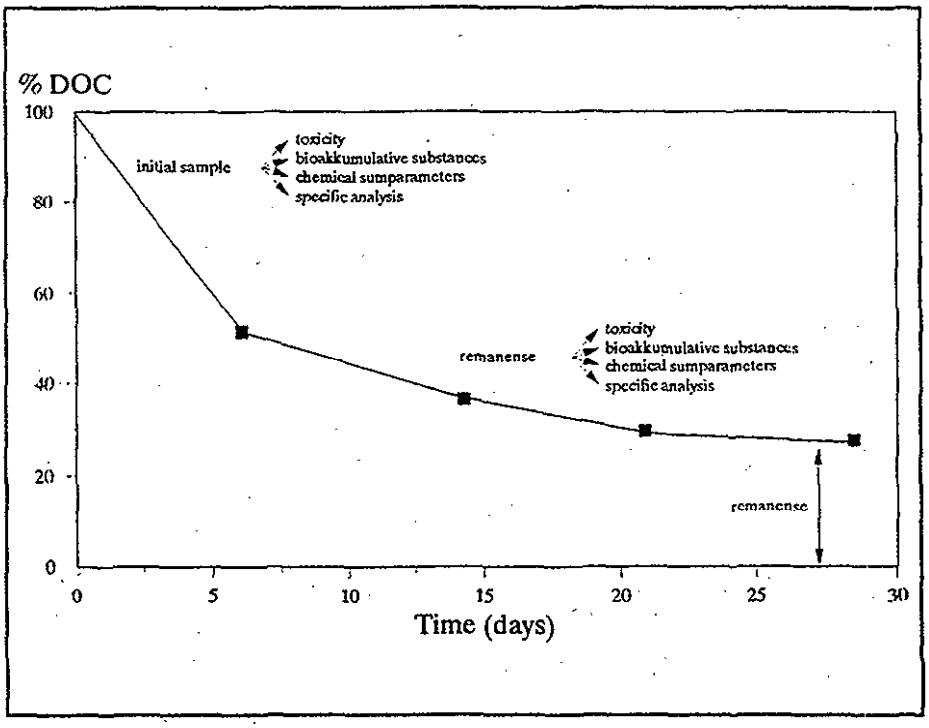

Figure 3.3.4 Degradation sequence for a mixture, measured by DOC, and tests of initial sample and remanence.

Sediment may be added in simulation tests as an extra biomass, if the objective of the test is to determine the speed of degradation in this part of the receiving environment. The progress of degradation can be followed in batch tests by making specific analyses or

by adding low concentrations of ^{14}C-marked substances to the wastewater. Batch simulations have an inherent source of error in that the nutrients and easily degradable carbon sources become used up in the course of the degradation, so that degradation may be limited by a lack of nutrients or primary substrate. In such cases the rate of degradation must be determined from the first part of the degradation process.

This source of error can be removed by using flow-through tests, for example in a chemostat, where fresh recipient water and wastewater are added continuously at relative concentrations corresponding to the expected concentration of wastewater in the recipient. The rate of addition determines the residence time in the chemostat. The rate of degradation is determined by comparing the concentration in the outflowing water with the concentration of the input. If the rate of degradation is low, a long residence time in the chemostat may be necessary in order to be able to determine the rate with sufficient accuracy.

Remanence characteristics

An investigation of the degradability of a wastewater sample also offers the advantage that changes in the characteristics of the mixture (toxicity, content of bioaccumulative substances) can be detected, while at the same time it may be possible to identify individual substances which contribute the toxicity or which are bioaccumulative. Even though the degradation test shows that the mixture has no undesirable characteristics in the form of toxicity or content of bioaccumulative substances, it is possible to identify undesirable persistent substances.

If at the same time the toxicity is followed continuously by screening with a sensitive organism, the point can be determined at which the toxicity has been reduced significantly. Specific analyses (or fingerprinting) can identify the substances that have been degraded in the time that has elapsed, and it therefore becomes possible to single out the substances which are the most important contributors to the toxicity of the mixture.

3.3.5 Evaluating degradability

Individual substances

Today information exists on the degradability of a great number of substances; in Denmark and other EEC-countries information on degradability is required for all new substances. Information on degradability - measured either in standardized screening tests or in environmental simulation tests - has been collected in references such as /9,10,33/. The information in Verschueren /33/ is not quality-controlled, however. More up-to-date information about the degradability of individual substances may be obtained from databases such as ECDIN, or from on-line bibliographic databases such as Biosis, Chemical Abstracts, Pollution Abstracts, and Aqualine. Using results from studies on individual substances, the substances identified in a wastewater mixture can be grouped according to their degradability. Since the characteristic "degradability" is qualitative, however, the information cannot be used to make a quantitative calculation of the rates of degradation and expected concentrations in the receiving water.

Within the group of readily degradable substances, however, it will usually be possible to use the actual results of the tests to group the substances on the basis of their half-lives (the time required for a halving of the concentration). However, this information is rarely reported when screening for degradability is carried out. The inherently degradable substances cannot be assumed to be degradable in the recipient.

Only results from simulation tests can be used to calculate rates of degradation and expected concentrations in the receiving water. The degradation rates can then be used to grade the substances in question.

In by far the greatest number of cases, traditional testing of substances for ready or inherent degradability only makes it possible to make a qualitative division of substances into classes. Using additional information on discharged quantities and toxic or bioaccumulative qualities, potentially problematic substances can be identified for further investigation, possibly by monitoring in the recipient or by means of simulation tests.

This strategy for investigating the degradability of individual substances in wastewater is defective in several ways. Experience has shown that it is only possible to identify up to about 10% of the organic matter in wastewater, even when detailed knowledge is available of the production processes and the chemicals used during the process. The remaining unidentified 90% can not be evaluated by this strategy. Nor does the strategy take into account the special circumstances prevailing in wastewater discharges which contain many carbon and energy sources.

Mixtures

The initial information about the degradability of a wastewater stream may often be limited to the ratio between the biological and chemical oxygen demands (BOD/COD). Information about the BOD/COD ratio is not sufficient to indicate whether the wastewater is degradable, unless at the same time there is good information about the individual substances in the wastewater. Even though the BOD/COD ratio may correpond to the normal figure for readily degradable wastewater, the wastewater may still contain substances which are not readily degradable.

The results of degradability studies carried out on mixtures can class the organic material in the wastewater into two groups, the readily degradable and the inherently degradable. These screening methods, like the screening methods for individual substances, cannot determine the quantitative speeds of degradation in the recipient, but the readily degradable fraction can be expected to disappear relatively quickly in the recipient.

The inherently degradable fraction is not necessarily degradable in the recipient, but the test method makes it possible to characterize the persistent fraction of the organic substances with regard to toxicity, bioaccumulative tendency, and chemical identity. In addition, the inherently degradable fractions of the organic material which are reponsible for undesired qualities such as toxic effects may often be identified.

Identified problem substances in the mixture can be tested in simulation tests using the mixture if sufficiently sensitive and specific analytical methods are available, or if the substances can be synthesized with a ^{14}C marker. In this way the quantitative speeds of degradation for individual substances in the mixture can be determined.

3.3.6 Examples of stabilization studies

Aerobic stabilization of mixtures has been used as a technique for characterization of wastewater. The following examples will be presented below:

- content of persistent nitrogen compounds in slaughterhouse effluent.
- content of bioaccumulative substances in wastewater from a papermill.
- content of toxic substances in wastewater after firefighting in a pesticide warehouse.

Persistent substances

This type of stabilization study has the objective of determining whether a wastewater sample contains persistent substances whose fate can be followed using an overall parameter. After stabilization the contents in the stabilized sample can be characterized in more detail in order to determine the origin of the persistent substances.

A grab sample of wastewater from a slaughterhouse and a sample of sludge from the treatment plant receiving the slaughterhouse wastewater /37/ were stabilized by adding sludge as seed (0.5 g DW/l) together with nutrient solution (1 ml/l) containing inorganic phosphate and ammonium, and 0.3 ml/l of the following stock solution:

15	g	NH_4Cl
33.4	g	$NaH_2PO_4 \cdot 2H_20$
8.5	g	KH_2PO_4
21.75	g	K_2HPO_4

Stabilization was carried out at 10 ± 1 °C. During stabilization, samples were taken for analysis of dissolved organic carbon (DOC), nitrite/nitrate-nitrogen (nitrate-N) and organic nitrogen (Kjeldahl-N÷ammonium-N). The results are shown in figure 3.3.5.

Most of the organic material in the wastewater degraded very rapidly and the DOC stabilized at a level of 7-8 mg/l. At the same time, the organic nitrogen fell to a level indistinguishable from zero, and nitrite/nitrate was formed. After a longer period of stabilization a tendency was seen to form small amounts of dissolved organic nitrogen as a result of degradation of dead microorganisms.

It was therefore concluded that the wastewater had a low content of persistent organic nitrogen compounds.

Bioaccumulative substances

This type of stabilization study has the objective of deciding whether a wastewater sample contains bioaccumulative substances which persist after degradation of the wastewater in the receiving water. If

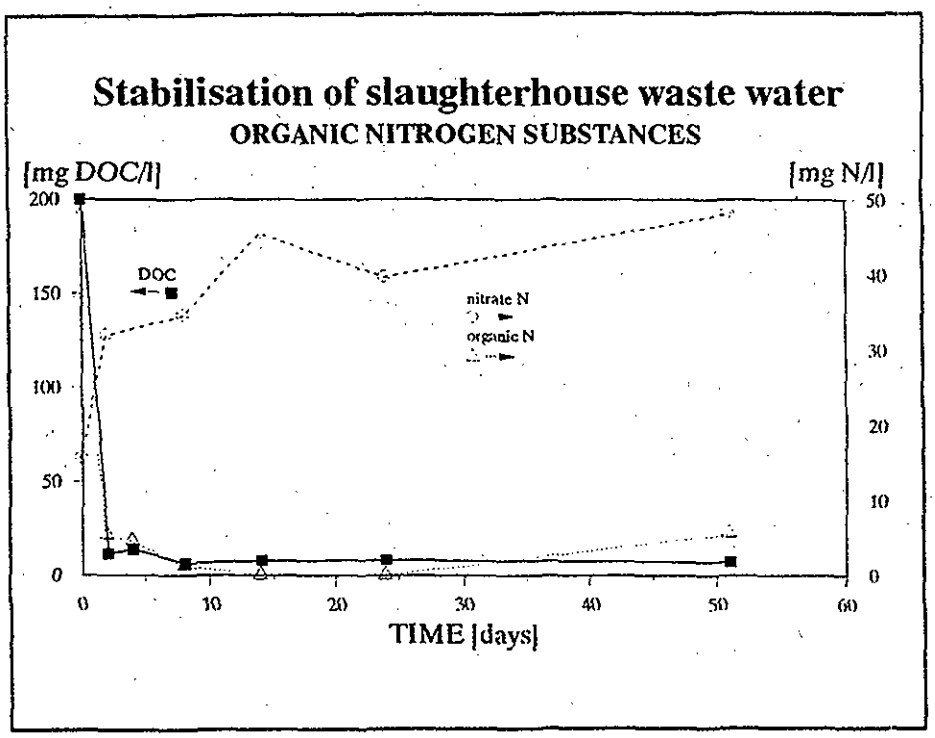

Figure 3.3.5 Removal of organic carbon and organic nitrogen by stabilization of wastewater samples, and formation of nitrite/nitrate.

this is the case, the substances can be characterized in more detail in order to identify the source of the bioaccumulative substances.

Three grab samples from a paper mill were mixed 1:1:1 and filtered to remove paper fibres and other particulate matter. The pooled sample was diluted 1:1 with receiving water and stabilized for one month at 16.5 ± 1 °C. Samples were taken at the beginning and end of this period for TLC-screening for content of bioaccumulative substances (substances with log P_{ow} greater than 3.0). DOC analyses were used to follow the progress of the stabilization.

No significant decline in the DOC content occurred in 27 days, and the organic content of the sample must thus be classified as very persistent.

At the start of the stabilization test the sample contained three potentially bioaccumulative substances with log P_{ow} of 6.5, 5.4 and 3.9 respectively. After stabilization, the two substances with the highest log P_{ow} values could not be detected. The third substance, with a log P_{ow} of 3.9, could be detected after stabilization, but at a far lower concentration.

It was therefore concluded that the wastewater sample contained three potentially bioaccumulative substances, but that none of these was truly bioaccumulative since all of them were degradable in the receiving water.

Toxic substances

This type of stabilization study has the objective of deciding whether a wastewater sample contains toxic substances which are persistent in the receiving water, and whether the toxicity of these persistent substances affects the degradation of other organic substances in the receiving water.

Wastewater from a firefighting action at a pesticide warehouse was mixed with receiving water from a nearby river at a concentration of 500 ml/l /39/. In addition, a solution was prepared using the same amount of receiving water topped up with wastewater and distilled water until the concentration of wastewater was 25 ml/l. This correponded to the EC80-value for the wastewater sample in the Microtox test. As additional seed material, sediment from the river was added to both wastewater samples at a concentration of 10 g DW/l. The samples were stabilized at 10 ± 1 °C.

During stabilization samples were taken for analysis of the DOC content and testing of toxicity using the Microtox test. The stabilization was considered complete when the decline in DOC was less than 10% after 5 days, and when the samples were no longer toxic in the Microtox test.

The results are shown in figures 3.3.6 and 3.3.7.

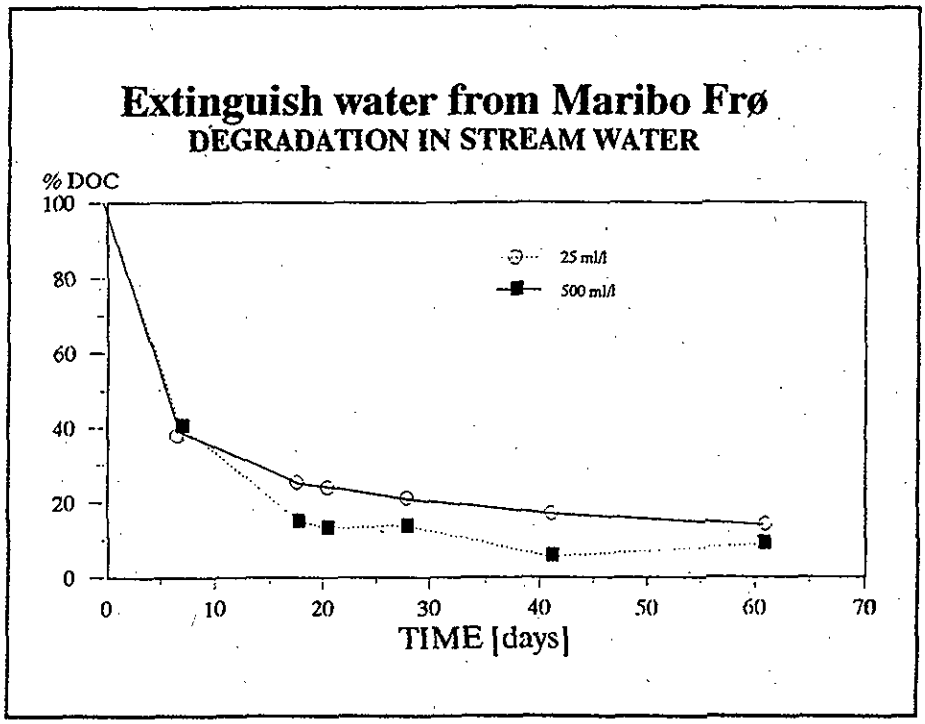

Figure 3.3.6 Course of stabilization measured as percentage of DOC at the two concentrations used.

As shown in figure 3.3.6, the organic matter in the wastewater was degraded at more or less the same rate, regardless of the wastewater concentration that was used. Although the wastewater was toxic in the Microtox test, high concentrations of wastewater did not seem to affect the microbial activity in the recipient so much that the degradation of organic material was affected. After 20 - 30 days the DOC of the wastewater samples was stabilized.

Figure 3.3.7 depicts the toxic units (1/EC20) of the undiluted firefighting water. The toxic units indicates the dilution that is necessary for avoiding toxic effects. As seen in figure 3.3.7, the toxicity of the wastewater stabilized at the higher concentration falls faster than the toxicity of the wastewater stabilized at the lower concentration.

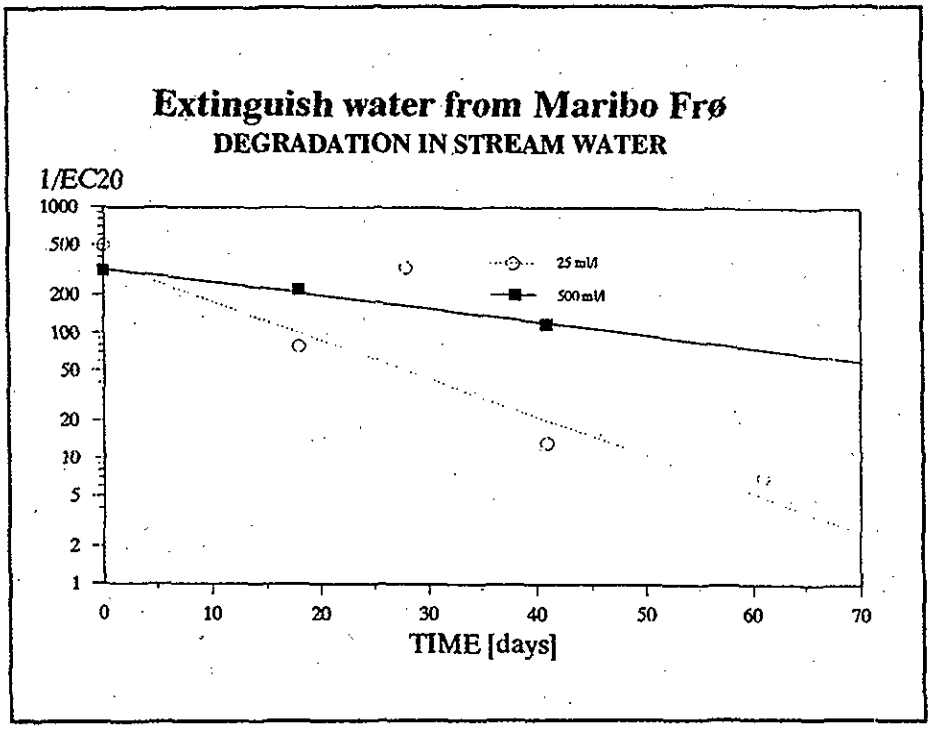

Figure 3.3.7 Course of stabilization measured as concentration at which inhibition commences (EC20) in the Microtox test.

An evaluation of the wastewater samples based solely on the organic carbon content would have indicated that the stabilization test could be terminated before the microorganisms in the recipient water had carried out any significant degradation of the toxic substances. This could have led to the faulty conclusion that the toxic substances were not degradable. Continuation of the tests on the basis of the toxicity measurements, however, showed that the toxic substances could be degraded, but at a low rate. The test showed furthermore that the toxic substances in the wastewater sample made up only a small part of the total organic content of the sample.

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3.4 Bioaccumulation

Because of bioaccumulation, some chemicals may be found in plants and animals at significantly higher concentrations than in the surrounding water. In consequence, a relatively short-lived exposure in the water phase may result in an "internal" load in the organism at a high concentration and over a longer period. Because of the increased concentration, substances with a bioaccumulative potential are there-

fore potentially capable of causing chronic effects, not only in the organisms directly exposed to the chemical, but also in organisms at higher levels in the food chain, including man. Bioaccumulation is therefore an important connecting link between surface water pollution and human exposure to xenobiotic substances.

Well-known examples of bioaccumulative substances are DDT, PCB, HCB (Hexachlorobenzene), heavy metals such as mercury, lead and cadmium, and organic metal complexes (methyl mercury, organotin compounds).

Traditionally, bioaccumulation is divided into two processes /28/:

- *Bioconcentration*: Elevated concentrations in organisms caused by uptake directly from the water phase by diffusion, adsorption, or active transport (direct bioconcentration) or by intake with food (indirect bioconcentration);
- *Biomagnification*: Food-chain-related bioaccumulation, in which the concentrations rise progressively from one link in the chain to the next.

In the following discussion, "bioaccumulation" will be used as a general term for xenobiotic substances which occur at higher concentrations in organisms than in their surrounding environment.

Bioaccumulation of chemicals was publicly recognized as an environmental problem in the 1960s when high concentrations of DDT, DDD and methyl mercury were found in fish and fish-eating birds. From the observation that the concentration of DDT rose progressively from one trophic level to the next (crustacea → fish → birds) it was assumed that biomagnification was of primary importance for bioaccumulation of chemicals.

In subsequent laboratory experiments using single species exposed either through the food or through the water medium, and also in direct food chain experiments in the laboratory, it has not been possible in the case of most substances to demonstrate that biomagnification is a significant accumulation process in aquatic environments (algae → crustacea → fish).

In a review published in 1979, Macek *at al.* /25/ analyzed data from bioaccumulation experiments using simple experimental aquatic foodchains, and concluded that only persistent chemicals with very high bioconcentration factors (BCF) $\geq 10^6$ and elimination times of 1 week or more could be expected to be biomagnified (DDT, PCB, dioxins and similar substances). For most other substances, bioconcentration (via water) was expected to be the most important mechanism.

It should be mentioned, however, that biomagnification is the most important bioaccumulation mechanism in terrestrial food chains.

The following factors are important for determining the bioaccumulative potential, especially for organic substances:

- low degree of polarization
- low solubility in water
- high lipid solubility

- low degree of biodegradability.

The fact that non-polar organic substances tend to bioaccumulate is related to the structure of organic membranes, which permit non-polar substances to pass, but act as a barrier towards polar substances. For example, a water/lipid emulsion of DDT will distribute itself at a ratio of 1 : 562,000 (water : n-octanol).

Uptake of heavy metals does not take place to any significant extent by passive diffusion, but occurs as an active process at sites on the membrane where ions such as Ca^{++} and Mg^{++} etc. are taken up. It is thus primarily those heavy metals which chemically resemble essential salts that are taken up. In the case of organo-metal complexes (and metallic mercury) the degree of lipophilicity (lipid solubility) determines the rate of uptake.

Substances with a high molecular weight will only be absorbed to a small extent as a result of purely physical processes, despite the degree of lipophilicity. Certain surfactants and dyes are good examples.

Substances which are biodegraded relatively quickly are not expected to pose any great risk of long-term effects, except in the immediate vicinity of a wastewater discharge. Substances of this type may also be expected to become metabolized in the organism and thus to be eliminated. Since bioaccumulation is only possible if the rate of uptake is greater than the rate of breakdown or excretion, easily metabolized substances are not normally expected to bioaccumulate.

Transport through biological membranes requires that the chemical is present in dissolved form. Environmental factors which reduce the amount of chemical in true solution will therefore reduce the rate of uptake (and also the toxicity) because of a reduction in the biologically available concentration. Important factors which can reduce the biologically available concentration are /35/:

- adsorption on suspended particles, humic acids and sediment
- formation of colloid particles
- formation of complexes and chelation (heavy metals).

In addition to their bioaccumulative tendencies, lipophilic substances also tend to adsorb to organic fractions in sediment, depending on the degree of lipophilicity /19/.

For example, the presence of humic acid reduced the rate of uptake of benzo(a)pyrene in fish, but did not affect the rate of uptake of anthracene, which is less lipophilic /34/. Similarly, eutrophication (increased algal growth) reduced the concentration of DDT in fish independently of the total concentration in the surrounding water /37/. It is well-documented that organic material and clay minerals can reduce the biological availability of heavy metals.

The bioavailability, and therefore also the bioconcentration potential, of substances of acid/base nature (amphoteric) varies with the pH of the medium. Since non-polar substances will normally be taken up more easily than polar ones, it is usually the acid form of organic substances that has the highest bioconcentration potential.

3.4.1 Bioaccumulation of individual substances

In connection with registration of new chemicals /27/, documentation of the bioconcentration potential of the substance must be submitted. In practice the so-called bioconcentration factor (BCF) is stated. BCF is defined as the concentration of the substance in fish in equilibrium with the surrounding water phase ($BCF = C_{fish}/C_{water}$). Internationally accepted methods have been developed for determining BCF experimentally. Two basic methodologies are used:

- experimental determination of BCF by exposure of fish
- calculation of BCF on the basis of experimental determination of the partition coefficient octanol/water (P_{ow}) and empirical relationships between BCF and P_{ow}.

Experimental determination of bioaccumulation of individual substances

The experimental methods accepted as international standards are all based on investigation of the substance using the so-called "One-compartment Concentration Model" /13,28/ and have as their objective the determination of the bioconcentration factor of the substance in fish (uptake via water).

In brief the methodology is to expose a population of immature fish to a constant concentration of the test substance for a period sufficient for achieving equilibrium ("steady state") between uptake and excretion of the substance in the test organism. After that the fish are transferred to water, and the excretion of the substance is followed for a period twice as long as the uptake period.

The ideal course of the experiment as determined by a number of analyses of concentrations in fish and in the medium during the period of the test is shown in figure 3.4.1.

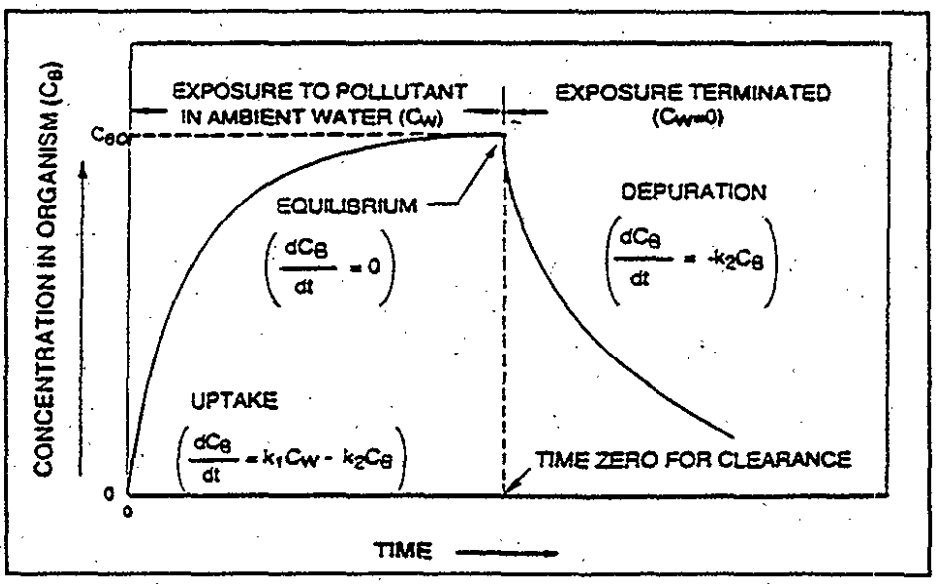

***Figure 3.4.1** Theoretical course of an experimental determination of bioconcentration in fish (from /9/).*

In the above-mentioned standard methods, BCF may be calculated directly assuming that the kinetics are 1st-order (one-compartment), i.e. that a simple diffusion model applies:

increase in concentration = rate of uptake - rate of excretion

or:

$$\frac{dC_F}{dt} = K_1 \cdot C_w - K_2 \cdot C_F \qquad (1)$$

where: C_F = concentration i fish (μg/g wet weight)
C_w = concentration i water (μg/ml)
t = time (days)
K_1 = 1.order rate constant for uptake
K_2 = 1.order rate constant for elimination

If we assume that "steady state" is reached, i.e. that the rate of uptake when all "binding sites" are occupied is of the same order of magnitude as the rate of elimination, then the increase in concentration dC_F/dt will approach "0"; in other words:

$$\frac{dC_F}{dt} = 0 = K_1 \cdot C_w - K_2 \cdot C_F$$

or:

$$K_1 \cdot C_w = K_2 \cdot C_F$$

or:

$$\frac{K_1}{K_2} = \frac{C_F}{C_w} = BCF \qquad (2)$$

Thus BCF can be described either as a relationship between the concentrations in the fish and in the water, or as the relationship between the rate constants for the uptake and the elimination of the substance. The assumptions are that "steady state" is achieved, and that the process can be described in terms of 1st-order kinetics.

A frequently used expression for the bioaccumulative potential of substances is the biological half-life ($T_{½}$). This is understood as the period of time required for the halving of a certain dose in the organism. $T_{½}$ may be calculated from the elimination constant (K_2):

$$T_{½} = \frac{\ln^2}{K_2} = \frac{0.693}{K_2} \qquad (3)$$

The experimental time required to achieve "steady state" is determined by K_2 ($T_{½}$) alone. The theoretical uptake curve for a number of values of K_2 are shown in figure 3.4.2.

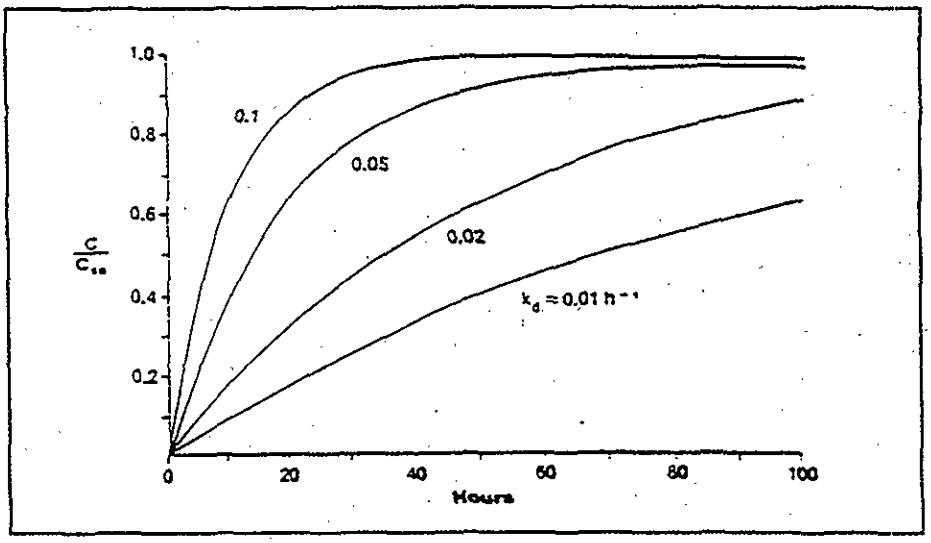

Figure 3.4.2 Importance of K_2 (K_d) for the time necessary for achieving "steady state" concentration in the organism under experimental conditions (1st order kinetiks) (from /35/).

The integrated accumulation function (1) and the known value of K_2 may be used to calculate the time required to achieve steady state.

For polychlorinated biphenyls (BCF $\approx$ 3000 in fish, $K_2 \approx$ 0.005 hour^{-1}), the 90% steady state will be achieved after about 20 days, while the corresponding period for hexachlorobenzene (BCF $\approx$ 10,000 in fish, $K_2 \approx$ 0.002 hour^{-1}) will be about 50 days.

The assumption that bioaccumulation can be described using 1st-order kinetics supposes that all the "compartments" in the organism take up and excrete the substance at the same rate. In principle this will only very rarely be true, but in practice the curves for the course of uptake and excretion can usually be *approximated* to 1st-order kinetics in those cases where one body compartment/tissue type is the most important for determining the kinetics (fatty tissue for lipophilic substances, liver/kidneys for certain heavy metals).

Whether 2nd or higher order kinetics are involved may be detected by investigating how well the data for excretion fit a semi-logarithmic linear regression. Figure 3.4.3 shows examples of data where 1st-order kinetics are *not* the best approximation, because the excretion period is clearly biphasic (2nd-order kinetics).

On this basis it is reasonable to suppose that two (or more) compartments with differing excretion rates (and presumably also differing rates of uptake) are involved. In such cases, the BCF may be determined from the relationship between C_{fish}/C_{water} at "steady state", or from the rate constants for uptake and elimination respectively in the two (or more) compartments /22, 35/.

Estimation of when an experimental "fish and water system" has reached equilibrium ("steady state") is especially difficult under the following conditions:

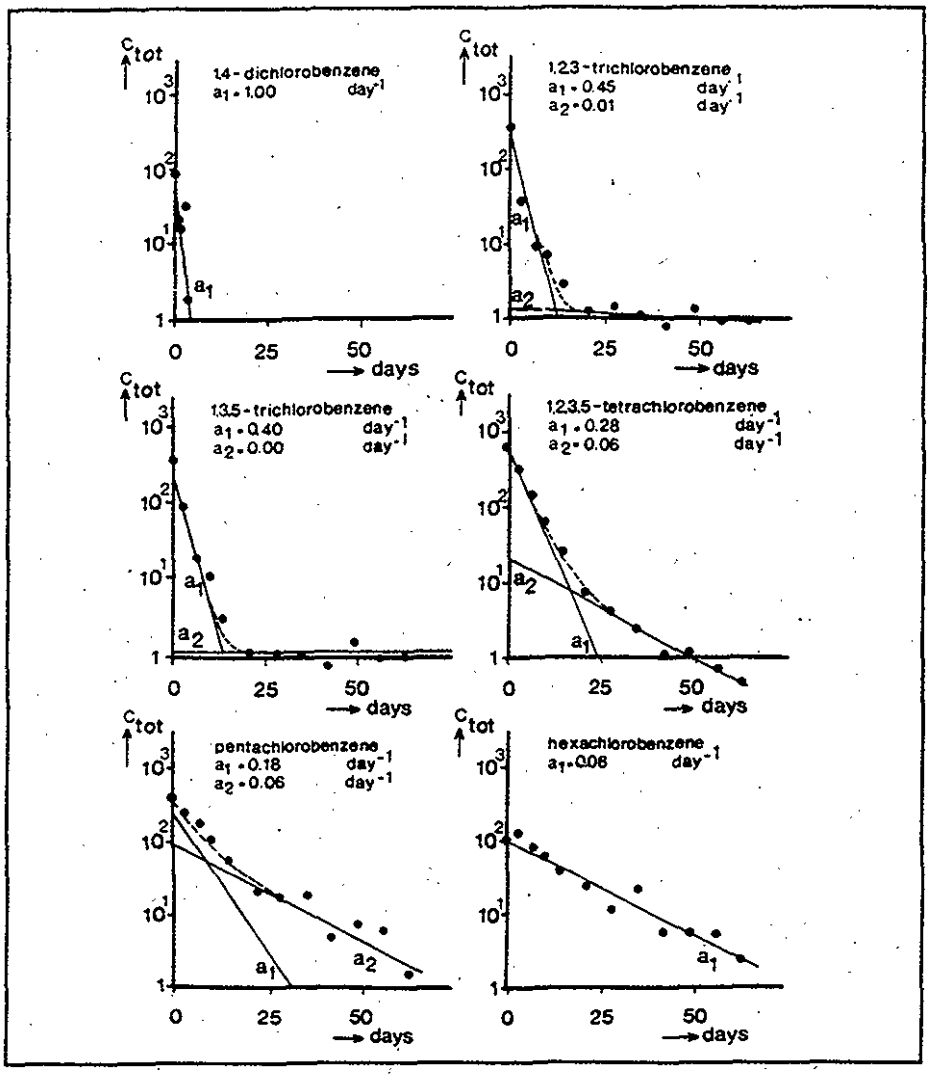

Figure 3.4.3 Elimination of chlorobenzenes from fish. C_{tot}: concentration in fish (µg/g lipid weight) (from /22/).

- several compartments participate in uptake/elimination of the substance
- induction of enzymes which can metabolize the substance
- use of fish with high growth rates relative to the test period.

For substances for which two or more compartments contribute at markedly different uptake/excretion rates it will be difficult to define when "steady state" has been reached, since a slight increase in concentration will follow after the steep initial uptake. Such a slow rate of increase may well be hidden by the experimental uncertainty or biological variation.

If the organism has active enzyme systems for metabolization and thus elimination of the substance in question, the rate of bioaccumulation will be lower than expected based on analogy. For substances for which metabolization can be initiated by induction of enzyme systems, an initial bioaccumulation will often be seen to be followed by a fall in the substance concentration (increased rate of elimination) in step with the synthesis of active enzymes. An example of this is shown in figure 3.4.4.

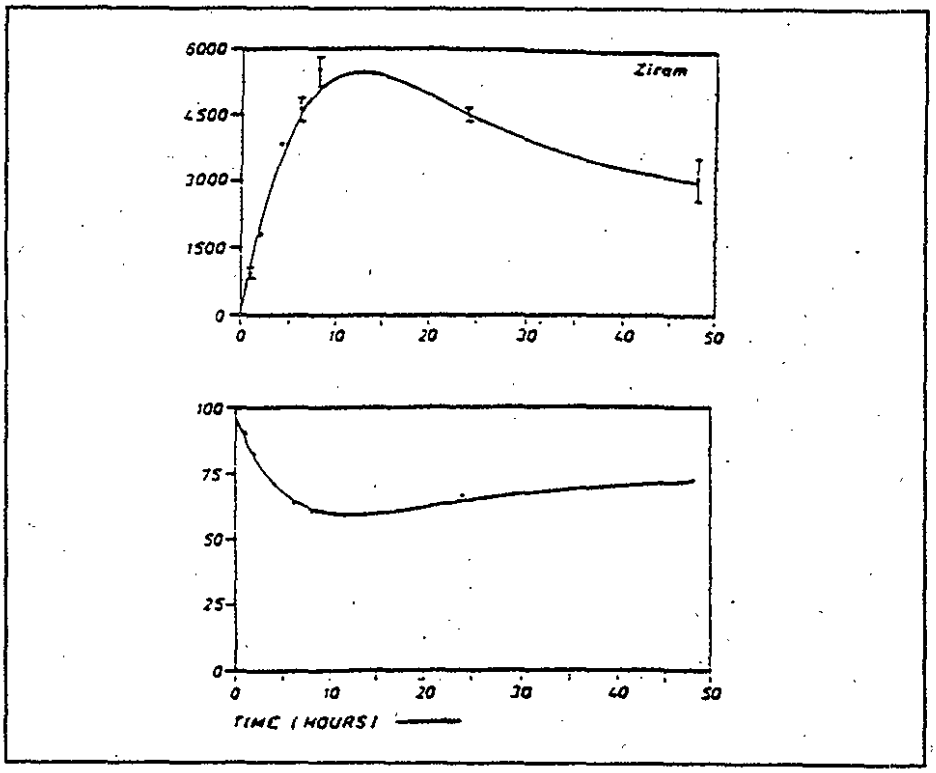

Figure 3.4.4 Bioaccumulation of ziram in rainbow trout.
Upper: concentration i fish (µg/g),
Lower: concentration i water (µg/l) /36/.

For substances with high BCF-values ($>10^5$), equilibrium will only be achieved very slowly, and quite a lengthy test period will be necessary. The growth of the test organisms during the lengthy test period may then "dilute" the substance concentration, and this may result in an apparent equilibrium. In such cases the experimentally determined BCF (C_{fish}/C_{water}) will be an underestimate.

Table 3.4.1 gives a number of examples of experimentally determined BCF-values based on kinetics (K_1/K_2) and on "steady state" (C_{fish}/C_{water}) methods.

In an EC ring test between 13 laboratories the average value and standard deviation of the individual laboratories' estimates of BCF for lindane was 422 $\pm$ 50% /20/. BCF-values determined by means of bioaccumulation experiments must therefore be expected to be rather inaccurate, if the result of this ring test is to be taken as typical of the "state of the art" for European laboratories (1985). The experience gained from this ring test was used to make significant clarifications and other improvements to the test (OECD 305E). The ring test also showed that a reduction in the uncertainty of the BCF estimate can be achieved by calculating the substance concentration in the fish on the basis of lipid weight rather than total weight. This indicates that the fatty tissue is the main site of lindane bioaccumulation. The amount of fatty tissue differs greatly between different fish species, and also depends on the state of nutrition and life stage of the fish. Although comparisons of the bioconcentration potential of different chemicals are therefore most precise when based on lipid

Table 3.4.1 Eksperimentally determined BCF-values based on kinetic and "steady state" methods. K_B = BCF (from /12/).

Substance	Kinetic K_B	"Steady state" K_B
DDT	52,358	100,000
Hexachlorobenzene	7,880	18,600
Tetradecylheptaethoxylate	850	700
Sodium dodecylbenzensul-fonate	286	20
1,4-Dichlorobenzene	215	60
Diphenyloxide	190	470
Tetrachloroethylene	39.6	49
Carbon tetrachloride	17.7	30

weight, the ecologically most relevant basis for comparisons of fish with a high lipid content (eel, herring, etc.) is total weight.

Bioconcentration factor based on empirical relationships (QSAR)

The tendency of organic substances to be taken up by living organisms is related to the ease with which the substance passes the cell membrane, and to the lipid solubility. Veith *at al.* /38/ reported a good correlation between the BCF-values and partition coefficients (n-octanol/water) of 84 chemicals (figure 3.4.5).

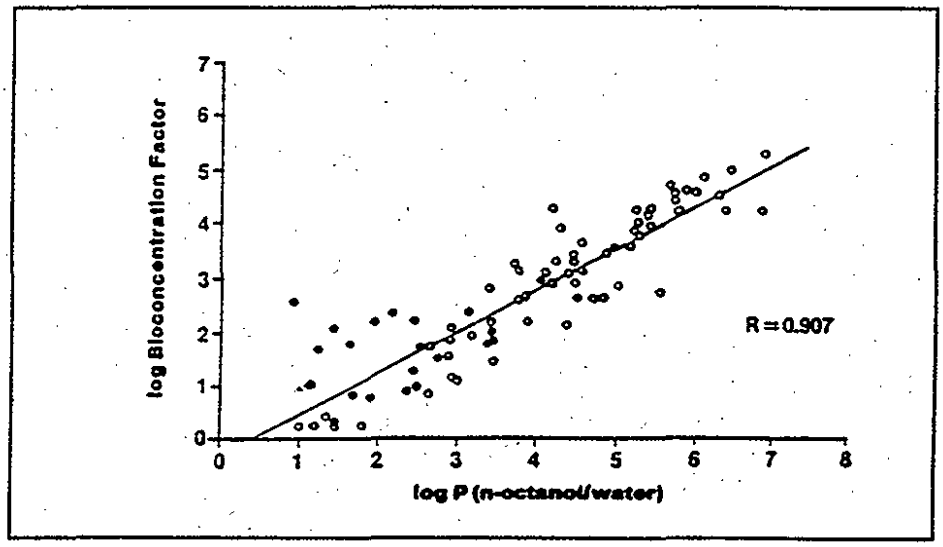

Figure 3.4.5: Correlation between bioconcentration factors in fish (BCF) and the n-octanol/water partition coefficient (P_{ow}) for 84 organic substances (from/38/).

The observed correlation may be described by the function:

$$\log BCF = 0.76 \cdot \log P_{ow} - 0.23 \qquad (r = 0.907).$$

For the interval $\log P_{ow} = 3\text{-}6$ this correlation makes it possible to calculate BCF on the basis of the value of P_{ow}.

Subsequent data correlations for specific groups of substances and specific organisms have given even stronger correlations. Thus the relationship between log BCF and log P_{ow} for chlorinated hydrocarbons and related substances in fish may be described by a polynomial function:

$$\log BCF = 6.8 \cdot 10^{-3} \cdot (\log P_{ow})^4 - 1.85 \cdot 10^{-1} \cdot (\log P_{ow})^3 + 1.55 \cdot (\log P_{ow})^2 - 4.18 \cdot \log P_{ow} + 4.79.$$

The curve representing this function is shown in figure 3.4.6.

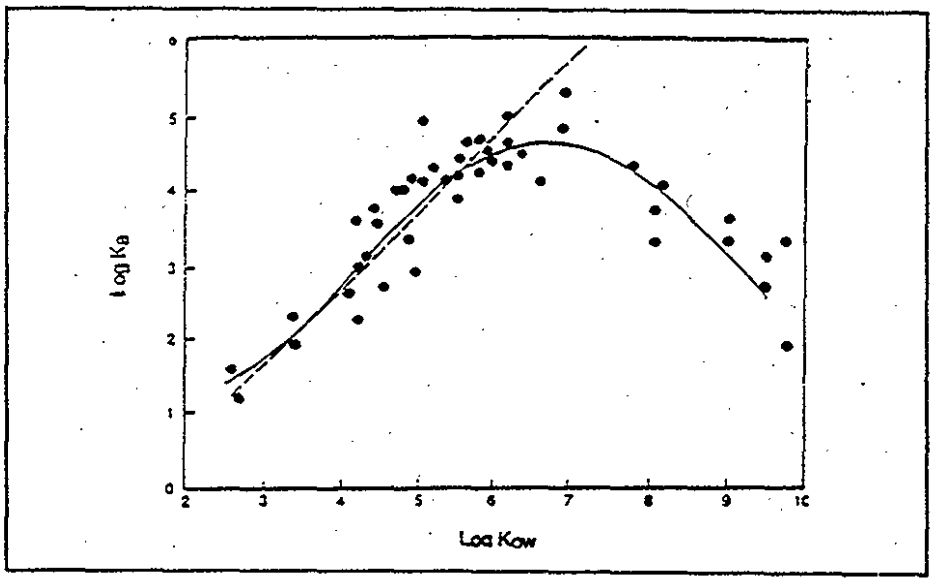

Figure 3.4.6 Relationship between BCF (fish) and P_{ow} for chlorinated hydrocarbons (and others) in the area log P_{ow} = 3-10. The stippled line represents substances with log P_{ow} = 3-6 (from /8/).

Connell & Hawker's data /8/ gave the following regression for the interval $\log P_{ow} = 3\text{-}6$:

$$\log BCF = 0{,}94 \cdot \log P_{ow} - 1.00.$$

The maximum for the polynomial function has been calculated to be $\log P_{ow} = 6.7$, corresponding to log BCF = 4.61. On the basis of Connell & Hawker's data it would not be advisable to extrapolate outside the interval on which the linear correlation is based. The reason why substances with extreme values for log P_{ow} bioaccumulate at lower rates than expected is thought to be that substances with high molecular weight have greater difficulty in penetrating the membrane.

An investigation of 150 organic substances representing various groups of substances revealed a parabolic relationship between the molecular weight and the bioconcentration factor (determined using fish) /2/. Bioconcentration was greatest for substances with molecular weights of 250-500, and with little or no bioaccumulation of substances with molecular weights <100 and >600. Later studies have confirmed this relationship /1,6/. The shape of the molecule (area and volume) also play a role in determining the rate of uptake /31/. A certain amount of uptake of high molecular weight substances could however occur through pinocytosis in the intestine /16/.

An apparently lower bioaccumulation than expected on the basis of P_{ow} values may also arise because of technical problems in the determination of both P_{ow} and BCF, since substances with very low solubilities are difficult to handle experimentally.

Table 3.4.2 lists a number of correlations that have been determined for various groups of substances using fish. Only critically validated results have been included /9/.

***Table 3.4.2** Examples of correlations between BCF and n-octanol/water partition coefficient (P_{ow}) for various groups of substances. BCF is experimentally determined in fish. N = number of data sets, r = correlation coefficient, $RP_{ow} = P_{ow}$ - range applied for the correlation (from /9/).*

Substance group	Regression	N	r	RP_{ow}	Ref.
Chlorohydrocarbons and PAHs	log BCF = 0.95·log P_{ow} -1.06	30	0.99	2-6	/19/
Different organic substances	log BCF = 0.94·log P_{ow} -1.19	49	0.89	-	/19/
Hydrocarbons and Chlorohydrocarbons	log BCF = 0.98·log P_{ow} -1.36	20	0.90	1.5-6.5	/12/
Aromatic substances	log BCF = 0.71·log P_{ow} -0.92	17	0.98	-	/22/

From the correlations reported in the literature it is seen that the strength of the correlations is increased if they are based solely on data from the same group of substances. Since the experimental methods for determination of both log P_{ow} and BCF have been improved greatly in recent years, it may be expected that the newer data generally give a basis for more reliable correlation formulae.

Relationships have also been reported for BCF and solubility in water. However, the quantity of data and the reliability of the correlations is relatively low so far compared with the P_{ow} / BCF correlations. Correlations for P_{ow} and BCF have also been reported for other types of organism than fish: microorganisms, algae, crustacea, polychaetes etc. - see for example /9/.

Two principally different methods are available for the determination of P_{ow}: the "shake flask method" and chromatographic methods.

In the shake flask method /2/ the test substance is shaken in an n-octanol/water system in a glass flask at a constant temperature. The system is shaken until the concentration of test substance in the two phases is constant. P_{ow} is calculated as the ratio between the concentrations in n-octanol and water.

This method is problematical, especially for strongly hydrophobic substances, where only a small proportion can be expected to enter the water phase. Some substances may become trapped in the water phase in the form of micelles. Adsorption to small impurities in the water phase can also disturb the concentration relationship and lead to considerable underestimates of P_{ow}. For the same reason, the method is only recommended for determination of P_{ow} in the interval log P_{ow} = 2-4 /29/. Recently, however, the method has been improved for application to hydrophobic substances, by substituting slow stirring for shaking. This reduces micelle formation /17/.

A number of chromatographic methods have been described in recent years, centering on thin-layer chromatography (TLC) amd high pressure liquid chromatography (HPLC).

The principle of the TLC method is to determine the mobility of the substance in a stationary phase. The mobility is expressed in terms of the retention coefficient (R_F), which on the basis of known values of R_F for substances of known log P_{ow} can be used to calculate the BCF. In contrast to the "usual" TLC technique, however, the stationary phase is hydrophobic, and the mobile phase hydrophilic (reversed phase chromatography). The TLC method can only give an indication of P_{ow}, since the determination of R_F is relatively imprecise.

The most widely used method today is based on HPLC determinations, since the retention time for substances can be determined rather precisely, and because the n-octanol/water system may be used directly /29/. The method is suitable for determinations of P_{ow} in the interval log P_{ow} = 0-6.

A number of methods have been developed in recent years for calculation of P_{ow} on the basis of molecular descriptors: fragment constants, structural factors, molecular binding strength etc. These methods are intended in particular for use in preliminary evaluations of the expected bioaccumulation potential of a substance.

For example, it has been found that the P_{ow} for aliphatic hydrocarbons increases with increasing chain length. For n-alkanes, the following relationship between P_{ow} and the number of carbon atoms in the molecule has been calculated /5/:

$$\log P_{ow} = 0{,}535 \cdot N_c - 0{,}302 \quad (r = 0{,}999)$$

where N_C = number of C atoms in the molecule.

For each carbon atom or CH_2-group the increase in P_{ow} is 0.535 units. This knowledge can then be used - cautiously - to predict the P_{ow} of a new substance, if the only difference from a substance

of known P_{ow} is in the chain length. Similar factors are now known for a great number of substituents in aromatic and aliphatic hydrocarbons. In an annex to /29/ a number of methods are described for calculating P_{ow} for various groups of organic compounds.

Bioaccumulation from sediment

In addition to their high bioaccumulation potential, lipophilic chemicals are also highly adsorptive towards organic fractions in sediment or suspended material, depending on the degree of lipophilicity (P_{ow}). Accumulation of organic substances in sediment from water may be described by the water/sediment organic carbon distribution coefficient (K_{oc}):

$$K_{oc} = \frac{C_s}{C_w \cdot f_{oc}}$$

where: C_s = concentration in the sediment
C_w = concentration in the water
f_{oc} = fraction of organic carbon in the sediment.

Some examples of empirical relationships between P_{ow} and K_{oc} are given in table 3.4.3.

Table 3.4.3 Correlations between sediment organic carbon/water (K_{oc}) and n-octanol/water partition coefficients (P_{ow}) (S_w: solubility in water) (from /9/).

Substance group	Regression	N	r	Ref.
Methylated and halogenated benzenes	$\log K_{oc} = 0.72 \cdot \log P_{ow} + 0.49$	13	0.95	/27/
Polyaromatic hydrocarbons	$\log K_{oc} = 1.00 \cdot \log P_{ow} - 0.32$	22	0.98	/27/
Triazines and nitroaniline hydrocarbons	$\log K_{oc} = 0.94 \cdot \log P_{ow} - 0.01$	19	0.95	/28/
Chlorohydrocarbons	$\log K_{oc} = 0.56 \cdot \log S_w + 4.04$	15	0.99	/28/

For using K_{oc} values to evaluate the expected bioaccumulation from sediment via P_{ow} calculations, it is assumed that only that fraction of the substance in question which is dissolved in the water phase is available for uptake in the test organism.

For amphipods, it was found that bioaccumulation of hexachlorobiphenyl in a water/sediment system did indeed take place solely from the water phase /24/. For filter-feeding organisms such as mussels, or sedimentivores such as polychaetes, however, a significant uptake through the alimentary canal can not be excluded.

Legal basis for regulation of use of chemical substances

According to the Danish "Law on chemical substances and products" /27/, documentation must be submitted for all new chemicals, including information on ecotoxicological properties such as bioaccumulation potential. For substances with log $P_{ow} > 3$, the accompanying administrative order requires experimental determination of BCF in fish, unless the substance can be shown to be "readily biodegradable".

These criteria are in accordance with the OECD recommendations. It should be noted that criteria such as these are currently (1990) the subject of discussion within the EC and OECD.

3.4.2 Complex mixtures

Evaluation of the content of bioaccumulative substances in wastewater is traditionally carried out using knowledge of the BCF for the individual substances detected in the wastewater. Recent investigations have shown, however, that this application of knowledge about substances in "pure" test solutions to situations where complex mixtures of chemicals are present is problematical, for the following reasons:

- the presence of organic material may reduce the bioavailability of hydrophobic chemicals by adsorption or complex formation, and thus reduce the actual amount of bioaccumulation. Obversely, an increase in sediment concentration may result in increased uptake in sediment-dwelling organisms.
- the presence of solvents/surfactants may increase the uptake of relatively hydrophobic substances.
- the presence of certain substances in the mixture may initiate or increase the rate of metabolization of another substance in some organisms.

Furthermore, there may be substances in the mixture with a high bioaccumulation potential which escape detection because of the methods used or because they are present at levels below the limit of detection.

Importance of suspended matter and humic acids for bioaccumulation

Depending on the lipophilic properties of the substances in question, and on the type of suspended material present (in particular, the organic content of the material), the uptake in fish may be significantly reduced. For lower chorinated benzenes and biphenyls with relatively high solubility in water (log $P_{ow} \approx$ 4-5) suspended material had no measurable effect on uptake in fish (guppy). For the more hydrophobic substances (log $P_{ow} \approx$ 5-7) uptake was significantly reduced. The proportion of chemical bound to the particles did not seem to affect the uptake in other ways (low bioavailability) /32/. Introduction of particulate material to a solution of hexachlorobenzene (log P_{ow} = 6.4) caused a significant increase (50-100%) in bioaccumulation in suspension-feeding bivalves. The content of particulate material had no influence on the bioaccumulation of lindane (log P_{ow} = 3.9). After introduction of particulates to the water phase (dynamic system), about 95% of the total amount of HCB was bound to the particles, whereas for lindane the proportion was only about 12% /14/.

The studies cited above show that the particulate content in complex mixtures may reduce the amount of bioaccumulation in fish,

but may increase the concentration in organisms which live by "eating particles" (filter- and suspension-feeders). A number of experiments have all noted that this effect is only observed for substances of relatively high lipophilicity (log P_{ow} > about 5). The composition of the particulate material (in particular organic carbon content) and its concentration are important factors influencing the amount of adsorption.

Dissolved humic acids have also been shown to have the same effect as suspended particles with regard to reducing the uptake of hydrophobic substances /3,26/.

Uptake of individual substances in relation to other substances in the mixture

At the present time only a rather limited data material exists concerning the uptake of individual substances from mixtures.

For phenol, uptake was reduced when this substance was present in a wastewater mixture (the water-soluble fraction). The rate of elimination was not affected. The reason was thought to be a concentration-related competitive uptake of other substances in the wastewater /11/. In an investigation on the bioaccumulation of anthracene in fish using a mixture of oil refinery wastewater, both uptake and elimination of anthracene were different in the mixture compared with the kinetics for anthracene in a pure solution. While the altered uptake rate was assumed to be due to interaction with suspended material, the increased rate of elimination was ascribed to increased metabolism of the substance, probably because of the induction by other substances of the so-called "mixed-function oxygenase" system /23/.

The methods available are based on the following principles:

- bioassay, particularly using fish and bivalves as biomonitors
- chromatographic methods (TLC- and HPLC-screening).

Biomonitors

Investigations of the heavy metal loads in the area close to wastewater discharges have been supplemented in a number of cases by laying out cages of mussels along transects from the discharge point (cage experiments). After various periods of time a proportion of the mussels are collected, and after a short period in clean water for depuration of heavy metals in the alimentary canal, they are analyzed for the heavy metals of interest.

In addition to the question of food purity standards the method has also been used to investigate the problem of chronic heavy metal loads in the environment. From the equilibrium concentrations in the organism and knowledge of the BCF for the substance, an "average" exposure concentration in the water phase can be calculated. The method is also useful for making estimates of bioavailabilities and fluxes from areas polluted with heavy metals. In addition to mussels, eels have also been used in cage experiments for monitoring heavy metal loads.

A number of complicating factors influence such *in situ* experiments:

- variable environmental conditions at different monitoring stations may affect the growth of the test organism and thus also the bioaccumulation.
- periods with poor food supply and other extreme environmental conditions may cause the mussels to cease filtration and thus stop the uptake of the test substance(s).
- variations in currents may complicate the evaluation of the causal relationships between the concentration in the organism and the source.

Investigations of accumulation in the laboratory do not suffer from the above-mentioned weaknesses, since a direct causal relationship can be established-on the other hand, the opportunity of applying the results directly to environmentally realistic situations is to some extent lost.

In situ monitoring studies for organic substances reported in the literature (using fish, mussels and other organisms) have primarily focussed on industrial discharges containing specific persistent and highly lipophilic substances such as PAH compounds, chlorinated hydrocarbons, etc.

There are no monitoring studies reported in the literature in which the ability of organisms to accumulate "unknown" lipophilic (persistent) substances is investigated in parallel with chemical analyses as a screening test for the presence of substances with unwanted environmental effects. In a few cases, investigations of this type have been carried out in Denmark as a sort of final approval of industrial wastewater discharges. The advantages of this sort of method is that substances which occur in the water phase at concentrations below the level of detection of the analytical methods used, may be bioaccumulated and in this way become chemically detectable. However, the outcome may often be difficult to interpret because of biological matrix effects.

Figure 3.4.7 shows an example of a bioassay using eel to evaluate bioaccumulative substances in biologically treated wastewater from A/S Cheminova /21/.

The investigation focussed partly on the substances which had previously been detected in the wastewater, and partly on gas chromatographic screening for any substances in the eels which had become detectable as a result of bioaccumulation. From a list of 21 substances, a total of 7 were detected in the eels after an exposure period of 28 days (triphosphoric acid esters). None of these had BCF values > 10 after 28 days. For all the substances the concentration in the eels fell during the test period, which suggested that the substances were metabolized. Gas chromatography also revealed a substance in the eels which had not been detected in the wastewater. The substance could not be identified precisely but by means of elimination tests it was found to have a biological half-life of 2-3 days, and therefore to pose little risk of bioaccumulation.

Figure 3.4.8 shows an example of uptake and elimination of one of the substances detected in the eels.

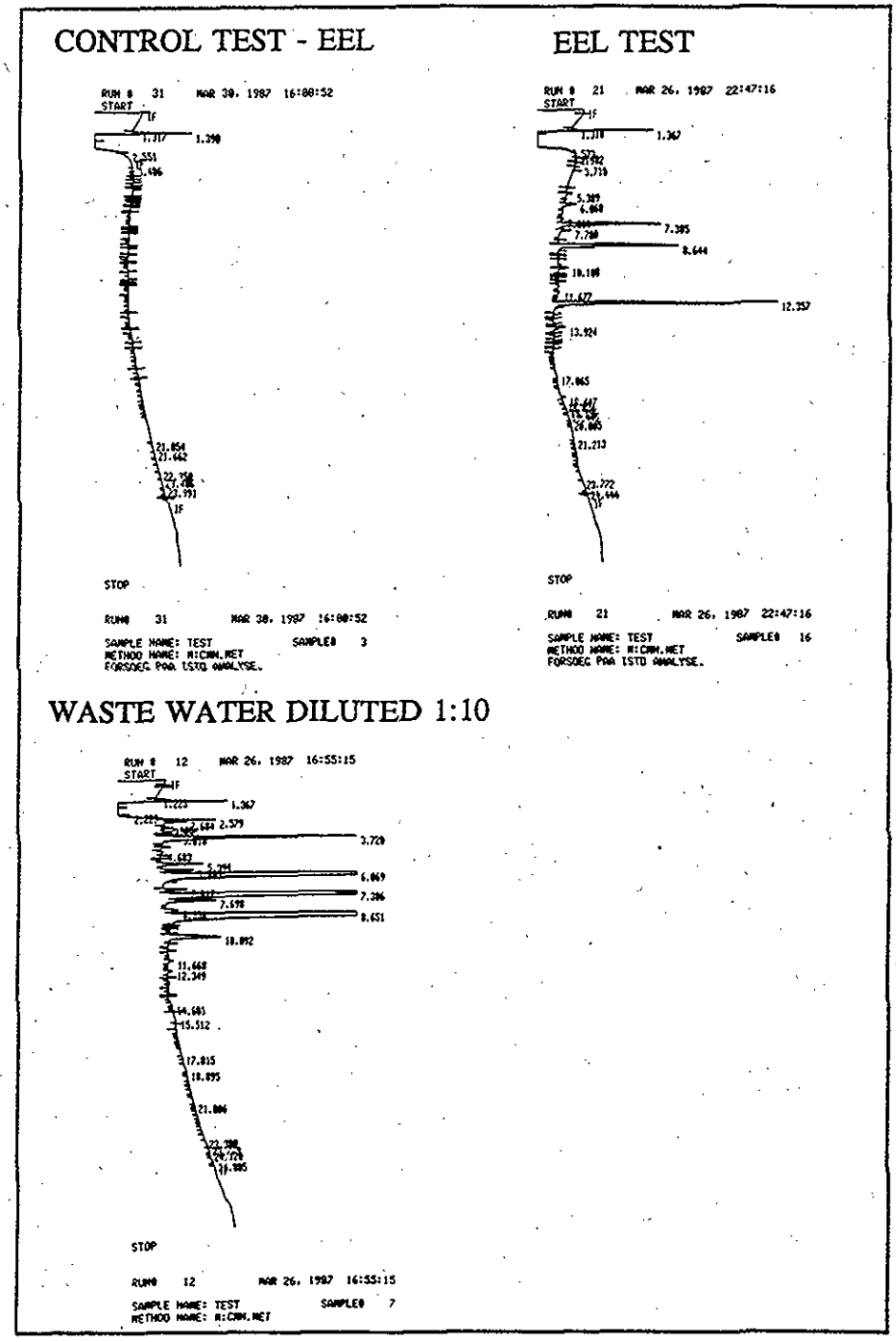

Figure 3.4.7 Gas chromatograms of wastewater (1:10) and eel exposed to wastewater and uncontaminated water respectively (from /21/).

Chromatographic methods

Thin layer chromatography (reverse phase TLC) is a well-documented and frequently used method for screening complex mixtures for substances which are potentially bioaccumulative /33/.

A hexane or pentane extract of the test substance is applied to the thin layer plate. The plate is eluted with an acetone/water mixture (70/30) and after drying the retention is measured using UV light. Simultaneous elution of standard substances of known P_{ow} permits the probable P_{ow}-values to be estimated for the test substances extracted from the wastewater sample. For closer identification of the

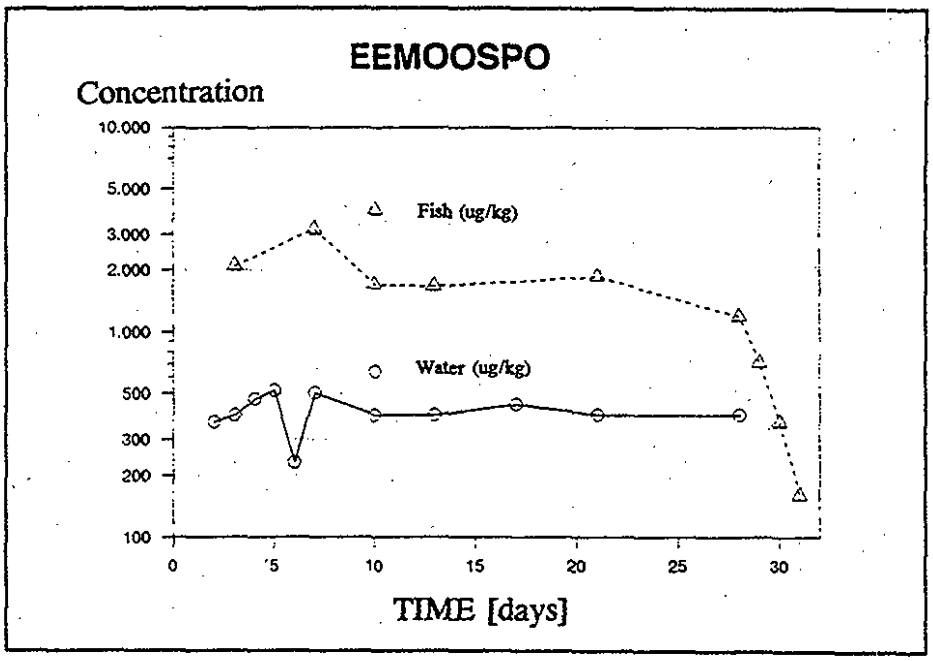

Figure 3.4.8 Uptake and elimination of a triphosphoric acid ester in eel exposed to biologically treated wastewater. After 28 days the eels were transferred to uncontaminated water (from /21/).

eluted substances, the "spots" may be scraped up and subjected to HPLC/MS or GC/MS analysis.

The method has frequently been used in Denmark for screening biologically treated wastewater. It should be added, however, that at the present time the method is only suitable for substances which absorb UV light. Furthermore, only substances which can be extracted by the extraction solvents used can be measured.

Some examples of TLC screening of percolate from a number of Swedish waste disposal sites are shown in figure 3.4.9 and table 3.4.4.

Table 3.4.4 Log P_{ow} values obtained from TLC-screening of a number of samples of percolate from waste disposal sites /10/.

Before stabilization						
Sample	No. of substs./ substance groups	log P_{ow} values				
		1	2	3	4	5
Brännbacken	5	6.35	5.79	5.44	4.82	5.56
Ulvberget	2	6.65	3.68	-	-	-
Råvsta	0	-	-	-	-	-
Atle	2	6.12	3.39	-	-	-
Rönneholm	0	-	-	-	-	-
Gräfsåsen	1	3.09	-	-	-	-
Träab	4	5.75	3.83	2.10	1.10	-
Måsalycke	1	2.26	-	-	-	-

HPLC may also be used directly to determine P_{ow} for the substances in mixtures /29/. The resolution of the chromatogram must be expected to be relatively poor, however, even in strongly diluted samples, because of the complexity of the mixture. In practice, therefore, it is usually only possible to determine the upper and lower limits of the P_{ow} of the mixture as a whole.

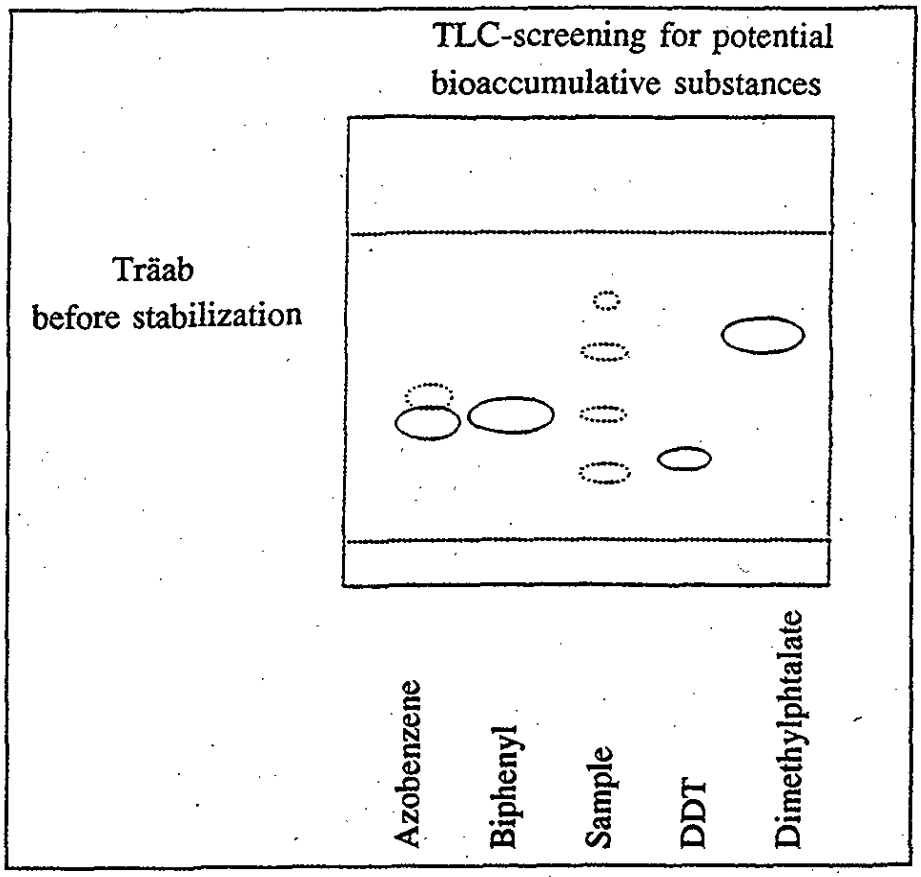

***Figure 3.4.9** Example of TLC-screening of percolate samples. "Copy" of UV-scanned thin-layer plate (from /10/).*

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4 Evaluation of ecosystem effects

4.1 Methods for evaluation of ecotoxicity

During the last 20 years, a number of international standard methods have been developed for investigating the toxic effects of chemicals on microorganisms, plants, and animals. This development has primarily focused on obtaining documentation of any adverse environmental effects of new chemicals, in order to regulate their use in relation to their environmental hazard.

Table 4.1.1 includes an overview of standard methods recognized by DS (the Danish Standards Institute), ISO, OECD and EC. The EC basic set of tests for investigation of new chemicals includes acute toxicity tests using fish, crustacea and algae. In addition, a number of methods are being developed for investigation of chronic effects in the same groups of organisms.

The international standard methods are often used as the framework for development of new methods using different test organisms. The methods are modified with due regard to the new organisms' biology and vital requirements, and in this way many new and reproducible methods have been developed in recent years using organisms from fresh water, brackish water and the sea. A selection of such methods is also included in table 4.1.1.

The use of biotests serves two general objectives:

- investigation of the "inherent" toxicity of substances and mixtures of substances (screening tests)
- investigation of the toxic effects of substances and mixtures of substances on specific environments or environmental compartments.

Biotests which are used for investigation of the "inherent" toxicity of substances or mixtures of substances should, if possible, fulfil the following requirements:

- the method should follow internationally accepted standard methods (ISO, OECD, EC)
- the method should give reproducible and comparable results
- test organisms should be available through the greater part of the year or be suitable for laboratory cultivation, while being sensitive towards a great number of chemicals.
- the method should be relatively simple to carry out at a moderate cost.

The requirements to be fulfilled by *screening tests* are thus very similar to the requirements applied to chemical standard methods.

On the other hand, evaluation of the possible *environmental effects* of substances or mixtures of substances (including wastewater discharges, etc.) is based on methods which have a greater degree of

Table 4.1.1 A Methods for testing effects of chemicals on aquatic organisms.

TROPHIC LEVEL	TYPE	PRINCIPLE	LEVEL*)	REFERENCES
Bacteria	Activated sludge	Simulation	A	EC /8/, OECD 303 /23/
		Inhibition of respiration (3 hour)	A	EC /8/, OECD 209 /23/ DS 297-298
		Inhibition of respiration (4 hour)	A	ISO DP 9509 /13/
	Photobacterium phosphorerum (Microtox)	Inhibition of luminiscens (5-30 min)	A	Beckmann Inc. /2/
	Pseudomonas putida	Inhibition of growth (72 h)	C	ISO DRAFT /13/
Algae	Phaeodactylum tricornutum (marin)	Inhibition of photosynthesis (6 h)	A	Kusk & Nyholm (1990) /16/
	Skeletonema costatum (marin)	Inhibition of growth (72 h)	C	EF /8/, OECD 201 /23/ Nyholm & Källqvist (1987) /21/ US-EPA (1989) /37/
	Dunaliella marina (marin)			
	Selenastrum costatum (fersk)			ISO/DIS 8692 /13/
	Nitcschia palea (fersk)			
	Algeplankton (fersk/marin)	Inhibition of photosynthesis (6 h)	A	Kusk & Nyholm (1990) /16/
Crustacean	Daphnia magna (fersk)	Immobilization (24-48 h)	A	EF /9/, OECD 202 /23/, ISO 6341 /13/
		Reproduction (21 days)	C	
	Gammarus pulex (fersk)	Lethal effect (96 h)	A	McCahon & Pascoe (1988a+b) /17, 18/
		Partial life-cycle	A	McCahon & Pascoe (1988a+b) /17, 18/
		Early life-stages (4-13 days)	SC	

*) A: Acute, C: Chronical, SC: Sub-chronical

Table 4.1.1 B Methods for testing effects of chemicals on aquatic organisms.

TROPHIC LEVEL	SPECIE	PRINCIPLE	LEVEL*)	REFERENCE
Crustacean (cont.)	Nitocra spinipes (brackish)	Lethal effect (96 h)	A	DS 2209 /5/ Renberg et al. (1980) /27/
		Partial life-cycle (12 days)	C	
	Acartia tonsa (marin)	Lethal effect (48-96 h)	A	ISO DRAFT /13/
		Life-cycle	C	Minshan & Møhlenberg (1991) /20/
		Egg production (5 days)	SC	Johansen & Møhlenberg (1987) /14/
	Chaetogammarus marinus (marin)	Lethal effect (96 h)	A	ISO DRAFT /13/
Insect larvae	Baetis rhodani	Lethal effect (96 h)	A	Williams et al. (1985) /42/
	Chironomus sp.	Lethal effect (96 h)	A	McCahon & Pascoe (1988a) /17/
Fish	Zebrafish Rainbow trout (a.o.)	Lethal effect (96 h)	A	EF /8/, OECD 203 /23/
		Lethal effect (14 days)	C	OECD DRAFT /23/ ISO DRAFT /13/
	Stickleback	Lethal effect (96 h)	A	DS F 86/185 /3/
	Cod	Lethal effect (96 h)	A	DS F 86/186 /4/
	Rainbow trout	Inhibition of growth (28 days)	SC	OECD DRAFT /23/ ISO DRAFT /13/
	Zebrafish Rainbow trout	Early-life-stages (ELS) (7-60 days)	SC	OECD DRAFT /23/
Plants	Allium	Inhibition of growth	SC	Fisksjø (1976) /11/
	Lemna minor	Inhibition of growth	SC	Taraldsen & Nordberg-King (1990) /30/
	Flax, cress	Root sprouting	A	OECD 208 /23/ US-EPA (1975) /31/

*) A: Acute, C: Chronical, SC: Sub-chronical

environmental realism, since it is important to be able to interpret the results in terms of the specific environmental conditions. At the same time the tests retain the controlled conditions of the laboratory so as to obtain reproducible results with a clear causal relationship. Thus species are used which as far as possible occur - or could occur - in the receiving water ("environmentally relevant tests", "simulation tests").

For example, the internationally standardized screening tests using fish include only freshwater species (zebra fish, rainbow trout etc.). For screening of discharges to marine recipients, an "environmentally relevant" test would use a marine species, and this requires modification of the standard method (cf. DS methods /3,4/).

Toxicity tests may also be classified according to the duration of the test in relation to the generation time of the test organism used. In *acute toxicity tests* the test organism is exposed for a relatively short time in relation to the generation time (short term exposure). The acute effects recorded during this type of test are usually effects which affect the survival of the organism (cf. table 4.1.1), such as:

- 48/96 hour lethal effects on crustacea and fish
- 6 hour inhibition of photosynthesis in algae
- 5-30 minute inhibition of light emission in *Photobacterium* ("Microtox").

In *chronic toxicity tests*, the organism is exposed for a significant part of the life cycle - sometimes for the entire life cycle. The types of effect that are recorded (end points, effect parameters) are often sublethal to begin with (reduced reproduction or growth, altered behaviour or development), but indirectly they can lead to increased mortality. For organisms with a relatively short generation time (bacteria, algae, some crustacea), chronic tests will usually cover one or more entire generations. For fish, which have a generation time of months or years, chronic tests usually cover life stages or phases which have been found to be particularly sensitive (early life stages, reproductive phase) or will focus on sublethal end points after a fairly long (> 14 days) period of exposure of juvenile or adult fish (growth or physiological end points).

In recent years there has been increasing interest in rapid methods for evaluation of "chronic toxicity". These so-called *sub-chronic* tests - or *chronic surrogates* - focus on life stages which have been found to be particularly sensitive (larvae of fish and crustacea) or on sensitive end points (such as size of offspring in crustacea or growth in juvenile fish). Usually the knowledge base for applying the results of such sub-chronic tests to evaluations of chronic effects has been inadequate; at best it relies on comparative laboratory studies, where the differences in effect concentrations for the two types of test have been shown to be "insignificant" (typically within a factor 2-3) for the same species using various test chemicals. For fish, for example, there is an extensive knowledge base for application of so-called "fish early life stage" tests (FELS-tests) as surrogates for whole life-cycle tests /15,19,43/.

4.1.1 Measurement of toxicity

The following section presents the principles for carrying out laboratory toxicity tests and a short description of frequently used methods in Denmark.

Test design

Investigation of the ecotoxicity of substances and mixtures of substances is carried out by exposing a group of test organisms in a series of dilutions of the test substance or mixture, under conditions which are as well-controlled as possible.

Each dilution is examined for well-defined effect parameters (death, immobility, growth, for example) at predetermined intervals, for example every 24 hours. A number of controls are included to ensure that any observed effects (or variations in effects) are not caused by other factors than the test substance. For the same reason, a number of key physical parameters in the test dilutions are also measured (oxygen saturation, temperature, pH, salinity etc.). These parameters must remain within relatively narrow limits, depending on the test organism used, to ensure normal responses.

On the basis of the recorded effect frequencies in the various dilutions, the effect concentrations (EC: effect concentration, LC: lethal concentration) are usually calculated for the 10, 50 and 90% mortality or effect level in the population. In addition, the highest tested concentration is often recorded at which no significant differences were observed in relation to the controls (NOEC: No Observed Effect Concentration), together with the lowest tested concentration at which the first significant effects were observed (LOEC: Lowest Observed Effect Concentration). Sometimes the calculated LC10 or EC10 is given as the LOEC.

The most recent OECD and EC standard methods for investigation of acute toxicity include the possibility of testing the chemical at one concentration only - the so-called "limit test". The purpose of this is to permit a briefer investigation of substances which on the basis of prior knowledge are expected to have low toxicity. The investigation is carried out at the highest soluble concentration (or 100 mg/l at most). One control is included. If toxic effects are observed at this concentration, a "normal" run of toxicity tests must be performed following the above-mentioned principles.

Three principal parameters are involved in ecotoxicity tests:

- the test organism
- the concentration and exposure time (= dose)
- the effect parameters or end points.

Test organisms

As mentioned above, only standardized methods are used for testing of chemicals. Thus the test organisms are "standardized" too: e.g. zebra fish, rainbow trout, *Daphnia magna*. There are two opposing tendencies in the choice of test organism.

On the one hand, populations of test organisms are standardized to be as homogenous as possible (low genetic variation). This will tend to produce less variability in the test results, and will increase the reproducibility and comparability between results from different laboratories (cf. optimization of chemical analyses). On the

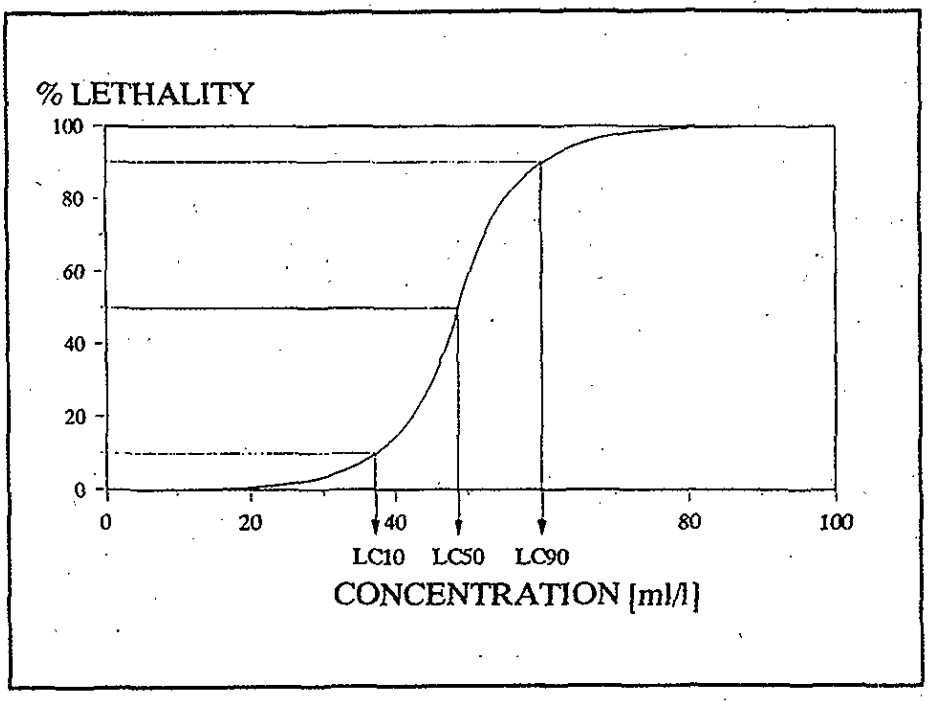

Figure 4.1.1 Theoretical relationship between exposure concentration and effect frequency (concentration/response curve).

other hand, the use of wild populations provides a better basis for determining NOEC-values for environmentally realistic conditions, but will lead to greater variation in the test results (greater genetic variation).

One decisive question in the choice of organism is the problem of whether the normal living requirements of the population can be maintained for a period of time which is at least as long as the duration of the test. Important factors relating to this question are:

- oxygen concentration
- temperature
- pH
- hardness
- salinity
- feeding requirements/light (algae)
- physical surroundings.

Knowledge of culture requirements is only available for a relatively small number of species. Standard organisms are typically robust towards variations in their surroundings, and are therefore easy to cultivate or maintain in the laboratory. With wild populations it can be particularly difficult to maintain satisfactory living conditions during long-term tests (chronic tests). As a general requirement, collected or purchased test populations should be acclimatised to the laboratory (test) conditions for 7-14 days before commencing a test. This serves to document that the population is thriving satisfactorily under laboratory conditions and to check on the state of health of the population.

It should be noted that "robustness" with regard to survival under laboratory conditions is not related to lower sensitivity towards toxic chemicals. There is no evidence for the often-heard assertion that eel and zebra fish, for example, are less sensitive than "finer" fish species.

During exposure it is important that the requirements of the test organism to the above-listed physico-chemical parameters are fulfilled. Requirements as to environmental realism, or to the nature of the wastewater to be tested may sometimes make it necessary to perform the test under conditions that are sub-optimal for the organism, but they should never lie outside the tolerance zone. In all standard methods a range is given for the maximum acceptable variation in these parameters (for example, pH ± 1-2 units, temperature ± 0.5-1°C). In short-term tests the organisms should never be fed unless their survival is dependent on this. The length of the standardized acute toxicity tests is determined partly by the requirement to avoid feeding (fish: 96 hours, daphnia 24-48 hours). In comparative studies with and without feeding, the same effect concentrations were measured in both types of test for a number of different substances.

The density of organisms per unit volume may be very important for some test species. For example, it is known that *Gammarus* becomes cannibalistic if the density is too high. For fish, a density below 1 g/l is recommended for static tests and 1-10 g/l for flow-through tests. Both too high and too low densities can lead to stress in fish: at low densities, pecking orders may arise, and at too high densities aggressive behaviour occurs.

Apart from the above-mentioned considerations as to genetic homogeneity, it is desirable to have a test population which is as uniform as possible with regard to sex, age, size, and physiological condition. It is known that physiologically stressed organisms are more sensitive than "normal" individuals, and that sensitivity generally falls with age. In particular, there can be great differences between juvenile and mature organisms, since the enzyme systems responsible for metabolism and detoxification of xenobiotic substances are not fully developed before the mature stage is reached.

Exposure (concentration/ exposure time)

In addition to the inherent properties of a substance, its toxic effect also depends both on its concentration in the medium and on the exposure time (the dose).

In order to achieve reproducible and "precise" effect concentrations it is therefore necessary - other things being equal - to define the concentration and exposure time very carefully, and to ensure that the variation in concentration is kept within relatively narrow limits.

According to the latest updates to the OECD standards, the reduction in test substance concentration during the test period must not exceed 20% of the starting concentration, and the variation in the average concentration must be within ± 20% (S.D.). If adequate data cannot be presented to document that these requirements have been met, chemical analyses should be performed to document the substance concentration in the water. This should also be done if it is suspected that the real concentration in the water is significantly lower

than the nominal (dissolved) concentration (OECD update proposals /24/).

The following factors are important for maintenance of well-defined and constant test substance concentrations:

- solubility in water
- adsorptive properties
- vapour pressure
- dissociation constant (pK_a)
- degradability

Organic compounds with low solubility (< 100 mg/l), relatively high vapour pressure (Henry's constant $> 10^2$ Pa·m³/mol), or tendency to adsorb to dissolved or particulate organic material (corresponding to a relatively high bioaccumulative tendency: log P_{ow} > approx. 4, cf. section 3.4) may be problematic with regard to maintenance of a stable concentration. The same is true of substances which may degrade to a significant extent during the test.

Although data may be available concerning the solubility of a substance, this information may be of doubtful value, especially for substances of very low solubility (< 1 mg/l). For these substances the actual solubility in the test solution may often be less than the figures stated in the references. Effect concentrations lying close to the limit of solubility may therefore represent significant underestimates if they are based on nominal concentrations.

Substances which adsorb to particles or to dissolved, high-molecular-weight substances are not biologically available to any great extent, particularly in the case of pelagic organisms. In the case of benthic organisms (filter- or suspension-feeders) the food may also contribute to the "dose", especially with strongly bioaccumulative substances (for example: uptake of heavy metals and organic compounds such as hexachlorobenzene by mussels). That part of the substance concentration which is in true solution will still be primarily responsible for a given toxic effect, however (cf. section 3.4). When testing strongly lipophilic substances, which are highly adsorptive, the effect concentration may be significantly underestimated if reliance is placed on the nominal concentrations; if chemical analyses are used the concentration may still be an underestimate since both the dissolved and the adsorbed fractions will be analyzed. The problem may be reduced by minimizing the amount of particulate material in the test medium (removal of food remains and faecal material), by correcting for the adsorbed fraction on the basis of the known adsorption coefficient and the content of particulate material in the test system (mg carbon/mg SS·l) (cf. section 3.2), and by reducing the content of particulate material - and thus the "non-available test substance" - by filtering or centrifuging prior to analysis.

For substances of low solubility in water, it may be necessary to use solvents or carriers in order to prepare a stock solution of sufficiently high concentration. Examples of frequently used solvents which because of their low toxicity and low vapour pressure are well-suited for toxicity tests are shown in table 4.1.2 together with their lethal effect level for fish.

Table 4.1.2 Solvents often used in toxicity tests. LC50-values for fish (fathead minnow) are shown (from /26/).

Solvent	LC50 (mg/l), fish
Triethyleneglycol	92,500
Dimethylformamide	10,400
Acetone	9,100
Dimethyl sulfoxide	33,500

In addition to the substances shown in table 4.1.2, methanol and ethanol are also used.

Most standard procedures indicate an upper limit for the concentration of solvent in the test solution of about 0.1-0.5 ml/l. If solvents are used, an extra control must also be included to document the non-effect of the solvent at the highest concentration used.

In later years there has been increasing criticism of the use of solvents. Solvents may act as carriers of substances through the cell membrane, and it is also doubtful whether the solubility of the test substance *in water* is really increased by the solvent. A better procedure is probably to prepare a saturated solution of the test substance in the test medium and to use this to prepare a range of dilutions, using chemical analysis to document the concentration of the saturated solution.

For pH-dependent dissociative substances the toxicity of the dissociated and the undissociated forms may differ greatly. One well-known example of this is ammonium and ammonia, where only ammonia is toxic. Chlorophenols have acid/base characteristics with the acid form as the most toxic. Generally, the form with the lowest degree of ionization is the most toxic, since non-ionized or weakly ionized molecules can most easily penetrate biological membranes.

In order to prevent a fall in concentration during the test period the solution may be partially replaced at regular intervals (*partial replacement*) or the organisms may be transferred to freshly prepared solutions in clean containers (*semi-static technique*). The latter method is preferable if the test substance degrades during the test period, since a significant part of the bacterial biomass will be removed along with the dirty container. If the test substance concentration cannot be maintained within the prescribed limits using the above-mentioned techniques, the *flow-through technique* must be used.

Measurements of the toxicity of *complex mixtures* presents particular problems with regard to maintenance of exposure concentrations within the limits prescribed by the standard method. It is rarely possible to ascribe the observed effect to some few substances in the mixture, and thus it is not possible to use chemical analysis to document that the quality requirements have been met.

Furthermore, the mixtures may contain substances with physico-chemical properties covering a broad range with regard to water solubility, adsorptive tendency, vapour pressure etc. In practice

this problem is dealt with by using semi-static or flow-through techniques and by covering the test containers to minimize evaporation of any volatile constituents.

Biologically treated wastewater will be considerably easier to handle than untreated effluent, since a significant reduction in volatile, adsorbable and easily degradable substances will take place during treatment.

Gentle aeration of both treated and untreated wastewater will often be necessary in order to maintain adequate oxygen tensions for the test organisms. Aeration can however affect the carbonate system, leading to unacceptable alterations in pH. A combination of gentle aeration and a flow-through system will often be the best solution. It should be mentioned that aeration is only acceptable when the toxicity contribution of volatile substances is negligible (for example when testing treated wastewater or chemical substances with low vapour pressure).

Storage of samples for biotesting at a later date should only take place at low temperature: < 5-8°C for short periods (24 hours) and < -18°C for longer periods. It is important to point out, however, that the toxicity of deep-frozen samples may change (fall) during storage, and that toxicity tests should therefore be made as soon as possible after sampling. In addition, samples must be stored in the dark to prevent photo-oxidation or algal growth. It should be noted that glass/polypropylene containers for sampling and storage should preferably be completely new. Even thoroughly cleaned containers can contain traces of substances (solvents, detergents) with sufficient toxicity to cause "effect-artefacts" in wastewater samples which in fact have low toxicity. The same principle holds for test aquaria or containers.

Wastewater samples are usually inhomogeneous mixtures containing both particulate material and substances at concentrations above the true limit of solubility (emulsions/stratified samples). Preparation of dilution series before biotesting means that the initial sample is diluted with non-polluted water, which can cause particle-adsorbed substances to be released, and emulsified substances to go into true solution. Since only the substances that are dissolved in water are responsible for a given biological effect, the ultimate consequence of these processes can be that the concentration in water of a number of the substances will remain constant in the highest wastewater concentrations, and therefore, if these substances are the toxic ones, that the test response will be the same. The method for obtaining representative sub-samples should therefore be chosen with care. Because of these changes in solubility- and adsorption equilibria due to dilution, any adjustments to the samples that are necessary to comply with the living requirements of the test organism should be carried out in the test solutions themselves and not in the initial sample.

In order to obtain reproducible and - in particular - comparable results from biotests on wastewater mixtures it is extremely important to consider and describe as precisely as possible all the important factors relating to sampling, transport, storage, pre-treatment and preparation of dilution series. As mentioned above, a thorough description may often be the only way of "documenting" the

effect concentrations achieved in the tests with regard to ensuring comparability with subsequent samples and tests.

Dilution water/medium water for dilution of wastewater-/chemical substances may either be prepared artificially from distilled water and salts etc., or it may be collected from a non-polluted locality or an area near the recipient which is not directly affected by the discharge. For chemicals testing, the use of artificial medium seems preferable, in order to ensure international comparability of data, but this is far from being straightforward for all test organisms. For example, it is not yet possible to obtain satisfactory reproduction in *Daphnia magna* in artificial medium.

For testing for effects of wastewater on a specific recipient, one should always use medium taken either from the recipient itself or from a comparable locality.

Effect parameters/endpoints

Toxicity is a rather poorly defined concept which covers a continuum from lethal effect to inhibition of specific enzyme systems with no apparent effect on the vital functions of the organism.

Unlike the task of human toxicology, the administrative application of ecotoxicology aims not at protecting individuals, but at ensuring "a versatile plant and animal life", i.e. at ensuring that the normal homeostatic function of the ecosystem is preserved. For example, demonstrating inhibition of the photosynthetic activity in one particular algal species does not necessarily mean that this effect is important for the plankton community in the free water masses, partly because natural physico-chemical variations in the environment may temporarily cause this type of effect too, and partly because inhibition of one species is not necessarily a good prediction of the effect on the plankton as a whole.

Another example is effects on growth of early larval stages of fish, which under laboratory conditions can be significant down to a few mm or mg relative to the growth of controls. Whether this statistical significance is also biologically significant is uncertain, since reduction in growth will also be caused by small changes in the natural environment relative to the optimal conditions for the species. Alterations to swimming ability, pigmentation, reflexes etc. will be more likely also to have biological significance, since they will affect the ability to find food and avoid predation, etc.

It must be concluded that some of the effects recorded in single species in the laboratory are only *indicative* of an effect, which *may* cause changes to the structure and function of the ecosystem.

Effect criteria in laboratory tests should ideally fulfil the following requirements:

- rapid and unambiguous scoring
- quantifiable
- reproducible
- biologically relevant

Effect parameters which include a subjective evaluation of the response are therefore not suitable. However, "subjective" observations are also important with regard to evaluating whether other

types of effects are being produced in addition to the unambiguous responses which the test aims at.

In acute toxicity tests the primary effect criterion is death. Completely objective determination of "death" in test organisms is rarely possible, however, and therefore for many organisms such as crustacea death is considered to be indicated by "lack of reaction to external physical stimuli". Immobility therefore becomes the criterion instead of lethality (IC = immobility concentration). In fish and crustacea tests any abnormalities appearing during the test period should also be recorded; these might include:

- reduced swimming ability
- alterations in pigmentation
- abnormal mucus production (irritative response)
- hyperventilation
- bleeding
- altered behaviour (such as aggressivity)
- reduced food uptake etc.

In acute toxicity tests the time-related development in the effect concentrations is also indicated by the LC10, LC50 and LC90 after each day's exposure. This can be used to indicate whether the effect concentration reaches a stable level during the test period or whether a lower effect concentration would be reached if the test were to be prolonged (cf. figure 4.1.2).

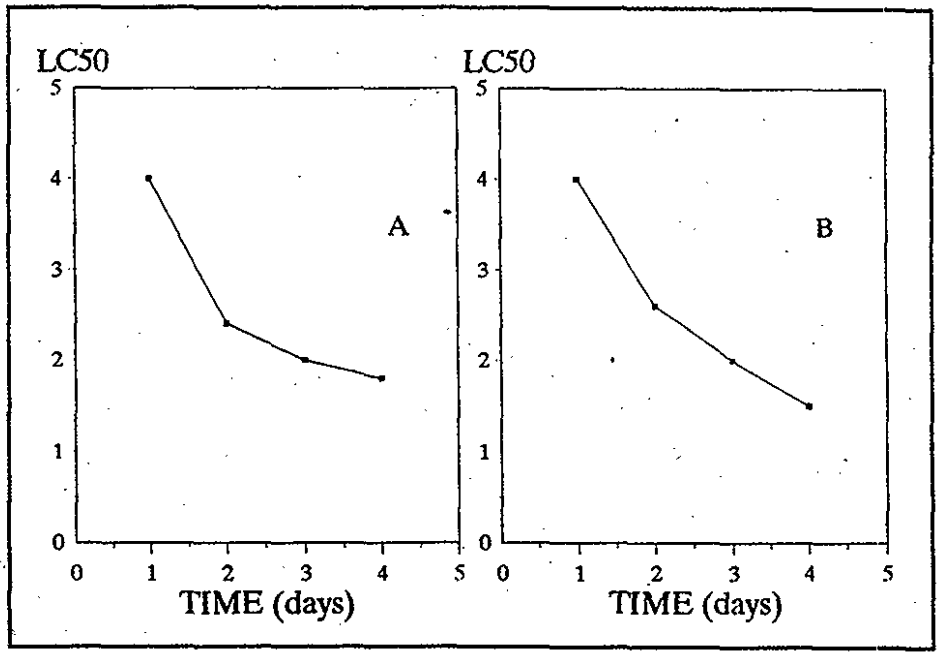

Figure 4.1.2 LC-values in relation to exposure time.

The time-related development in effect-concentration represents in fact an indication of whether the dose corresponding to the exposure concentration has in fact been delivered in the test organism during the test period. A continuing fall in effect concentration (figure 4.1.2 B) may be expected for substances which bioaccumulate, and

which during the short time period of the acute toxicity test do not achieve equilibrium in the organism relative to the exposure medium; a similar fall may be expected for substances which penetrate the cell membrane very slowly.

Chronic effect parameters are generally less well-defined, and in addition to "all or nothing" responses (dead/not dead), graduated responses such as length or weight may also be used. The latter can be subjected to normal statistical analysis (testing of hypotheses). Examples of frequently-used end points are growth, mortality of various life stages, brood size, and morphological or physiological alterations.

As with acute toxicity tests, the results of chronic toxicity tests are presented as concentration/response curves. Only in a few cases is it possible, however, to calculate EC50 values from the test data. In the case of wastewater samples this is not even desirable, since for an environmental interpretation of the results the NOEC/-LOEC values are more useful.

Data processing

Regardless of the effect parameter that is followed in a toxicity test, the relationship between the response of the test organisms and the concentration of test chemical or mixture will in theory follow a sigmoid curve (cf. figure 4.1.1). The classical theory underlying this curve is that the response data are normally distributed, and that there is a lower concentration limit below which a given effect will not be observed in the population (0% response), and an upper concentration limit above which all test organisms exhibit the given effect (100% response). Depending on the size of the group of organisms exposed at each level of concentration, and the interval between these, the toxicity data will in practice follow the sigmoid curve with greater or lesser accuracy.

For economic and ethical reasons the number of organisms exposed at each level of concentration is held to a minimum. In acute tests with fish, 10 individuals per concentration level is the norm, and in the latest update proposals for OECD standards (1989-90) 7 fish per concentration level are proposed. For corresponding daphnia tests, 20 individuals per concentration are used. In standard methods, 5 concentrations and one control are recommended as a minimum. Because of the theoretical form of the concentration/response curve, the concentrations should be chosen from a geometrical series, which according to various standards should not be greater than a factor 1.8 (= $\sqrt[4]{10}$, covering one decade with 5 test concentrations) or 2.2 (= $\sqrt[3]{10}$, covering one decade with 4 tests).

The lowest tested concentration should preferably reveal a zero response and the highest concentration a 100% response, which in theory leaves the 3 intermediate concentrations to indicate the graduated response - in practice often only 1-2 of these.

Because of these limitations, to which should be added the genetic variation of the population and the variation in the water-dissolved concentration, the approximation to the theoretical curve is often relatively imprecise.

In the standardized methods the maximum permissible mortality in the control population is 10%. If mortality occurs below this

level, the responses in the test concentrations can be corrected using Abbott's formula /10/ before proceeding with data processing.

If a sigmoid concentration/response relationship does not seem to be indicated by a simple non-transformed plot, further data processing is meaningless. In such cases the effect concentration should be given as the interval between 0 and 100% response.

Data showing a clear concentration/response relationship, i.e. at least 2 response values lying between 0 and 100%, may be treated statistically. For tests in which the observed effect gives an "all or nothing" response (lethality), the effect concentrations (EC10, EC50, EC90) may be calculated using probit analysis /26/. Using this method the data from the *progressive* part of the concentration/response curve is linearized in a logarithmic (concentration) probit (response) system, as shown in figure 4.1.3.

Probit analysis presupposes that response data is approximately normally-distributed (may be tested by means of the chi^2 test).

In addition to the linearized concentration-response curve, the confidence limits of the curve may also be calculated (figure 4.1.3). It will be seen from the figure that the surest estimate of the toxicity (least variation) - other things being equal - will lie at the EC50 value. There is thus good reason to use EC50 values if reproducible and comparable data is needed (for comparison of toxicity of various substances, estimation of temporal variation in wastewater toxicity, comparison of wastewater discharges).

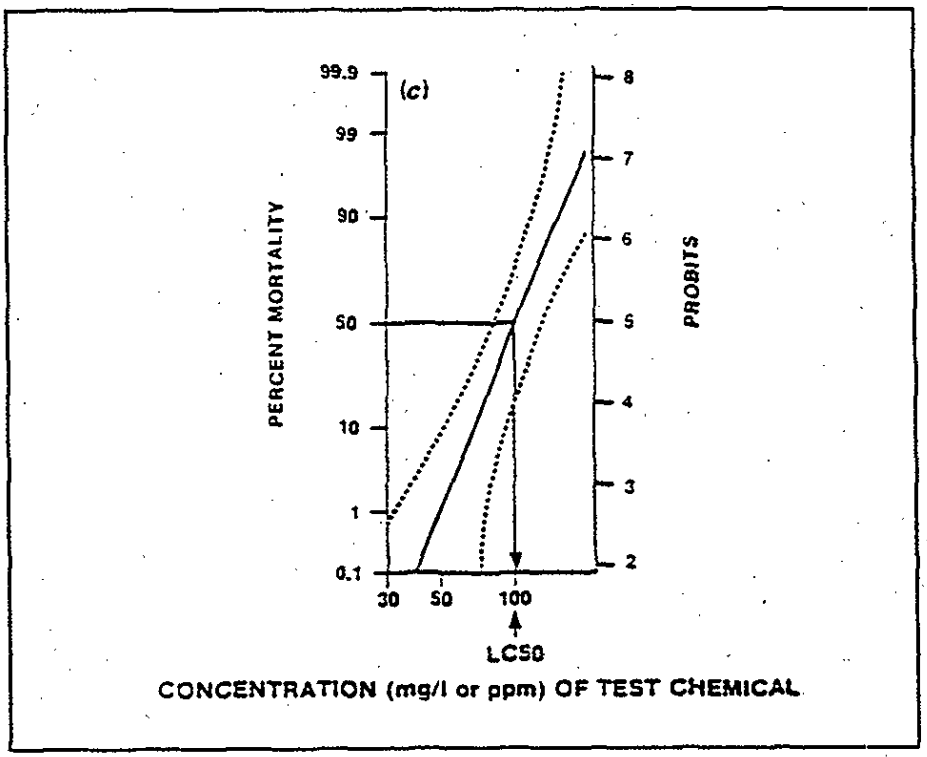

Figure 4.1.3 Probit analysis of concentration-response data /26/.

Today commercially available computer programmes can calculate effect concentrations by probit analysis /28,36,38/.

In addition to probit analysis a number of other methods are available for transformation of concentration/response data. In particular, the Spearman-Karper method /12/ should be mentioned as an alternative to probit analysis if there is low probability that the data is normal-distributed (a non-parametric method).

Processing of data from chronic tests is often focused on establishing NOEC- and LOEC-values. The reason for this is partly that chronic tests - also when used for environmental hazard classification purposes - are better suited for establishing zero-effect levels in the environment, and partly that effect concentrations above the 50% level are seldom observed for other end-points than lethality.

The fundamental principle in statistical estimation of LOEC (and thus also NOEC) is a comparison of the response at each test concentration with the control response in order to reveal any significant difference, and whether a significant difference may be ascribed to the test chemical/mixture, or to chance biological variation.

Statistical analysis is usually performed in the following steps:

1) Transformation of data by means of probit analysis or "arcsine square root" transformation. The latter method is preferred since it gives a uniform distribution of the variance of the individual estimates, and because the distribution of the transformed data approximates better to the normal distribution (permits use of "ordinary" parametric statistics).

2) The t-test is used to evaluate any differences existing between the individual control replicates (and where appropriate, comparison with control with added solvent). If there is no significant difference, the control replicates may be pooled for evaluation of the response in the individual test concentrations.

3) The responses in the individual test concentrations are then subjected to Dunnett's test procedure /6,7/, starting with the highest concentration. This procedure is analogous to the ordinary t-test, but unlike this gives equal weight to all significance tests in a single series of samples (multiple comparison tests). Williams /40,41/ has developed an improved test procedure. In this method the individual test responses are tested against the control value as before, but in addition the method ensures that the response data follow a monotonically increasing scale in relation to the test concentration. In both methods the LOEC is determined at the 5% level of confidence ($P = 0.05$). The NOEC value will be the next concentration level (a factor 1.8-2.2 lower than the LOEC value). Dunnett's test is available as a PC-programme /33/.

In tests involving several effect parameters, for example lethality and body weight, brood size etc., it is recommended that the first statistical test is probit analysis with regard to lethality. As the

second step, an examination is made of the test concentrations at which there was no significant lethality in relation to the control, by means of a significance test using Dunnett's or William's test. The advantage of this sequence is that the significance tests are then carried out on approximately the same brood size.

Before testing weight and length data, these should be checked for homogeneity (Bartlett's test) and normal distribution (Shapiro-Wilk's test) /25,29/.

Figure 4.1.4 presents a summary of a statistical test strategy which is used in the USA /35/.

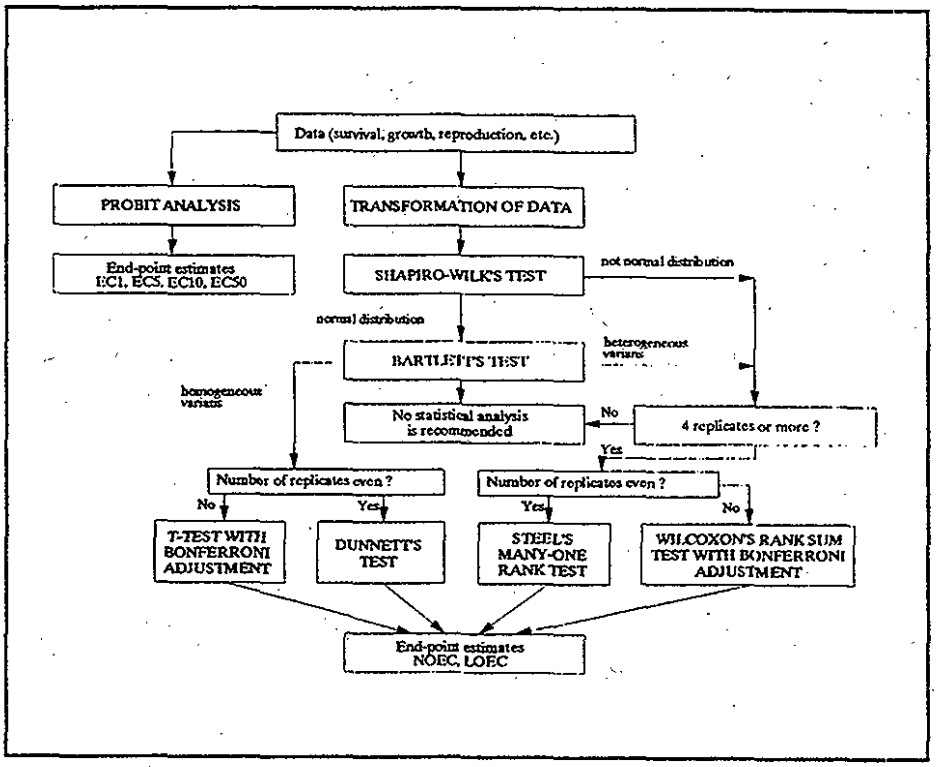

Figure 4.1.4 *Flow sheet for statistical treatment of data /35/.*

Statistical analysis should only be used on data which on the basis of an initial expert assessment *indicates* toxic effects, and it is unnecessary if the data show pronounced effects in relation to the control values. Statistical analysis is not conclusive in itself, but should always be followed up by expert evaluation of the *biological* significance of the results.

4.1.2 Brief description of biotests for acute toxicity

This section describes some of the methods frequently used in Denmark:

Microtox

This method uses inhibition of light emission by the marine bacterium *Photobacterium phosphoreum*. The test is performed using a specially designed apparatus (Beckman Microtox Assay System /2/). Light emission is measured photometrically using a suspension of bacteria

in 2 ml. cuvettes (at 15°C, pH = 7.2 and 20 ‰ NaCl). Measurements are taken after 5, 15 and 30 min. After each reading the concentration/response curves are plotted and the EC-values read off or calculated statistically. The highest concentration that can be tested is 500 ml/l.

Activated sludge, inhibition of respiration

Investigation of the effect on the respiratory activity of activated sludge is carried out following Danish Standard (DS) 297 if the test material contains volatile components (closed system). In other cases DS 298 is followed.

As starting material, secondarily precipitated activated sludge is used, taken from a wastewater treatment plant which predominantly handles domestic wastewater. The rate of respiration is measured by an oxygen electrode after 0.5 and 3 hours incubation in a suspension of sludge and synthetic wastewater with the addition of increasing amounts of the test wastewater/test substance (minimum of 5 concentrations). In addition to a control, the test is also performed with a reference material (e.g. 3,5-dichlorophenol) and an abiotic control.

The results are used to calculate/read off values for EC20 and EC50 as a minimum.

Activated sludge, inhibition of nitrification

Investigation of the inhibitory effect on nitrifying bacteria is carried out on sludge with an initial nitrification rate of about 26.5 mg N/g·h.

From the known content of NH_3-N (added or initially present in the wastewater) the concentration of NO_3/NO_2-N produced after 4 hours' incubation is calculated. The nitrification rate is expressed as NO_3/NO_2-N produced per gram suspended material per hour.

In addition to control and at least 5 test concentrations, a test using a reference material (e.g. alkylthiourea, 100% inhibition at 11.6 mg/l) is included. Wastewater can be tested at concentrations of up to 400 ml/l.

Algal photosynthesis test

Inhibition of algal photosynthesis is tested using the normal methods for measurement of primary production.

The test can be made on single species in the exponential growth phase, or on plankton samples from fresh or salt water.

The algae are incubated for 6 hours in growth medium containing ^{14}C-marked bicarbonate at constant temperature and light intensity. In addition to a control (5 replicates) and test concentrations (3 replicates), the test is also performed using a reference substance such as potassium dichromate.

The photosynthetic activity of the individual samples is measured using a scintillation counter.

The highest concentration that can be tested is 200-300 ml/l.

Crustacea, Nitocra spinipes or Acartia tonsa: lethality

Nitocra spinipes and *Acartia tonsa* are both marine crustaceans (copepoda) that can be cultivated in the laboratory.

Nitocra is a sediment-dwelling species under normal conditions, and tolerates relatively large variations in salinity (it may be cultivated in the laboratory at 9 and 15 ‰). *Acartia* is a planktonic species and is widespread in Danish waters. It is cultivated at 28 ‰.

For acute toxicity tests large copepodites and adults are used. 20 animals are used for each concentration (4 replicates, 5 animals each). In addition to test concentrations and a control, a test series using the reference substance potassium dichromate is also used (*Nitocra*: LC50 = 20-50 mg/l (9‰) og 40-70 mg/l (15‰), *Acartia*: LC50 = 10-30 mg/l (28‰)).

The test is performed at 20°C (*Nitocra*) or 18°C (*Acartia*) with a duration of 96 hours.

In contrast to *Nitocra*, *Acartia* must be fed during the test. Mortality is counted in each test glass every 24 hours, and on the basis of the results the LC10, LC50 and LC90 values are calculated by probit analysis.

Crustacea, Daphnia magna: immobility

Daphnia magna, which may be cultivated in the laboratory, is a natural component of plankton in lakes and ponds, feeding on phytoplankton. Daphnia reproduce under favourable conditions by parthenogenesis; under unfavourable conditions (food, temperature) males appear and reproduction is sexual. Juveniles, < 24 h old, are used for the test which has a duration of 24-48 hours. No feeding is done during the test, but prolongation after 48 hours will make feeding necessary in order to ensure satisfactory survival in the controls.

A total of 20 animals (4 replicates) is used for each concentration. A test on a reference substance such as Potassium Dichromate is also included (IC50 (24 hours) = 0.9-2.0 mg/l).

The test is performed at 20°C and 150 mg $CaCO_3$.

After 24 and 48 h the number of immobile animals in each test glass is counted. Immobility is used as effect criterion instead of lethality, since "death" is difficult to see in daphnia. The IC values are calculated by probit analysis.

Fish, Zebra fish, rainbow trout etc.: lethality

Zebra fish may be cultivated in the laboratory, while rainbow trout are obtained from commercial fish farms. The test is performed with juvenile fish (rainbow trout 4-6 cm, zebra fish approx. 3 cm).

Before commencing the test the fish are acclimatized for at least 7 days to observe for any signs of disease or poor condition.

20 fish are used for each concentration and control, and no feeding is done during the exposure time which is 96 hours. Mortality is recorded every 24 hours, and on the basis of these results the effect concentrations are calculated by probit analysis.

4.1.3 Brief description of biotests for chronic toxicity

Algae, inhibition of growth

Examination of growth inhibition (rate of growth) in algae is performed on populations which initially are in the exponential phase of growth. The exposure time (72 hours) is considerably longer than the generation time of the algae and the test is thus equivalent to a life-cycle test.

The physico-chemical parameters during the test are specific for the species being tested.

In addition to test concentrations (3 replicates at each one) and control (3-5 replicates) a reference substance such as potassium dichromate is also included. The highest concentration of wastewater that may be tested is 200 ml/l.

Growth in the algal population is measured by fluorescence or electronic particle counting (Coulter Counter).

Effect concentrations are calculated by probit analysis or significance test.

Crustacea, Daphnia magna: life cycle test

The test involves exposure of juvenile organisms, < 24 h old (F_0), and terminates by counting the number of offspring from this generation (F_1) (21 days in all).

10 individuals are used for each concentration (control: 20 animals). The test organisms are fed with algae (*Chlorella*) during the test.

During the test a record is kept of:

- Lethality in the F_0 generation - 3 times weekly
- Time from start of test until appearance of F_1 generation
- Brood size of F_1 generation(s).

The effect concentrations for lethality may be calculated using probit analysis; the other effect parameters are calculated using significance analysis.

Crustacea, Nitocra spinipes: reproduction

This test consists of exposure of females with egg-sacs, and their offspring, to the test substance for 12 days. 20 individuals (4 replicates) are used per concentration and control. The organisms are fed with finely ground fish food during the test.

The following parameters are measured:

- Survival of mother organisms (F_0)
- Brood size (F_1)
- Survival and age distribution of nauplii and copepodites (F_1)

The lethality scores are subjected to probit analysis; the other data are assessed by a significance test.

Crustacea, Acartia tonsa: life cycle and egg production

3-week old females are exposed for 8 days (approx. 20 individuals per concentration, 4 replicates). Offspring are followed through egg development, hatching, and juvenile stage to sexual maturity, approx. 14 days in all.

Instead of this life cycle test a shorter version is often used, which only includes recording of egg production (exposure of females and counting eggs produced during 5 days).

In the life cycle test the following parameters are counted:

- Survival of the F_0 generation (mother animals)
- Brood size (F_1)
- Survival and age distribution of juveniles (F_1)
- Growth and number of mature animals (F_1)

Results are treated as for *Nitocra spinipes*.

Fish, Zebra fish, rainbow trout etc.: Early life stage test (ELS)

Examination of effects on early life stages involves exposure of eggs immediately after fertilization until the end of the egg-sac stage (short-term method: Short Term Fish Early Life Stage test, ST-FELS), or through a shorter or longer part of the juvenile stage (FELS test).

A significant (practical) difference between the two variants is that feeding is not necessary in the ST-FELS test (cultivation methods only available for rather few species) and that the ST-FELS test is significantly shorter.

20-30 eggs are used for each concentration.

The following effect parameters are followed (ST-FELS):

- Survival of eggs and larvae
- Duration of the egg stage
- Larval growth rate
- Larval development (deformity)

Data processing is carried out in two stages. First the effect concentrations for lethality and deformities are calculated by probit analysis. In the test concentrations for which survival and development are not affected, significance analysis is employed to test for effects on larval growth and duration of egg stage.

4.1.4 Reproducibility of screening tests

In Denmark it is customary to check the reproducibility of screening methods by regular testing using a reference substance. This improves the possibility of comparing results obtained at different times at the same laboratory.

Only a few actual ring tests or intercalibrations have been performed in Denmark (or internationally). Only tests of this type can provide the foundation for comparison of data obtained at different laboratories.

The most frequently used reference substances are all metal salts: zinc chloride, potassium dichromate, copper sulphate. These substances fulfil all the requirements to a reference material:

- well-defined level of toxicity
- easily handled, i.e. readily soluble in water, low adsorptivity and volatility
- persistent
- long shelf-life at room temperature.

Below is shown a survey of the results achieved using potassium dichromate in a number of different test systems at one particular laboratory (Water Quality Institute (VKI), ATV) in 1989-90 (table 4.1.3).

It will be seen that the EC50 value is usually determined with greater precision than the EC10 value.

Table 4.1.3 EC10- og EC50-values in various test systems using $K_2Cr_2O_7$ (and $ZnSO_4$ for the Microtox test). In addition to means are indicated: 1) mean of the confidence intervals (C.I.) expressed as percentage of EC, and 2) the coefficient of variation of the mean.

Method	No. of tests (N)	EC10 Mean value (mg/l)	EC10 95% CI % EC10	EC10 Var. coeff. % EC10	EC50 Mean value (mg/l)	EC50 95% CI % EC50	EC50 Var. coeff. % EC50
Bacteria							
Microtox (5 min)	3	15	16	19	53	7	14
Algal photosynthesis							
Skeletonema (marine)	5	1.5	61	25	4.0	39	14
Phaedactylum (marine)	5	4.9	180	61	33	18	19
Dunaliella (marine)	3	263	49	19	715	26	12
Nitzschia (freshwater)	3	0.08	69	5.6	0.38	28	13
Crustacea, acute							
Daphnia (48h) (freshwater)	4	0.6	94	6.1	1.2	50	17
Nitocra (96h) (marine)	6	14	112	29	30	48	27
Acartia (96h) (marine)	4	4.5	111	49	10	52	31
Fish, acute							
Zebrafish (96h)	3	-	-	-	310	34	8.5
Rainbow trout (96h)	2	-	-	-	535	(88)	(2.0)

The coefficient of variation for EC50 determinations ("reproducibility") is $< 20\%$ for all tests except for *Nitocra* and *Acartia* ($\leq 30\%$). This variation includes biological variation and uncertainties in parameter determinations as well as errors arising in the preparation of exposure medium (weighing, pipetting, and preparation of dilution series). The average confidence level (95%-level) includes only the biological variation and uncertainties in parameter determination.

The most important reason why the coefficient of variation for mean LC50 values is usually much smaller than the corresponding confidence limits is presumably that the probit method overestimates the uncertainty, since the sigmoid concentration/response curve is only linearized around the 50% value, not at the extremities (0 and 100%),

which are included in the data material for the probit analysis. The stated variation coefficients are therefore regarded as giving a more realistic picture of the reproducibility of the quoted tests. Table 4.1.4 gives a similar example of reproducibility of three test methods at a laboratory in the USA.

***Table 4.1.4** Reproducibility of 3 acute toxicity tests using 3 reference chemicals (from /32/).*

Testorganism	Reference toxicant					
	SDS		NaPCP		Cd	
	N	CV(%)	N	CV(%)	N	CV(%)
Pimephales promelas (Fathead minnow) (96h)	9	22	12	40	9	96
Daphnia pulex (48h)	10	43	14	36	9	21
Daphnia magna (48h)	8	29	13	10	8	72

a. *SDS* = *Sodium dodecyl (lauryl) sulfate*
NaPCP = Sodium pentachlorophenol
Cd = Cadmium
N = Number of tests
CV(%) = *Coefficient of variation = (standard deviation · 100)/mean*

b. Data provided by Philip Lewis and James Dryer, Aquatic Biology Section, EMSL-Cincinnatti, and taken in part from Lewis and Weber, 1985.

It will be seen from this table that data for *Daphnia sp.* are comparable to the figures given in table 4.1.3 for crustacea, in particular when it is recalled that 2 of the 3 reference chemicals used are considerably more difficult to handle than potassium dichromate. By way of comparison it could be pointed out that in chemical analysis of organic compounds variation coefficients of approx. 20% are obtained in analysis at the level of detection.

4.1.5 Data sources

More than 80.000 chemicals are produced commercially today, and the number is steadily increasing /22/. Only a relatively small number of these chemicals are sufficiently well described to permit evaluation of their ecotoxicological characteristics.

Information on the toxicity of specific substances has been compiled from the scientific literature in the following data sources:

- handbooks
- scientific review articles/monographs
- data banks
- reference data bases comprising scientific literature

OECD has compiled a list of the sources available in 1986, covering all four of the above types. The list is only available on microfiche or in hard copy, and comprises 230 handbook titles, 401 monographs/reviews, and 58 databanks/databases /22/.

Handbooks

Rapid access to information is available through handbooks, but the data should be treated with caution for the following reasons:

- Data is often collected from reviews of the literature, and simple transcription errors are frequent ("third-hand data")
- The most recent literature will not be covered
- Data collection may be relatively uncritical. Users have no means of checking the quality.

For these reasons the most widely used handbook in Denmark for information on ecotoxicology, Verschueren: Handbook of Environmental Data on Organic Chemicals (1983) /39/ should only be used for a preliminary screening of information, and it should not form the sole basis for decisions relating to the toxicity of a substance.

Reviews and monographs

These contain data which usually have been subjected to expert evaluation, and they usually draw attention to suspect data. On the other hand, reviews are relatively difficult to use as look-up references.

Electronic databases

Computerized databases on the ecotoxicity of chemicals must be regarded as the best updated sources of information available today. A great number of databases are available on-line at relatively modest expense.

While databases generally supply references to the original literature, databanks represent "handbooks" in computerized form (e.g. ECDIN), and like handbooks the quality of information may be more or less well-assured. One exception is the databank "AQUIRE" developed by US-EPA, and now available in a PC-version (diskette-version). At present (1990) AQUIRE is being linked to US-EPA's QSAR programme (Quantitative Structure Activity Relationships), so that QSAR may be used for an initial evaluation of substances for which there is no experimental information available in AQUIRE.

4.1.6 Prediction of toxicity/QSAR

It is well known that the toxicity of the members of certain groups of substances is correlated to some extent with the molecular structure or to the number of substituents in it - for example, the degree of chlorination.

Thus for chlorophenols the toxicity increases with the number of chlorine atoms in the phenol ring and with the number of phenol rings in the molecule.

This empirical knowledge has been utilized in a number of cases to permit cautious estimates of the toxicity of a substance where little or no data has been available (*analogical* prediction). This procedure has also been employed to predict the degradability of some substances.

During the last 10 years a considerable effort has been deployed, particularly in the USA, to examine and document the physico-chemical properties and molecular structure of chemicals, and to relate these parameters to the toxicological characteristics: so-called "structure-activity relationships (*SAR*) or "quantitative structure-activity relationships" (*QSAR*). The development of QSARs has especially been centered on evaluations of bioaccumulative tendency (cf. section 3.4), toxicity, and physico-chemical characteristics. The development of QSARs for toxicity proceeds in principle along the following lines:

- critical evaluation of available toxicity data for substances in the common group (primarily LC50 values)
- collection and evaluation of physico-chemical and stoichiometric data such as octanol/water partition coefficient (P_{ow}), solubility, molecular weight (MW), molecular structure (substituted groups, aromatic rings, number of carbon atoms etc.)
- calculation of mathematical correlations: iteratively with evaluation of outliers with regard to special or specific biological action mechanisms
- documentation of the resulting correlations using additional substances from the group: again in an iterative process with optimization of the correlations.

Clearly, the strength of such correlations increases with the amount of data on which they are based and - most important - with the adequacy of the explanations for the data which does not fit into the correlation (outliers).

As of 1988, US-EPA had developed about 50 QSARs, covering about 40 groups of organics /34/ of the following three types:

a) Neutral organic chemicals, which are expected to have a general action mechanism (narcotic effect), such as solvents. The following groups are covered:

   - alcohols, ketones, ethers, alkyl halides, acrylohalides, aromatic and aliphatic hydrocarbons, sulphides and disulphides.

   A total of 9 QSARs have been developed for this group: see examples in table 4.1.5.

b) Organic substances with more specific action mechanisms/-toxicity in addition to those of the general group: substances with reactive groups (possibly after metabolic activation), ionisable functional groups (such as phenols and anilines), specific structural relationships leading to specific effects (e.g. alkyl phosphate esters, which inhibit acetylcholinesterase). The following groups are covered:

- acrylates, methacrylates, aldehydes, anilines, benzotriazoles, esters, phenols and epoxides.

Each of the above-mentioned groups has its own QSAR, and presumably a group-specific action mechanism, even though this mechanism is only known for a minority of the groups /1/. Table 4.1.5 also includes examples from this group.

c) Surfactants, including the following groups:

- anionic (linear alkylbenzenesulphonates, LAS), non-ionic (alcohol ethoxylates), and cationic (linear N-alkyl quaternary ammonium compounds).

It should be noted that the QSARs developed by US-EPA are *extremely variable in quality*, ranging from well-documented and highly significant relationships, to relationships which are purely indicative because of the modest amount of data and weak correlations. QSARs can therefore only be used at present to *indicate* a level of toxicity within the specific relationships on which the correlations are based.

Table 4.1.5 A Examples of QSARs reported in US-EPA 1988 /34/.

I: Neutral organic substances:	
Organism & endpoint	Fish, 96 hours LC50 (mol/l)
Correlation	$LC50 = - 0.94 \cdot \log P_{ow} + 0.94 \cdot \log (0.000068 \cdot P_{ow} + 1) - 1.25$ $N = 60, r^2 = 0.942$
Groups of chemicals	Alcohols, ketons, ethers, alkyl halides, acryl halides possibly also: aromatic hydrocarbons, halogenated aromatic and aliphatic hydrocarbons, sulphides, and di-sulphides
Limitations	$\log P_{ow} < 5.0$
Organism & endpoint	*Daphnia sp.*, 48 hour LC50 (µmol/l)
Correlation	$\log (1/LC50) = 0.91 \cdot \log P_{ow} - 4.72$ $N = 19, r^2 = 0.992$
Groups of chemicals	aromatic hydrocarbons, halogenated aromatic and aliphatic hydrocarbons. possibly also: alcohols, ketons, ethers, alkyl halides, acryl halides, sulphides, and di-sulphides
Limitations	$\log P_{ow} < 5.0$
Organism & endpoint	Planktonic green algae (fresh) 3 hour EC50 (µmol/l) photosynthesis
Correlation	$\log EC50 = 8.865 - 1.0446 \cdot \log P_{ow}$ $N = 74, r^2 = 0.865$
Groups of chemicals	alcanes, cyclic alcanes, polyaromatic compounds, chlorinated hydrocarbons. possibly also: alcohols, ketons, ethers, alkyl halides, acryl halides, aromatic hydrocarbons, halogenated aromatic and aliphatic hydrocarbons, sulphides and di-sulphides.
Limitations	$\log P_{ow} < 8.0$

Table 4.1.5 B Examples of QSARs reported in US-EPA 1988 /34/.

II: Specific acting substances:	
Organism & endpoint	Fish, 96 hours LC50 (µmol/l)
Correlation	$LC50 = - 1.46 - 0.18 \cdot \log P_{ow}$ $N = 10, r^2 = 0.627$
Groups of chemicals	Acrylates
Limitations	$\log P_{ow} < 5.0$, $MW < 1000$ Allyl acrylates: expected to be 30 times more toxic than calculated from correlation
Organism & endpoint	Fish, 96 hour LC50 (µmol/l)
Correlation	$\log (1/LC50) = 0.46 \cdot \log P_{ow} - 3.04$ $N = 11, r^2 = 0.824$
Groups of chemicals	phenols, chlorphenols possibly also: halogenated and substituted phenols
Limitations	$\log P_{ow} < 5.0$, pH = 7.8

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4.2 Assessment of toxicity of chemical substances

A large volume of data has gradually been built up concerning the toxicity of chemical substances to a large number of different aquatic organisms. These data are available in databases, reference works, and in the scientific literature. The majority of data are the results of tests for toxicity towards a limited number of species under short-term exposure in the laboratory (LC50 or EC50 values).

One of the purposes of investigating the toxicity of substances is to determine the size of the effect at different concentrations, and ultimately to determine a "No Effect Concentration" (NEC) or a water quality objective setting a concentration at which there is only a small risk of damage to the ecosystem. Since in the great majority of cases there is only limited data available for the substance in question, there is a need for a series of methods for extrapolating NEC values from the available data set. These extrapolation methods will be presented and discussed in the following section.

4.2.1 Extrapolation between different types of effect on the same species

In many cases it is wished to determine the toxicity caused by long-term exposure to a chemical substance using data on toxicity after

short-term exposure, which may be the only data available. A number of comparisons between acute and chronic toxicity will be described here.

Slooff *et al.* /16/ calculated the relationship between acute and chronic toxicity for the same species on the basis of data for the effects of 164 substances on fish and daphnia. The resulting correlation equation was:

$$\log NOEC = -1.28 + 0.95 \cdot \log L(E)C50$$

95% of the NOEC values lay within an uncertainty factor of 25.6 (figure 4.2.1).

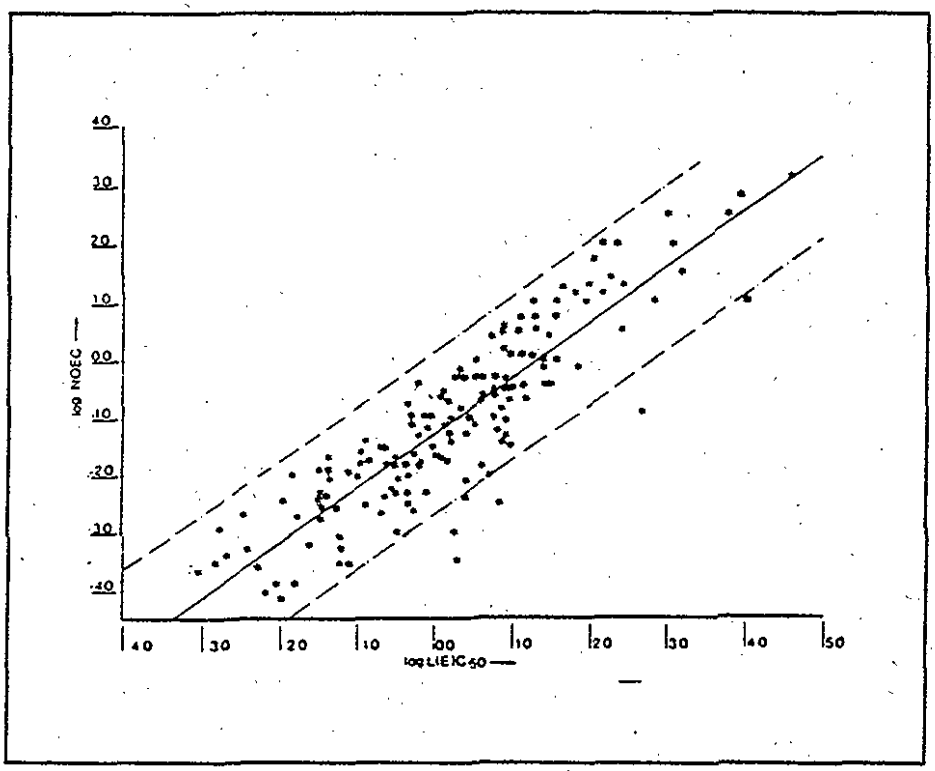

Figure 4.2.1 Correlation between acute and cronic toxicity towards the same species based on 164 data pairs. The dotted lines represent 95% safety margins /16/.

Suter & Rosen /18/ compared the acute toxicity with the MATC (Maximum Acceptable Toxicant Concentration) values for a number of substances relative to appr. 10 marine species, of which the sheepshead minnow (*Cyprinodum variegatis*) and the crustacean *Mysidopsis bahia* provided 88% of the data sets. The MATC is defined as the geometric mean between the lowest concentration giving a statistically significant effect and the highest concentration which gives no effect on survival, growth or reproduction at any stage of the species' life cycle. The following extrapolation equations were worked out for fish and crustacea respectively:

$$\log MATC = -0.60 + 0.98 \cdot \log LC50 \text{ (fish)}$$

$$\log MATC = -0.88 + 1.00 \cdot \log LC50 \text{ (crustacea)}$$

95% of the MATC values lay within an uncertainty of 18.6 (= $10^{1.27}$) and 7.9 (= $10^{0.90}$) respectively.

The extrapolation equations in these two papers have a slope of appr. 1.0, but the intersection with the y-axis is lower in Slooff *et al.*'s equation. This may be because this equation uses the NOEC whereas Suter & Rosen calculate the MATC, which is larger than the NOEC. Thus there appears to be good agreement between the results of these two exercises.

Kühn *et al.* /9,10/ have carried out acute and chronic tests on *Daphnia* using 73 different environmentally relevant chemicals from a wide range of different groups of inorganic and organic substances. The lowest concentration found to cause an effect in the chronic tests (mortality, rate of reproduction, generation time) after 21 days was taken to be the NOEC. The ratio between the 24 h EC50 and the NOEC (acute/chronic ratio, A/C ratio) was calculated for each substance and was found to have a mean of 140 with a standard variation of 410. The ratio was highly variable with a range of 2 to 3000.

On the basis of the above data it must be concluded that there appears to be no simple relationship between acute and chronic toxicity. The relationship also varies according to the type of chronic toxicity that is studied, since the development of more sensitive test parameters for chronic toxicity will naturally result in an increased A/C ratio.

The above comparisons between acute and chronic toxicity have been made using well-defined substances. Only few comparisons have been made of acute and chronic toxicities of complex samples (such as wastewater). On the basis of a relatively small data-set on the acute and chronic toxicity of complex wastewater, US-EPA has recommended /4/ using a factor of 10 for calculating chronic toxicity on the basis of acute toxicity values in cases where no chronic toxicity tests have been performed.

4.2.2 Comparison of acute toxicities for different species

A considerable number of comparisons have now been made of toxicity of a given substance towards different species, including studies with the purpose of determining whether some species are generally more sensitive than others, since such species would be useful as test organisms.

Slooff *et al.* /16/ have compiled a large set of data on toxicity of 15 substances towards 35 freshwater species. On this basis they have attempted to calculate transformation factors for calculation of the LC50 for a chosen species from data taken from other species. Such a calculation can only be made on the basis of a very high application factor, since the differences between the sensitivity of the various species towards the same substance are considerable (from 4 to 1985). The spread of the differences is shown in figure 4.2.2. It was also concluded that the taxonomic difference between the species was not reflected in the actually measured difference in toxicity, and that the use of one single indicator organism to test unknown toxic substances therefore seems to be impossible.

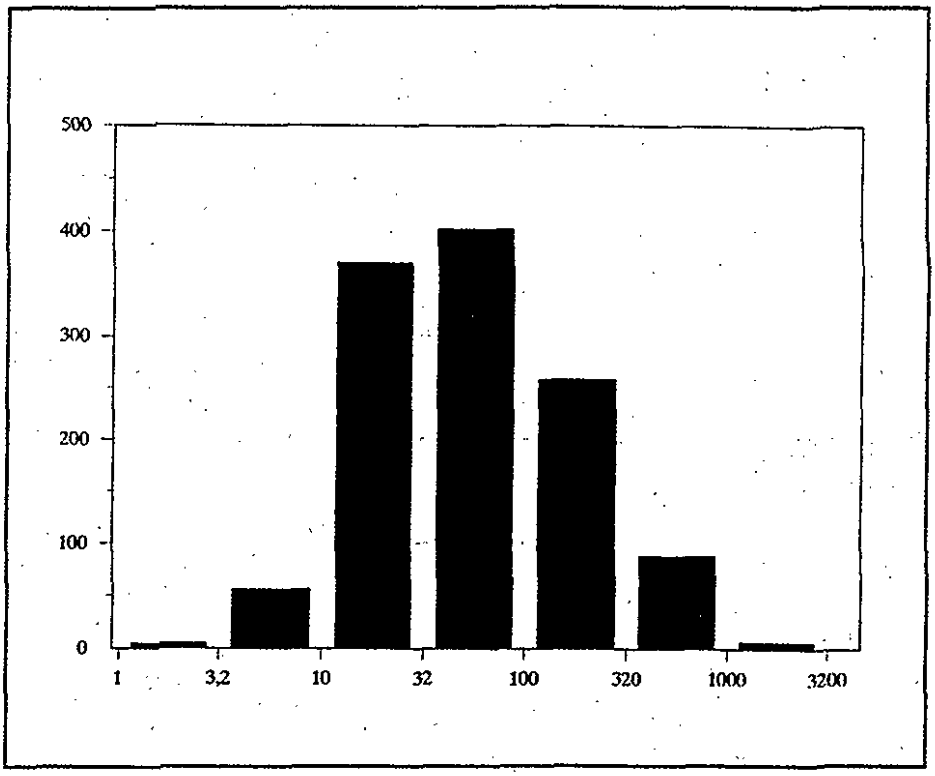

Figure 4.2.2 Distribution of uncertainty factors for the variation af acute toxicity between various species, based on data for toxicity of 15 substances to 35 freshwater species /16/.

In an earlier investigation of the toxicity of 15 substances towards 22 freshwater species, Slooff *et al.* /15/ also concluded that the effect concentration for even the most sensitive of 3 test organisms in a standard test combination (planktonic algae, crustacea, fish) would be more than 10 times higher than the effect concentration for the most sensitive of the 22 tested species.

LeBlanc /11/ has examined the correlation between species using a data set of acute toxicities of appr. 50 substances and appr. 10 test species. For organic non-pesticides, of which the majority were non-specific narcotically acting substances, the toxicity in closely related species was more or less the same. The correlation weakened with increasing taxonomic distance between the species. For pesticides, there was a high correlation between toxicity in closely related species, but it was not possible to predict the toxicity towards species belonging to a different taxonomic group (for example, extrapolation from fish to crustacea or algae). For metals a correlation was found between fish and daphnia, which were the only groups of organism studied. For all other substances, there was a high correlation between the acute toxicities for closely related species. In contrast to Slooff *et al.* /16/, therefore, LeBlanc found a correlation between acute toxicities for some types of chemical substances (organic non-pesticides and metals) but only within a rather limited number of species.

Suter & Rosen /18/ also compared a large number of data sets and concluded that the toxicities towards sheepshead minnow were representative of the toxicity towards other species of fish. For

crustacea, however, it was not possible to identify a representative species, since the sensitivity towards various toxic substances was highly variable.

Holcombe *et al.* /7/ have also compared acute toxicity data sets - in this case mainly EC50 values for fathead minnow, *Pimephales promelas*. The toxicities of a total of 64 organic substances towards 44 freshwater organisms, mainly fish and crustacea, was compared. Out of the total 309 data sets a correlation could be established for acute toxicity values. The correlation equation was determined to be:

$$\log LC50(all) = -0.42 + 0.91 \cdot \log LC50(\text{fathead minnow})$$

The figure for the 95% protection level was not stated. Holcombe *et al.* /7/ themselves consider that the method can be used for initial risk evaluation, and that with the addition of data for other groups of organisms and for chronic toxicity, a tool can be created for calculation of concentrations which will give protection to a specific proportion (for example 95%) of the species in an ecosystem.

In a comparison of the sensitivity of various species towards 73 priority substances, US-EPA concluded /4/ that the most sensitive of the species *Daphnia magna, Pimephales promelas* and *Lepomis macrochirus* had an LC50 value lying within a factor 10 of the most sensitive of the other tested species. This was the case for 71 of the 73 tested substances. The number of species tested was not reported, however.

It is characteristic of most of the above-mentioned studies that they primarily use data for fish and crustacea for their comparisons and attempts to describe correlations between the sensitivities of different species. The investigation covering the most taxonomically varied range of test organisms is that of Slooff *et al.* /16/. Here the toxicity of 15 substances towards 35 species from 11 taxonomic groups was compared.

A standard set of toxicity tests often consists of 3 acute toxicity tests using a planktonic algae, a crustacean, and a fish. The sensitivity of these species in relation to that of other species can be evaluated if a sufficiently large data set is available. Slooff *et al.* /15/ have compared a large quantity of data concerning toxicity of 15 different chemicals tested on 22 different freshwater species from 9 different taxonomic groups.

If a standard set is chosen consisting of *Selenastrum capricornutum, Daphnia magna* and *Poecilia reticulata*, the ratio between the lowest effect concentration for these three species and the lowest effect concentration for any other species will give an index of the usefulness of the standard set (see table 4.2.1).

Table 4.2.1 Ratios between lowest effect concentration for S. capricornutum, D. magna and P. reticulata and the lowest effect concentration among the other 19 species (from /15/).

Substance	Lowest EC (standard set) / Lowest EC (other species)		Lowest EC (standard set) / Lowest NOEC (other species)	
Mercury(II)chloride	1.7	(D)	3.8	(D)
Cadmium nitrate	1	(D)	1.8	(D)
n-Propanol	22	(A)	22	(A)
n-Heptanol	8.8	(A)	8.8	(A)
Ethylacetat	1.7	(F)	2.1	(F)
Ethylpropionat	8.9	(F)	8.9	(F)
Acetone	97	(A)	97	(A)
Trichlorethylen	2.2	(D)	3.8	(D)
Allylamin	34	(F)	34	(F)
Anilin	6.4	(D)	9.1	(D)
Benzen	12	(D)	17	(D)
Pyridin	13	(A)	13	(A)
o-Cresol	1.4	(D)	2.5	(D)
Salicylaldehyd	3.9	(F)	3.9	(F)

A,D, and F indicate that the alga, the daphnia, respectively the fish is the most sensitive of the three species of the standard set.

As seen in the table, the ratio between the lowest acute toxicity within the standard set and the lowest acute toxicity for other tested species varies between 1 and 97 (mean = 15, s.d. = 25), while the ratio between the lowest acute toxicity within the standard set and the lowest NOEC for the other tested species varies between 1.8 and 97 (mean = 16, s.d. = 25). Which species of the standard set is most sensitive varies evenly between the three species. The reason for the similarity between the ratios for acute toxicity and the NOEC is that the most sensitive species is often a microorganism (bacteria, algae, protozoa) in which there is no difference between acute toxicity and NOEC in the tests that are used, because of the short generation time.

On the basis of the above-described studies it must therefore be concluded that there is no single "most sensitive species", and that a standard test battery consisting of an algae, a crustacean and a fish is not suitable either for indicating the general sensitivity of other species. Cairns & Niederlehner /2/ arrive at the same conclusion after a review of various attempts to compare the sensitivity of different species. In other words, it is not possible to predict whether a given species belongs to the most or least sensitive with regard to a particular substance. The best way to increase the certainty that the tested species reflect the sensitivity of relevant species living in the recipient water body will therefore be to increase the number of species tested.

4.2.3 Extrapolation methods for calculation of protection levels

The consequence of the conclusion of the previous section, that no "most sensitive species" exists, must be that the available test results are to be regarded as more or less random samples from the total quantity of effect concentrations for all species in the ecosystem in question. On the basis of this assumption, a number of different extrapolation techniques have been developed for calculating the concentrations which will give a specified level of protection for the ecosystem and its species. In this section a number of available extrapolation techniques are described and compared with the aim of establishing their suitability for evaluating the environmental effects of chemical substances.

Environmental Concern Level

Under the terms of the Toxic Substances Control Act (TSCA) the US-EPA has developed a method /3/ for calculating the "Environmental Concern Level" (ECL) using measured or calculated data from registrations of new chemical substances, with the aim of protecting human health and the environment. The method may be used to identify the concentration of a chemical that will cause undesirable effects in the environment, and to select chemicals which ought to be tested (additionally) under the requirements of TSCA. The method is thus intended for use in an initial environmental risk assessment, and the calculated ECL is therefore not a "safe" concentration, but on the contrary a concentration which, if exceeded, will cause undesirable effects on populations for at least 95% of the time /12/. The advantage of the method is that it is extremely simple to use and requires only a small data set, since ECL is calculated by dividing the results for acute or chronic toxicities by an application factor (see table 4.2.2).

Table 4.2.2 Application factors for calculation of "Environmental Concern Level" /3/.

Available data	Application factor (AF)
1 acute L(E)C50 or QSAR	1000
Lowest of 5 L(E)C50 for invertebrates and fish	100
Lowest cronic NOEC for most sensitive of above	10
Ecosystem tests	1

In December 1990 OECD organized a workshop on extrapolation methods /23/, where the US-EPA ECL method was discussed amongst others. It was decided to modify the method to include more

than just fish and invertebrate data; algal toxicity data would also be included (see table 4.2.3). This would make the method more appropriate, since this would allow the data submitted in the minimum data set or used for environmental hazard classification (cf. section 4.1) to be used directly for calculation of the ECL.

Table 4.2.3 OECD application factors for calculation of "Environmental Concern Level" /23/.

Available data	Application factor (AF)
Lowest L(E)C50 or QSAR calculation for acute toxicity	1000
Lowest acute L(E)C50 or QSAR calculation towards at least alga, crustacea, and fish	100
Lowest NOEC value or QSAR calculation for cronic toxicity towards at least alga, crustacea, and fish	10

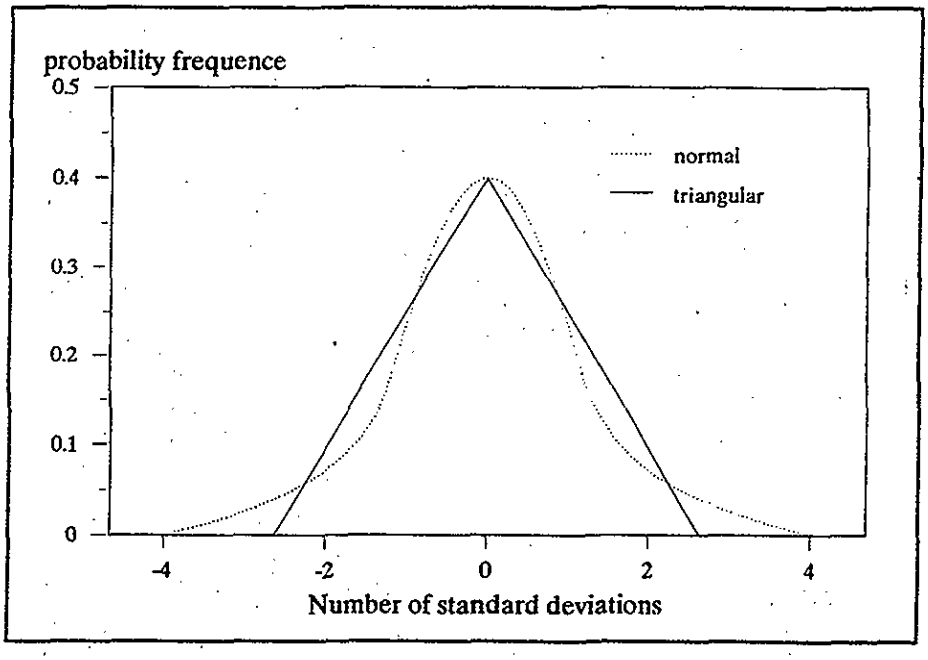

***Figure 4.2.3** Comparison between normal and triangular distribution of effect concentrations.*

Water Quality Criteria

US-EPA has also developed a method /5/ for calculation of the "Final Acute Value" (FAV) of chemicals in surface waters, based on data on the acute toxicity of the substance in question, ("Mean Acute Value", MAV), either towards individual species or defined as the mean acute toxicity towards species belonging to the same taxonomic group. The method has been developed further /17/ for calculation of "Final Chronic Value" (FCV). By means of this method, "Ambient Water Quality Criteria" (AWQC) may be calculated for 1 hour (acute) and 96 hour (chronic) periods from a data set consisting of test results for at least 8 different animal species, mainly fish and crustacea.

The FAV is defined as the concentration that protects at least 95% of the ecosystem species against acute toxic effects. It is also supposed that the available MAVs are random samples of all the species in the aquatic ecosystem, and that the effect concentrations for the species are log-triangularly distributed (figure 4.2.3). This distribution function is not scientifically based, and it presupposes implicitly that a 100% safe concentration level exists at which there are no toxic effects on any species. Furthermore it may be concluded that the method in general will be sensitive towards the discovery of new sensitive species, since this will result in a relatively large reduction in the FAV.

Kooijman's method

Kooijman /8/ has presented a method for calculation of "Hazardous Concentration for Sensitive Species" (HCS) on the basis of information on LC50 values for a number of species. The HCS is defined as the concentration at which the probability that the LC50 for the most sensitive species is lower than the HCS is equal to an arbitrary value δ_1, i.e. usually 5 or 10%. Thus:

$$P\{LC50(\text{most sensitive species}) < HCS\} = \delta_1$$

The basic assumption of Kooijman's method is that the observed LC50 values are random and independent samples from an ecosystem consisting of n species, whose LC50 values are distributed log-logistically. Data in Kooijman's paper seem to confirm this assumption for the substances examined (figure 4.2.4). However, it cannot be evaluated whether the variation of the sensitivity of species towards substances with a specific mode of action (pesticides, for example) follow this distribution. Whether the organisms tested may be considered to represent random and independent samples cannot be decided either on the basis of the material presented. Finally, it is assumed that the variance of the individual measurements of LC50 for each species is negligible compared with the variance of the LC50 values for different species. This assumption may be assumed to be correct.

In general Kooijman's method produces a value for HCS which is very sensitive towards the lowest measured LC50 value, so a very sensitive species will produce a large reduction in the value of the HCS.

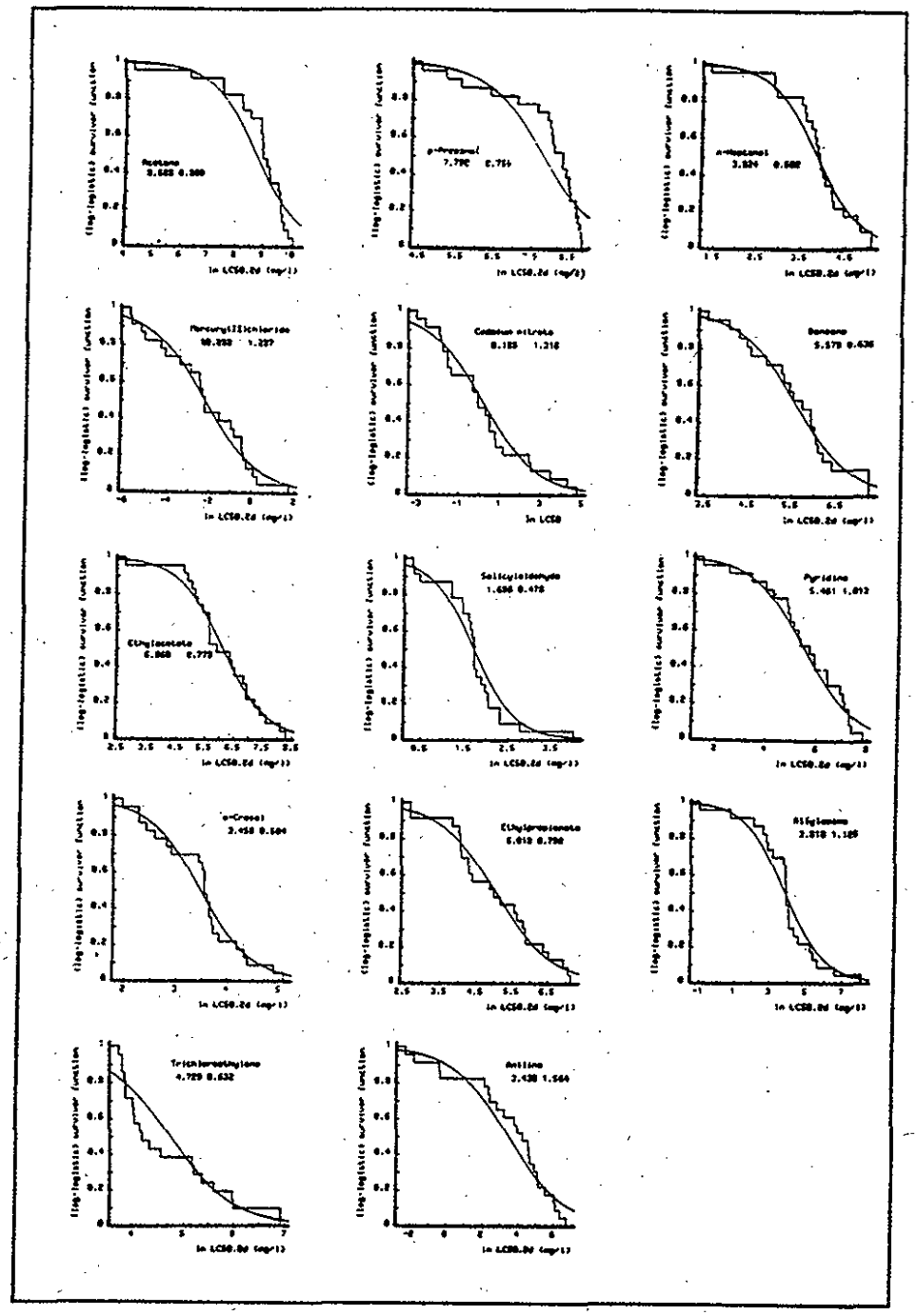

Figure 4.2.4 Correlation of LC50-values among various species for 14 different substances /8/.

van Straalen & Denneman's method

In contrast to Kooijman, van Straalen & Denneman /20/ have attempted to develop a method for calculating the concentration at which p% of the species in an ecosystem will be affected by a toxic substance. This concentration is called "Hazardous Concentration for p% of Species" (HC_p). p may be set at 5%, for example, so that 95% of the species will be protected if the concentration in the environment is kept below HC_p. In contrast to Kooijman, van Straalen & Denneman use chronic effects, and HC_p is therefore defined as follows:

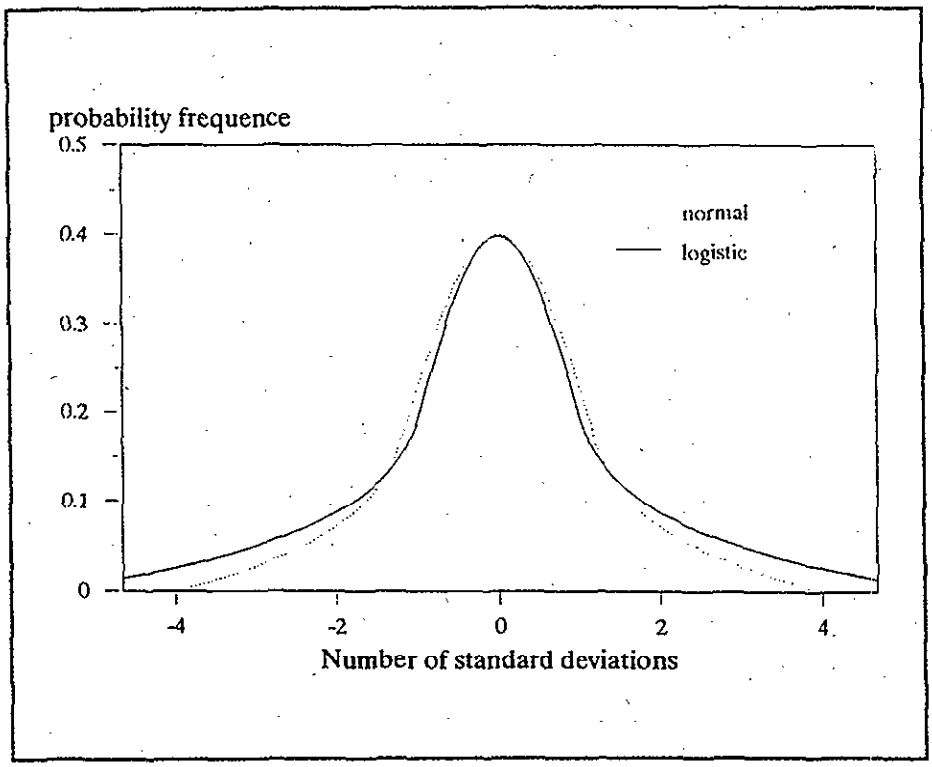

Figure 4.2.5 *Comparison between the normal and the logistic distribution of effect concentrations for various species.*

$$P\{\text{chronic NOEC} < HC_p\} = \delta_1 = p/100$$

By means of the formula

$$HC_p = \exp(x_m)/T$$

HC_p may be calculated on the basis of the mean (x_m) of the logarithm of the individual NOEC values and a factor T, which may be regarded as an extrapolation factor, which is dependent *inter alia* on the variance of the NOEC values, a computer-simulated correction factor d_m depending on the number of tests, and the protection percentage (100-p%).

Isolated extremely sensitive species will not produce such large reductions in HC_p as in HCS, since HC_p does not aim to protect the p% most sensitive species. Whether the chosen level of protection is adequate can not be decided easily, since amongst the p% most sensitive species there may be species of vital importance to the composition and function of the rest of the ecosystem, or species of great commercial interest etc.

The Netherlands environmental research institute RIVM is currently engaged in further development and improvement of Kooijman's and van Straalen & Denneman's extrapolation techniques on the basis of statistical considerations /1/. The preliminary results of the statistically more correct method show that van Straalen & Denneman's HC_p values are in fact overestimated by a factor of about 2.

Wagner & Løkke's method

Wagner /21/ and Wagner & Løkke /22/ have also produced a further development of van Straalen & Denneman's extrapolation method. As in the Dutch method, a concentration K_p is calculated at which p% of the species in the ecosystem may be expected to be affected by the toxic substance in question. The method rests on more or less the same assumptions as van Straalen & Denneman's, except that the effect concentrations for the species in the ecosystem are assumed to be log-normally distributed instead of log-logistically. The logistic distribution is largely identical with the normal distribution, but the latter has the advantage of being considerably better described and in many cases available in table form. For this reason it is not necessary - as for van Straalen & Denneman's method - to use Kooijman's /8/ computer-simulated correction factor (d_m).

K_p is calculated from the formula:

$$K_p = \exp(x_m)/T$$

which corresponds to that of van Straalen & Denneman, but without the need for computer simulations to calculate the factor T.

The extrapolation factor T

Both in Wagner & Løkke's method /22/ and in van Straalen & Denneman's method as modified by Aldenberg *et al.* /1/ it is possible to evaluate how the calculated protection concentration depends on the number of tested species and the variation in their sensitivity. In these methods it is possible to distinguish between objective parameters which are given in the data set available, and subjective parameters, whose values may be chosen at will.

The data set includes three different objective parameters: the number of test results (m), the mean toxicity (x_m), and the standard deviation of the data set (s_m). The subjective parameters, whose values may be chosen at will, include the level of protection (100-p) and the probability of overestimating this (δ_2).

In both methods the extrapolation factor T is defined as:

$$\ln T = s_m \cdot k$$

where k is a factor depending on m, p and δ_2. The value of k for different values of p and δ_2 may be derived from tables for the normal distribution, or it may be calculated by computer simulations, as done by Aldenberg *et al.* /1/.

In figure 4.2.6 the value of ln T is illustrated schematically as a function of m and s_m. It will be seen that the extrapolation factor T will be very large for small data sets with large standard deviations, but the value falls with increasing numbers of test results and a lower standard variation in the data set.

4.2.4 Evaluation of the extrapolation methods

Of the above-mentioned extrapolation techniques the two American ones are used in practice today: for example Water Quality Criteria have been set for almost 100 different chemicals using the method described by Stephan *et al.* /17/. Furthermore, the USA water quality

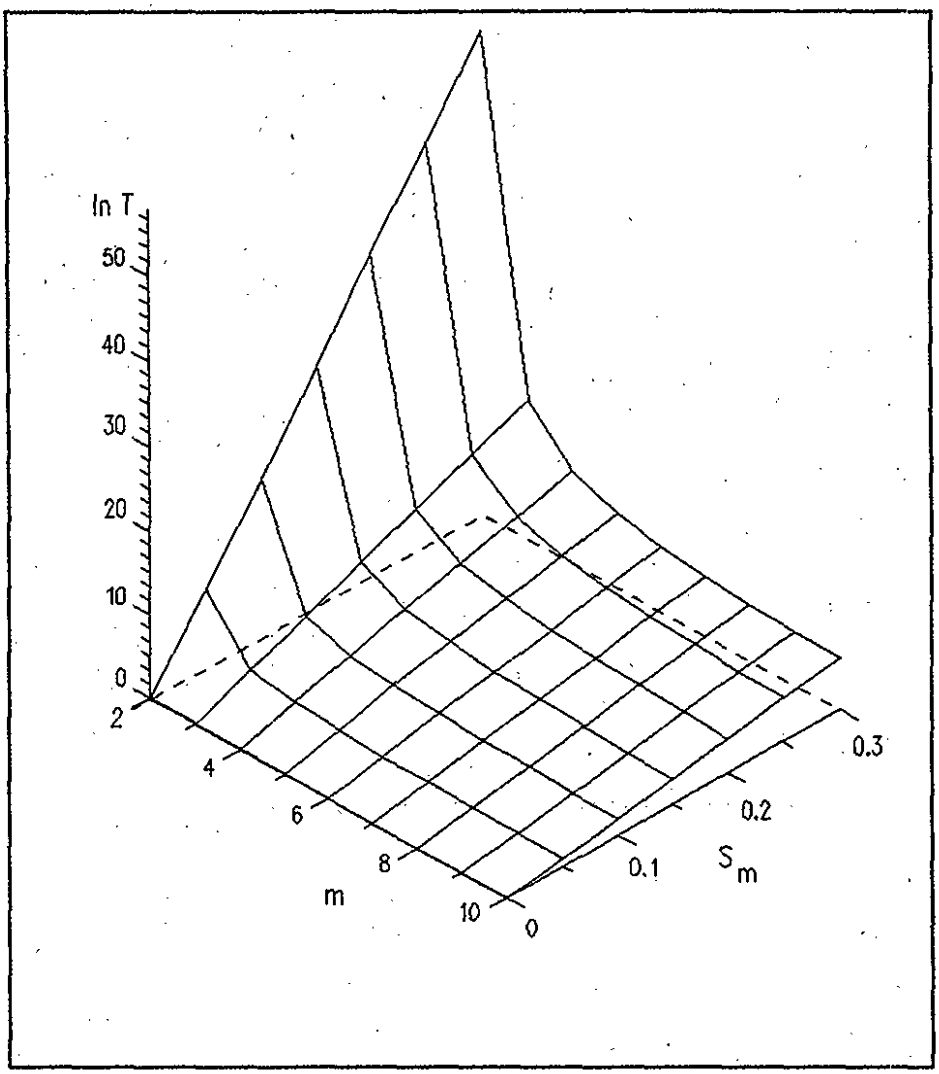

***Figure 4.2.6** Ekstrapolation factor T as a function of the number of test results (m) and the standard deviation of these (S_m).*

criteria are widely used in other countries, since they are regarded as being reasonably well-documented.

Theoretically speaking, van Straalen & Denneman's method /20/ and Wagner & Løkke's method /22/ must be admitted to be more soundly based, since they presuppose a log-logistic and log-normal distribution respectively, and also assume that there is no lower effect limit, in contrast to the method of Stephan *et al.*, which presupposes that effect concentrations may be described by a log-triangular distribution, and thus that there is a lower limit below which no effects on organisms occur. The use of van Straalen & Denneman's method is recommended by the Netherlands environmental protection authorities /6,19/, but as yet no thorough investigations have been made to establish whether this method, or that of Wagner & Løkke, is usable in practise. In the following sections the four different extrapolation methods will be evaluated and compared.

Comparison of extrapolation methods

Comparison of the extrapolation methods described above may be undertaken by applying the various methods to the same data set and

comparing the results. Table 4.2.4 shows the results of extrapolations of one data set consisting of toxicities of 8 different substances towards 11 different freshwater organisms under chronic exposure (NOEC values). The data set has been compiled by Slooff & Canton /14/. The extrapolations were performed by Okkerman *et al.* /13/ and Wagner /21/ respectively.

***Table 4.2.4** Comparison of extrapolation methods applied to the same data set, consisting of NOEC-values for 8 substances tested on 11 different freshwater /14/. Calculations by Okkerman et al. /13/ og Wagner /21/. All concentrations are µg/l.*

	ECL	FCV	HCp	L	K_p {δ=0.05}	K_p {δ=0.10}	K_p {δ=0.15}
$K_2Cr_2O_7$	10	42	29	20	13	21	31
NaBr	1000	52,000	1000	200	290	620	1100
TPBS	32	430	110	41	52	83	120
2,4-DCA	3.2	23	25	8.0	10	17	26
p-NT	32	1500	230	100	120	180	240
DNOC	3.2	320	10	2.7	3.7	6.8	11
Dimethoat	3.2	20	3.0	0.35	0.57	1.5	3.4
Pentachlor-phenol	0.32	8.0	1.2	0.32	0.45	0.85	1.4

In order to compare directly the results of the extrapolations, they are expressed as the ratio between the individual results and K_p, calculated for $\delta = 0.05$ (table 4.2.5).

***Table 4.2.5** Comparison of results obtained from various extrapolation methods applied to the same data set consisting of NOEC-values for 8 substances tested on 11 different freshwater species (from /14/). The comparison is expressed as the ratio between ECL, FCV, HC_p, L and K_p and K_p calculated for $\delta = 0,05$ (From /13, 21/).*

	ECL	FCV	HCp	L	Kp {δ=0,05}	Kp {δ=0,10}	Kp {δ=0,15}
$K_2Cr_2O_7$	0.77	3.2	2.2	0.77	1	1.6	2.4
NaBr	3.4	4000	3.4	0.69	1	2.1	3.8
TPBS	0.62	8.3	2.1	0.79	1	1.6	2.3
2,4-DCA	0.32	2.3	2.5	0.80	1	1.7	2.6
p-NT	0.27	13	1.9	0.83	1	1.5	2.0
DNOC	0.86	86	2.7	0.73	1	1.8	3.0
Dimethoat	5.6	35	5.3	0.61	1	2.6	6.0
PCP	0.71	18	2.7	0.71	1	1.9	3.1
Mean	1.6	520	2.9	0.74		1.9	3.2
S.D.	1.9	1400	1.1	0.07		0.36	1.3
Max. value	5.6	4000	5.3	1 0.83		2.6	6.0

The K_p value has been chosen because, as described above, it is the scientifically best established estimate of the concentration which will protect 95% of the species of the ecosystem in question. Furthermore, $\delta = 0.05$ has been chosen since it corresponds to a probability of 95% that K_p has not been overestimated, since in such a case the level of protection would be less than 95%.

Table 4.2.5 shows the ratios between the K_p values calculated for $\delta = 0.10$ and 0.15, and the K_p values calculated for $\delta = 0.05$. It will be seen that the K_p values increase with increasing values of δ, which of course can be ascribed to the fact that the K_p values are calculated with decreasing probability. In general there is no great difference between calculations of K_p at 85, 90 or 95% probability.

The other two methods based on statistical principles; van Straalen & Denneman's HC_p value and the further development of this by Aldenberg *et al.* /1/ (the L-value) also produce results with very little scatter, in which the HC_p values are always rather larger than the corresponding K_p value and more or less equal to K_p calculated for $\delta = 0.15$. The L value is always appr. 25% less than K_p.

Compared with these largely identical statistically-based results, the two American methods produce rather more variable concentrations. The calculation of FCV forms the basis for US-EPA's Water Quality Criteria, which like the statistically-based methods are claimed to provide protection for 95% of the species in an ecosystem. However, in spite of this, the FCV values on average lie considerably higher than the K_p values, and the ratios are also very variable.

The reason why the FAV/FCV figures lie generally higher than the K_p and HC_p values must be that there is a difference in the assumptions underlying the calculation methods, since a triangular distribution will implicitly signify the existence of a lower limit below which there is no toxic effect on any organism in the ecosystem (see figure 4.2.3). This assumption is not necessary when log-normal or log-logistic distributions are used, and therefore the K_p and HC_p values will generally be lower than the FAV/FCV values. It has not been proved that such threshold values for toxic effects on species in ecosystems exist - on the contrary, an increase in the amount of data almost always results in a lowering of the LOEC value.

The ECL values are grouped fairly evenly around the K_p values within a factor of about 5. The ECL values are by definition concentrations which may be expected to result in damage to the ecosystem, but despite this the ECL values are always lower than the FCV values, which by definition signify protection of 95% of the species in the ecosystem. As already mentioned, the ECL values are not very much different from the K_p values, which also by definition should protect 95% of the species. However, this should be viewed in light of the fact that the ECL method was developed to screen substances on the basis of very little data. Okkerman *et al.* have calculated ECL values for the 8 substances on the basis of data on their acute toxicities for only 3 species. In general this calculation did not give very different results.

On the basis of the results presented above it may be concluded that the calculated ECL values, with some uncertainty, correspond approximately to the calculated HC_p values, and that the ECL

value will presumably be adequate to give an estimate of the concentration which, when exceeded, should give grounds for concern. Thus it cannot be ruled out that US-EPA's extrapolation technique of 1984 may be just as adequate as Wagner & Løkke's and van Straalen & Denneman's methods.

Naturally, it is impossible to draw far-reaching conclusions from this comparison, which as mentioned is based on a relatively small amount of data. However, it must be concluded that the extrapolation methods of van Straalen & Denneman /20/, Aldenberg *et al.* /1/ and Wagner & Løkke /22/ give results which lie very close to each other.

Importance of the amount of data for the size of the safe concentration level

As mentioned above, both Aldenberg *et al.*'s /1/ and Wagner & Løkke's extrapolation methods are based on the assumption that the various sensitivities (for example the LC50s) of the species in the ecosystem towards the substance in question are log-logistically or log-normally distributed, and that the species whose effect concentrations are used for the calculation are randomly chosen from the ecosystem species. As shown in figure 4.2.4, the first assumption appears to hold for the 14 investigated substances, and unless special circumstances come into play it must be assumed to hold generally. The second assumption, of randomly selected species, must also be assumed to hold, even though when planning test programmes, species from different organism groups are usually selected, even though the test organisms are also selected for their ability to survive under laboratory conditions.

Assuming these assumptions to be valid, the level of confidence of the calculated values of HC_p and K_p can then be calculated using statistical techniques. Generally it will be true that the larger the data material, the more precise the calculation of HC_p and K_p. Contrariwise, the less data that is available, the greater the uncertainty that will attach to the calculated value of HC_p and K_p. Since there will normally only be a small amount of data available concerning the toxicity of a given substance, a discussion of the importance of the size of the data material will now be presented.

Okkerman *et al.* /13/ calculated the HC_p values for 8 substances on the basis of a data set containing chronic toxicity results for 11 different species (cf. figure 4.2.7). A general tendency may be seen for an increasing data quantity to produce higher HC_p values. If HC_p is calculated from a standard test battery consisting of an algae (*Scenedesmus pannonicus*), a crustacean (*Daphnia magna*) and a fish (*Oryzia latipes*), the HC_p value for 6 of the 8 substances will be underestimated by a factor ranging from 5.9 to 910 compared with the HC_p value calculated from all 11 species.

However, for 2 of the substances the HC_p value was overestimated by factors of 2.5 and 7.7 respectively. In both cases this is due to the fact that the variation between the toxicities towards the test organisms was less than between the toxicities towards the other species.

On the basis of the available data, the best estimate of the "true" concentration at which 95% of the species are protected will be HC_p calculated from all the available data ($HC_p(11)$). The importance

of the quantity of data available may then be evaluated for each substance in the data set by calculating the ratio between the HC_ps calculated for 3, 5, 7, and 9 species respectively, and $HC_p(11)$ (see table 4.2.6). The $HC_p(3)$ calculation includes the data for algae, crustacea and fish, and in the other calculations, data is added for species from other taxonomic groups. It will be seen that the ratio for each individual substance gradually approaches 1 as the number of included test results increases. This will also be the case for the average of the ratios. It will also be seen that the ratio for individual substances will be very variable when the amount of data is small.

The relationship between the maximum and minimum ratio for the 8 substances is an expression of the uncertainty of the HC_p calculation on the basis of the different sized data sets. It will be seen that this uncertainty will be very large if HC_p is calculated on the basis of only 3 effect concentrations, whereas the uncertainty falls when the amount of available data increases.

On this basis it may be concluded that when data from only three species are used, there will be a large uncertainty attached to the calculation of HC_p using van Straalen & Denneman's method. By increasing the quantity of data to cover 5 or more species, the uncertainty falls significantly.

***Table 4.2.6** Importance of the amount of data for the size of HC_p, expressed as the ratio between $HC_p(n)$ and $HC_p(11)$ (Calculated from data in /13/).*

	HCp(3)/HCp(11)	HCp(5)/HCp(11)	HCp(7)/HCp(11)	HCp(9)/HCp(11)
$K_2Cr_2O_7$	0.016	0.16	0.45	0.97
NaBr	0.032	0.12	0.37	0.77
TPBS	2.5	0.51	0.49	0.84
2,4-DCA	0.012	0.076	0.36	0.68
p-NT	0.17	0.19	0.40	0.70
DNOC	0.098	0.061	0.30	0.61
Dimethoat	0.0011	0.067	0.50	1.37
PCP	7.7	0.072	0.33	0.53
Mean	1.3	0.16	0.40	0.81
S.D.	2.7	0.15	0.074	0.26
Max. ratio	7.7	0.51	0.50	1.37
Min. ratio	0.0011	0.061	0.30	0.53
Max./min.	7000	8.4	1.7	2.6

In table 4.2.7, Wagner & Løkke's method has been used to perform the same calculations as in table 4.2.6, where van Straalen & Denneman's method was used. It will be seen that the ratios are smaller in every case, and especially when the quantity of data is small, the ratios are very extremely small. Wagner & Løkke's method

is thus much less robust than van Straalen & Denneman's when the amount of data available is small.

The same is true of Aldenberg *et al.*'s improvement of van Straalen & Denneman's method, since as already mentioned there is not much difference between this method and Wagner & Løkke's.

The general conclusion must be that van Straalen & Denneman's /20/, Aldenberg *et al.*'s /1/, and Wagner & Løkke's /22/ extrapolation techniques should not be used on small data sets, since the results in such cases will be very dependent on whether the effect concentrations for the "randomly" chosen species lie close together or further apart. In addition the protection concentration will in many cases be extremely low because of the large T-values.

Table 4.2.7 *Importance of the amount of data for the size of K_p expressed as the ratio between $K_p(n)$ and $Kn_p(11)$ (Calculated from data in /22/).*

	$\frac{K_p(3)}{K_p(11)}$	$\frac{K_p(5)}{K_p(11)}$	$\frac{K_p(7)}{K_p(11)}$	$\frac{K_p(9)}{K_p(11)}$
$K_2Cr_2O_7$	$0.58 \cdot 10^{-6}$	0.022	0.25	0.85
NaBr	$0.16 \cdot 10^{-6}$	0.012	0.13	0.55
TPBS	0.25	0.16	0.27	0.71
2,4-DCA	$0.71 \cdot 10^{-6}$	0.0073	0.18	0.56
p-NT	$6.8 \cdot 10^{-6}$	0.053	0.25	0.59
DNOC	$5.9 \cdot 10^{-6}$	0.0035	0.11	0.43
Dimethoat	$0.03 \cdot 10^{-9}$	0.0033	0.18	1.04
PC_p	0.98	0.0049	0.11	0.33
Mean	0.15	0.033	0.19	0.63
S.D.	0.34	0.054	0.065	0.23
Max. ratio	0.98	0.16	0.27	1.04
Min. ratio	$0.03 \cdot 10^{-9}$	0.0033	0.11	0.33
Max./min.	$33 \cdot 10^{+9}$	48	2.5	3.2

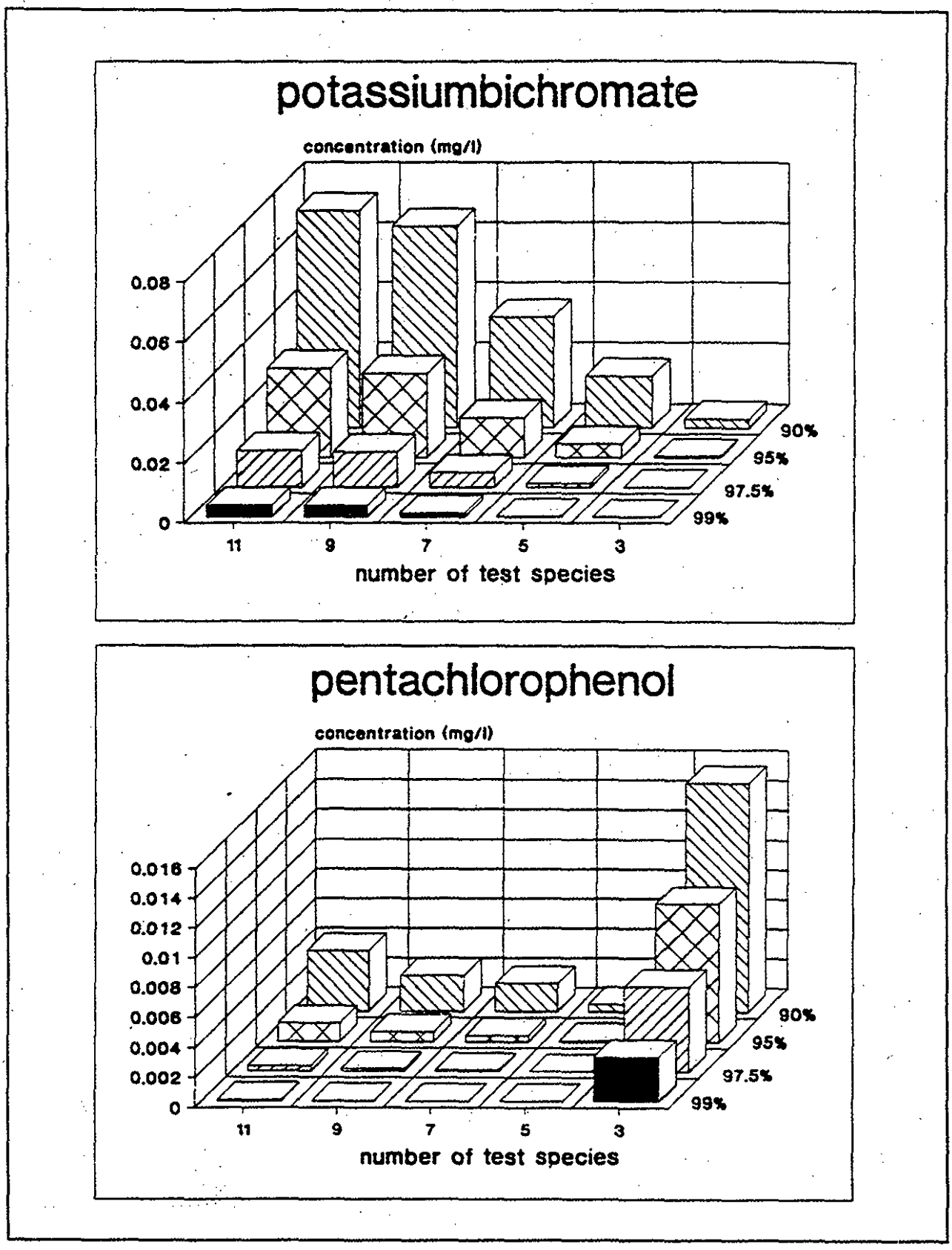

Figure 4.2.7 (Semi)cronic NOEC-values for ecosystems for 2 substances calculated according to van Straalen & Denneman /20/ based on a different number of species and on different protection levels /13/.

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4.3 Evaluation of the toxicity of complex mixtures

In practice it is extremely rare for an ecosystem or a recipient to be affected by one hazardous substance alone. In wastewater discharges there will usually be several known or unknown hazardous substances present. In this section methods will be discussed for evaluating the effects of such complex mixtures.

4.3.1 Toxicity of mixtures

In a mixture consisting of several different toxic substances, the resulting toxicity will depend on the mode of action of the individual substances and on the interactions between the substances. Joint action toxicity is best illustrated as in the following table (after /9/):

***Table 4.3.1** Classification of interacting toxicity (joint action) from /9/.*

	Similar	Dissimilar
Non-interactive	Simple similar	Independent
Interactive	Complex similar	Dependent

If two or more substances act in the same way and possibly also at the same site, this is a case of *similar action*. The opposite situation is *dissimilar action*. If the toxic effects of the individual substances are independent of the presence of other substances, then they are *non-interactive*. If the substances influence each others bioavailability or toxic effect, they are *interactive*. Examples of this are synergism and antagonism. As shown in table 4.3.1, combinations of these categories produce four different types of joint action between substances.

These types should however be viewed as the extreme cases in a continuum of different degrees of joint action.

These relationships may also be described graphically (figure 4.3.1) and mathematically, and development of the theory of joint action has developed mainly within the field of pharmacology. The following section presents a brief account of the theoretical background.

Figure 4.3.1 /9/ shows various possible combinations of joint action of a mixture of two different toxic substances. The curves in the figure are "isoboles", where the resulting toxicity of the mixture is equally great at every point on the curve.

The simplest form of combined effect of two different toxic substances is the *simple additive effect* (*concentration-additive* or *simple similar action*), where the two substances act in the same way, and where the resulting toxicity therefore only depends on the combined molar concentration of the two substances. This is depicted by curve I in figure 4.3.1.

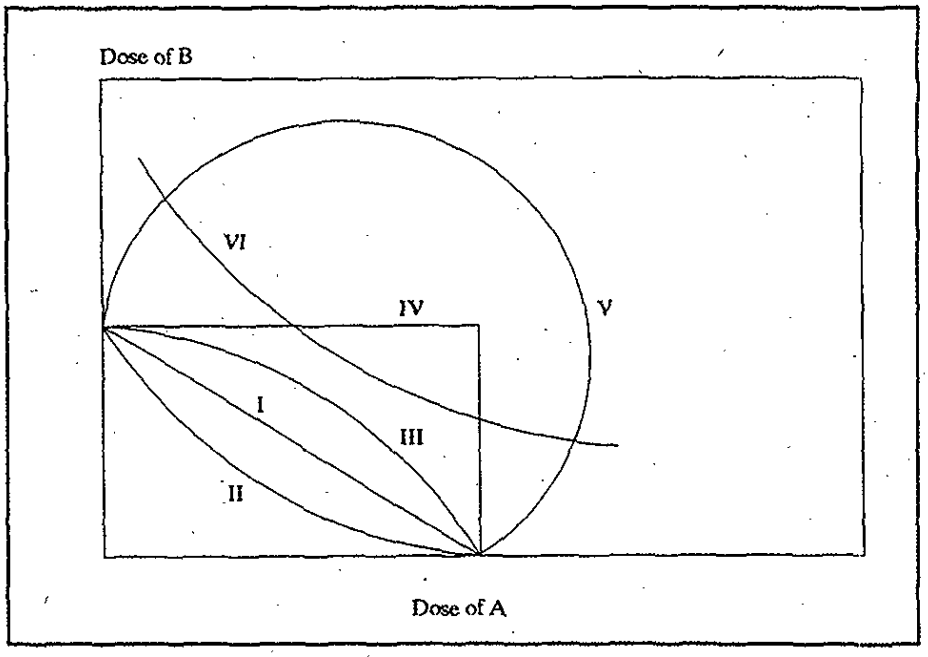

Figure 4.3.1 *Isoboles for interacting toxicity between two substances. See text for explanation /9/.*

In cases where the two substances increase each others action (*synergistic effect*, curve II), a smaller combined concentration or dose will be required to achieve the same toxic effect.

If the combined effect is smaller than the sum of the effects of the two substances independently, but greater than the individual effect of either substance, this is a case of a *partly additive effect* (curve III).

If the substances act completely *independently* of each other, i.e. the effect of substance A is independent of the concentration or dose of substance B, and the opposite is also the case, then the effect will be as shown by curve IV.

Antagonistic acting substances (i.e. the substances must be applied at concentrations higher than the effect concentrations for the individual substances in order to achieve the same effect) are represented by curve V.

Finally, curve VI depicts a *dependent effect* (*coalitive action*), where neither substance is toxic in itself, but where the combination of the two substances has a toxic effect.

For estimating the combined toxicity of a mixture of two or more known substances, the assumption that the toxicities are *concentration-additive* will give the most conservative estimate of the combined toxicity, if the possibility of synergistic effects is discounted. Measurement of the contribution of the individual substances to the combined toxicity is expressed in *toxic units*, TU, which are defined as the relationship between the actual concentration of the substance and the effect concentration (such as LC50); i.e. $TU_i = C_i/LC50_i$. The combined toxicity af a mixture of several known substances at known concentrations may thus be calculated as the sum of the TU_i. In those cases where the toxicity of the individual substan-

ces is concentration-additive, the toxicity of the mixture (TU_M) may be calculated as:

$$TU_M = \Sigma\, TU_i = \Sigma \frac{C_i}{LC50_i}$$

TU_M is defined as $1/LC50_M$ (= 100 [%]/$LC50_M$ [%] = 1000 [ml/l]/$LC50_M$ [ml/l]). The $LC50_M$ of the mixture may thus be calculated as $LC50_M = 1/\Sigma\, TU_i$, if it is assumed that the individual substances' contributions to the combined toxicity is concentration-additive.

For interpreting the results of investigations of the toxicity of mixtures, the sum of TU_i is a very insensitive indicator of the joint action. In the effect concentration of the mixture (EC50 or LC50 etc.) the toxicity of the individual substances is additive if the sum is equal to 1. If it is greater than 1, the toxicity is partly additive or antagonistic, and if it is less than 1, the toxicity is synergistic.

In mixtures of substances whose toxicity is partly additive, the sum of the TU of the individual substances at the effect concentration will not give any indication of the type of joint action which is involved (cf. figure 4.3.1). To solve this problem, Könemann /10/ has proposed a "Mixture Toxicity Index" (MTI) which describes the degree of joint action quantitatively. The MTI is defined as follows:

$$MTI = 1 - \frac{C_i}{\log(\Sigma\, TU_i / \max TU_i)}$$

where max TU_i is the greatest toxicity contribution from any individual substance. In table 4.3.2 the relationship between MTI and the various types of joint action toxicity is shown.

Table 4.3.2 Relationship between MTI and the interacting toxicity (from /10).

MTI	Interacting toxicity (from /9/)
< 0	Antagonism
= 0	Independent action
0-1	Partly additive action
= 1	Concentration addition
> 1	Synergism

Calculating the MTI-value at the effect concentration (EC50, LC50 etc.) of a mixture of known substances in known concentrations will therefore give an indication of the type of joint action that occurs between the substances in the mixture.

4.3.2 Investigations of the toxicity of known mixtures

In recent years a number of investigations have been published concerning the toxicity of known mixtures. In the experimental studies, *equitoxic mixtures* have mainly been used. These are mixtures in which the concentration of the individual substances are at the same percentage of that substance's effect concentration (LC50), or in other words the TUs of all substances are the same and thus, each substance is contributing equally to the joint toxic effect.

Synergistic effects

Only few references have been found describing observation of synergistic effects. Warne *et al.* /13/ measured the individual toxicities of a number of substances from crude oil towards a mixed culture of marine bacteria, and also measured the toxicity of equitoxic mixtures of the substances. The toxicities of mixtures of homologous substances could generally be interpreted as being concentration-additive, wheras mixtures of substances from different homologous series generally seemed to display synergistic effects, with an increase in toxicity of up to 4 times compared to a simple additive effect.

de Zwart & Slooff /3/ investigated the toxicity of a great number of mixtures towards the frog *Xenopus laevis* and identified synergistic, concentration-additive, and partly additive effects. A mixture of heavy metals and a mixture of amines both showed a synergistic effect.

Concentration-additive toxicity

In a great number of experiments, the toxicity of mixtures has been found to be best described as a simple addition of the individual toxicities of the substances in the mixture. This is especially the case for substances which have a non-specific action mechanism but are generally membrane-damaging (narcotic or anaesthetic action) /1,4,5,10/. In mixtures of 50 different substances it was found that each substance contributed to the total toxicity, even when the concentration was as small as 0,25% TU /4,5/. The NOEC for *Daphnia* could also be calculated by concentration addition in mixtures of up to 25 narcotic-acting substances /7/.

In a study by Hermens & Leeuwangh /6/, the toxicity of mixtures of substances with different specific modes of action could be described as approximately concentration-additive, although the total toxicity tended towards partial additivity.

Partially concentration additive toxicity

Hermens *et al.* /8/ studied the effect of mixtures of narcotic-acting substances on the reproduction of *Daphnia magna*, and found that the toxicity of the mixture appeared only to be partially concentration-additive. However, the total toxicity approached complete additivity with increasing numbers of substances in the mixture (from 5 to 25 substances).

The toxicity of mixtures of substances having specific action mechanisms was found by Könemann /10/ to be partially concentration-additive in LC50 determinations for guppy. Deneer *et al.* /4,5/ found partial addition in a study of the effect of mixtures of substances with different action mechanisms on the growth of *Daphnia magna*. Since they previously had examined the acute toxicities of a mixture of the same substances, and here had found a clear concentra-

tion-additive effect, Deneer *et al.* concluded that the use of more specific and more sensitive response parameters would lead to a lower degree of additivity.

Independent response and antagonistic response

No references have been found describing independent responses or antagonistic responses between toxic substances. However, there are many examples of investigations which found that the bioavailability of toxic substances is reduced by the presence of other substances in the test mixture (for example, complex formation by humus substances and heavy metals).

Conclusion

On the basis of the above material it may be concluded that the effect of substances with the same toxicological mode of action ("simple similar action", cf. table 4.3.1) may be explained as a consequence of the combined concentration of the substances, regardless whether specific or unspecific toxicity is involved. This is the case, for example, for substances with a non-specific narcotic action. It has been estimated that up to 60% of all chemical substances only exhibit non-specific toxicity; for such substances the joint action toxicity can be calculated simply by adding the toxicity contributions of the individual substances (TU).

If a number of substances have different mechanisms of action, and therefore different toxicological endpoints, the joint action toxicity will in the most extreme case be independent (cf. table 4.3.1). This will probably only happen in theory, however, since it is hard to imagine that the toxic effect of a specifically-acting substance will not lead to a weakening of the general health of the organism (stress), and thus make it more sensitive to other substances, even though these may have other action mechanisms. For this reason the joint action in these cases can probably be described as partially concentration-additive. Assuming that there are only a limited number of different specific action mechanisms it can be proved mathematically that MTI will approach 1 when increasing numbers of substances are involved, which corresponds to the joint action effect approaching the concentration-addition type.

A description of the joint action will also depend on how specific the measured effect is. The effect "death" is very unspecific, since it may be the result of a great many different effects, which in combination express themselves as the death of the organism. On the other hand, effects of increasingly sub-lethal character, for example at sub-organism level, will have an increasing specificity (for example, inhibition of reproduction, MFO-induction, enzyme inhibition). It must therefore be assumed that the joint action toxicity will be less toxicity-additive when sub-lethal effects are measured, than when lethal effects are measured.

4.3.3 Investigations of the toxicity of complex mixtures

In recent years, in connection with official approval of industrial wastewater discharges in Denmark, a considerable number of investigations have been made into the toxicity of the whole effluent, in order to decide the dilution necessary so that toxic effects would not be expected to occur in the receiving water. Only in a very few cases,

however, have so many investigations been carried out that it is possible to evaluate the relationship between the toxicity of the whole effluent and the toxicity of the individual substances in the effluent. In Denmark it is probably only the wastewater from A/S Cheminova that has been characterized to such an extent. This procedure will therefore be presented below as an example.

Example: wastewater from A/S Cheminova

The most thorough and extensive investigation of industrial wastewater in Denmark has been carried out on the effluent from A/S Cheminova. The investigation was summarized in the environmental approval issued by Ringkjøbing County Council under the powers of §38 of the Environmental Protection Act /11/. All the calculations presented below are based on the data in this approval.

The enterprise discharges an average of 3,000-3,500 m^3 wastewater per day. The effluent contains approx. 100 different chemical substances, of which the majority are xenobiotic substances. Since 1966 the toxicity of the whole effluent towards guppies has been measured daily in order to test whether the wastewater conformed to the requirements of the discharge permit. In recent years the LC50 has been higher than 100 ml/l. The acute toxicity of a great number of the substances in the whole effluent has also been tested by means of the guppy test.

From a knowledge of the concentrations of the individual substances in the effluent, it is therefore possible to calculate the toxicity contributed by each substance (TU_i). The calculations use conservative estimates, i.e. they use the lower figure for the volume of effluent discharged (3000 m^3/d) and the greatest estimate of the concentration (highest figure of Cheminova's and Ringkjøbing C.C.'s estimates of discharges quantities per day). It is possible to calculate the toxicity contributions of 70 substances to the whole effluent. The sum of the contributions of the individual substances is found to be 11.6; in other words, if the toxicities are concentration-additive, then a dilution of the effluent by a factor 11.6 would give a concentration that was just at the acutely toxic concentration for guppy.

This result may then be compared with the fact that the whole effluent showed no toxicity towards guppies at a dilution of 10 times (100 ml/l). The County Council considers that the whole effluent is acutely toxic to guppies at a dilution of about 5 times (Christensen, pers.comm.). However, this toxicity is probably due to the salinity of the effluent, which corresponds more or less to normal seawater (Bastholm, pers. comm.). Since the acute toxicity of the whole effluent to guppy is thus incompletely known, a calculation of MTI will therefore be very uncertain. However, with this proviso the MTI for the toxicity of the whole effluent to guppy may be calculated to be about 0.6.

It may therefore be concluded that the toxicity of the whole effluent from A/S Cheminova can be accounted for on the basis of the toxicity of the individual substances, but that the toxicities of these substances are only partially additive. Of course, considerable uncertainty attaches to these calculations. Since the concentrations of the individual substances in the whole effluent have been calculated conservatively (see above), the sum of the toxicity contributions will

tend to be overestimated. On the other hand, only toxicity contributions from 70 of the approximately 100 substances in the effluent have been included, and this implies that the sum of the toxicity contributions will be underestimated. In addition, the whole effluent contains a quantity of particulate material to which some of the substances identified in the wastewater may adsorb. If this occurs, they will only be partially bioavailable, and will not contribute to the toxicity of the whole effluent to so great an extent. This will lead to an overestimate of the wastewater toxicity if addition of toxicity is assumed to be the rule.

The calculated NOEC-values for the individual substances have also been compared with the NOEC for the whole effluent in order to evaluate whether addition of toxicity occurs /2/. As will be seen from figure 4.3.2, it was concluded that several of the substances are present in the effluent in such high concentrations that they alone can account for almost all the toxicity of the whole effluent.

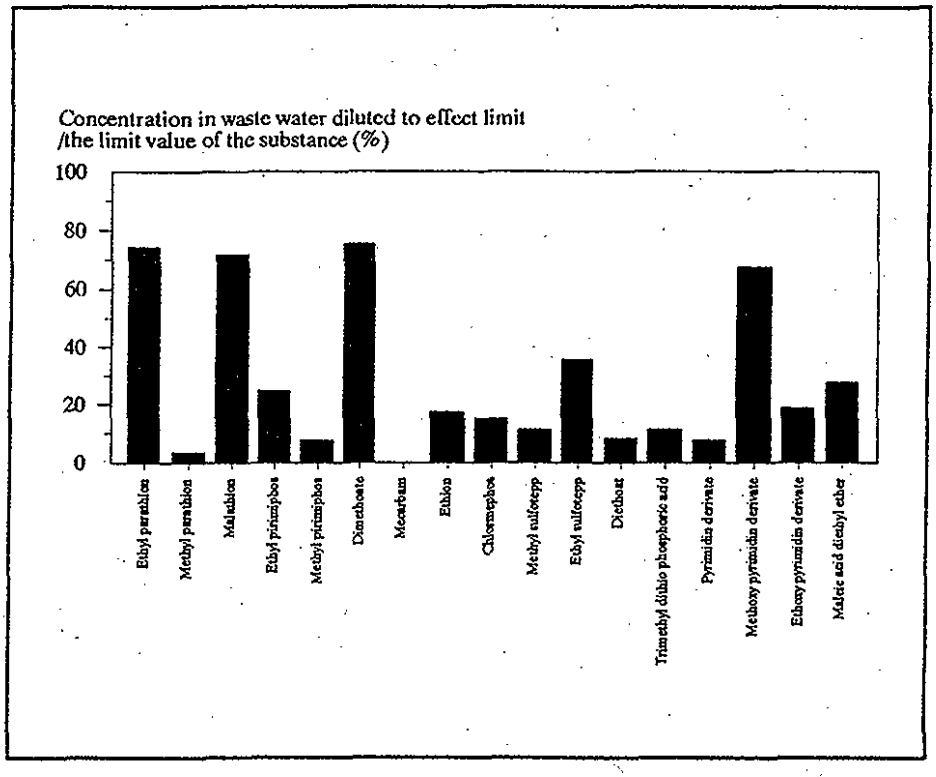

***Figure 4.3.2** Toxicity balance for wastewater from A/S Cheminova. 17 substances, making out the majority of toxicity, are included.*

Ringkjøbing County Council has estimated the NOECs for 18 substances in the wastewater from AS Cheminova. On the basis of the concentrations of these substances in the wastewater, the TU of 17 substances can be calculated. The sum of the TUs was found to be about 50,000: this means that, if the toxicities of the individual substances were additive, the wastewater would need to be diluted 50,000 times before reaching a concentration at which no toxic effects occurred. This should be contrasted with the fact that the lowest ob-

served effect concentration for the whole effluent in practice was found to be at a dilution of 6,600 times. This suggests that the toxicity is only slightly additive, and that in a few cases an antagonistic effect may be operating, since the TU for two of the substances is greater than 6,600.

However, an evaluation such as the above raises a number of problems. In the first place, the NOEC for the individual substances is calculated on the basis of available data, which are of highly variable quality. In all cases, though, data were available for acute toxicity to guppy, and in several cases the NOEC has been calculated from these data using an application factor of 10^4-10^5. It must be assumed that such NOECs are realistic. The estimate of NOEC for the whole effluent is based on the lowest *observed* effect concentration - a dilution of 6,600 times - at which an avoidance response was elicited in shrimp. If the NOEC for the whole effluent is *calculated* in the same way as for the individual substances, by taking the NOEC for acute toxicity to guppy - a dilution of appr. 5 times - and using an application factor of 10^4, an NOEC value of an appr. 50,000 times dilution is arrived at, corresponding to the sum of the TU for the individual substances.

It is clear that there are so many uncertainties attaching to these estimates that it is impossible to draw firm conclusions about the possibility of adding the TUs of the individual substances, calculated from NOEC data, in order to reach a meaningful figure for the NOEC for the whole effluent. In this connection it is important to consider whether the same types of effect are being compared. For example, it is not very relevant to compare *calculated* NOEC values, i.e. concentrations which are not expected to cause any sort of effect, with a particular type of *observed* effect, such as - in this case - an avoidance reaction.

So many tests have been carried out on the whole effluent from AS Cheminova (see table 4.3.3) that it is possible to calculate the 95% protection concentration using Wagner & Løkke's method /12/. Using the acute LC50 values the 95% protection level for acute effects is found to be 0.2 ml/l, and using EC50 values the protection level for chronic effects is found to be 0.06 ml/l, corresponding to a dilution of about 17,000 times if untreated effluent is being discharged. This dilution is of the same order of magnitude as the dilution that was estimated to be necessary to reach a NOEC level assuming additive toxicity.

4.3.4 Conclusion

The estimation of the toxicity of complex mixtures of chemical substances is obviously carried out best and most accurately by performing a series of toxicity tests on the mixture itself (see section 4.1). However, for a preliminary evaluation of the toxicity of a complex mixture, a knowledge of the concentrations and effect concentrations of the individual substances in the mixture will give a lot of information. A reasonable and perhaps conservative estimate of the toxicity of the complex mixture towards a particular species may be obtained by adding the toxicity contributions of the individual substances in the

Table 4.3.3 Acute and cronic toxicity of whole effluent from A/S Cheminova towards a number of test organisms /11/.

Species	LC50 (ml/l)	EC50 (ml/l)
Algae		
Natural plankton		3.3
Skeletonema costatum		1.2
Katodinium rotundum		4.8
Ditylum brighwelli		> 5.9
Pavlova lutheri		27
Protozoans		
Uronema marinum		0.4
Crustaceans		
Nitocra spinipes	20	
Acartia tonsa	3	3.3
Praunus flexuosus	3.7	7.7
Mysidopsis bahia	1.6	
Crangon crangon	13.6	0.5
Fish		
Herring	6.7	
Eel	33	
Flounder	25	0.8
Guppy	> 100	0.8

mixture, assuming that all the substances of toxicological importance in the mixture are known.

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4.4 Prediction of no-effect-concentrations on the basis of laboratory tests

One of the reasons for using laboratory toxicity tests on chemical substances and wastewater discharges is the wish to predict the no-effect levels for the receiving environment. Since laboratory tests can only encompass a limited range of effect concentrations on a limited

number of the relevant ecosystem species, a precise prediction of no-effect levels in the environment is impossible - in theory at least. Experience has shown, however, that laboratory tests may be used to find a concentration level below which toxic effects will not be expected to appear. The use of laboratory tests will thus continue to be necessary, and it is therefore also necessary to attempt to decide how well laboratory tests combined with extrapolation techniques can succeed in predicting effects on natural ecosystems.

4.4.1 Effects on ecosystems

Parameters which may be used to describe the state of the ecosystem may typically be classified as structural or functional parameters /1/.

Effects on structure: Effects on the structure of an ecosystem are a reflection of effects on populations and in the final analysis of effects on individuals. In order for effects on organisms to produce a structural effect, such as a change in species composition for example, the effects must be so large that they affect the fitness of the species (i.e. the ability of the species to compete successfully with other species within the ecosystem). This need not necessarily result in changes in the functional parameters of the ecosystem, since other species can take the place of the affected species within the ecosystem.

Effects on function: Effects on the function of the ecosystem must also reflect effects on individual organisms (for example, reduced photosynthetic activity or slower mineralization), but the effects need not necessarily be reflected in a change in the species composition of the ecosystem, since the various species may still be present in the same numbers despite the toxic effect.

Some types of ecotoxicological effect will manifest themselves primarily as structural changes, while other types of effect will manifest themselves primarily as functional changes. Presumably the precise type of effect will depend on a combination of the substance or wastewater in question and the recipient affected.

At first glance it would seem that effects on the functional parameters of an ecosystem would be less sensitive than effects on the ecosystem's structure, since the difference in sensitivity between different species will presumably rapidly result in differences in species composition. On the other hand, however, it is more time-consuming to detect such changes in the environment than to detect functional effects.

The following section will present a number of studies of the effects on ecosystems produced by chemical substances and wastewater discharges respectively, in order to decide whether it has been possible to predict the environmental effects on the basis of the laboratory tests.

4.4.2 Investigation of effects of chemical substances on ecosystems

Okkerman *et al.* /3/ have examined a large number of multi-species and ecosystem experiments in order to find ecological "No Observed Effect Concentrations" (NOEC) for chemical substances. The NOEC values thus obtained were compared with the toxicity of the substances

towards individual species and with NEC values calculated using various extrapolation methods.

A literature search produced about 2,000 references to multi-species and ecosystem experiments. Of these experiments only 16 were judged to be of such a type and quality as to permit calculation of a reasonably reliable NOEC value. The results are shown in table 4.4.1.

For 8 of the substances it was possible to compare the sensitivity of the species which were most sensitive to these substances in the multi-species experiments, with the corresponding sensitivities of the same or similar species in single-species chronic tests. The ratios between the NOEC values in multi-species and single-species tests are shown in figure 4.4.1. The ratio for the 12 most sensitive organism groups varies between 0.3 and 50, and for 9 of the organism groups between 0.3 and 3.3.

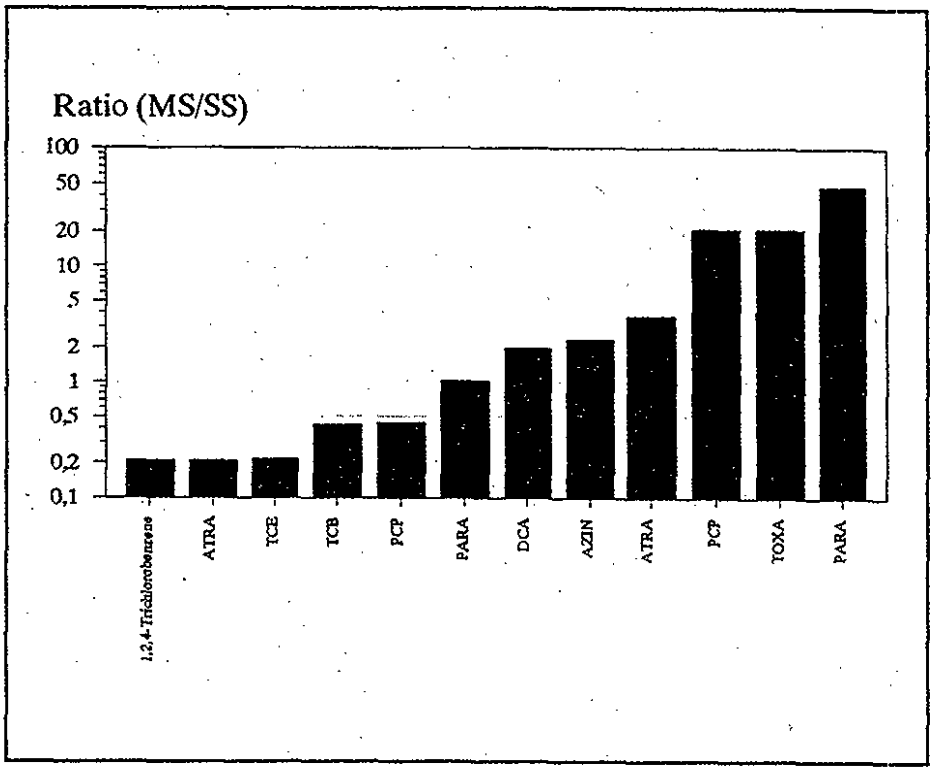

Figure 4.4.1 *Ratios between NOEC-values for multispecies respectively single species experiments /3/.*

Table 4.4.1 Test results from multi-species and ecosystem experiments /3/.
Explanation to abbrevations conc. taxonomic groups is given in table 4.4.2.

Chemical	Use	Test type	Qua-lity	Taxonomic groups tested	Most sensitive group	Crite-rion	Total no. of species	NOEC (µg/l)
azinphosmethyl	I	F	R	Chl,B,C,O,Di,G	B	D.R.	11	0.25
parathion	I	F	R	Cy,Pr,B,C,O,Ep,Di, Od,He,Co,H,Ol,G	B	D.	14	0.1
atrazine	H	L	R	Cy,Chl,Pr,R,N,O,C,Ol	Cy,Chl	D.	6	5
1,2,4-trichlorbenzen	S	F	R	Cy,Cr,Chr,Chl,Eu,B	B	D.R.	32	57
3,4-dichloranilin	In	F	R	B,C,O,Di,G	B	D.R.	12	12
diflubenzuron	I	L	R	Ba,Chr,My,Ep,Pl,Di, Tr,Co,Ol,G	*	D.	28	0.1
methylparathion	I	F	R	Ma,Chl,Chr,B,C,Ep, Di,G	Ep,Di	D.R.	13	0.1
trichlorethylen	S	F	LR	Cy,Chr,Cr,Pr,B	B	D.R.	12	2.8
pentachlorphenol	Im	F	LR	Chl,Ma,B,C,Ep,Od,Co, Di,He,Ol,G	Chl,Ma	Biom.	15	20
permethrin	I	F	LR	R,B,C	B,C	D.R.	16	0.023
trifluralin	H	F	U	Cy,Chr,Chl	**	D.	6	10000
dichlobenil	H	F	U	Cy,Chl,Chr,Ma,R,O,M,I,P	Ma	D.R.	18	5
		L	U	Ba,Chr,Chl,Pr,R,B	B	D.	7	≥ 160
endosulfan	I	L	U	Ma,G,P	**	Mort.	3	≥ 19
malathion	I	L	U	Ma,G,P	**	Mort.	3	≥4500
toxaphene	H	L	U	Ba,Chl,Pr,R,B	**	D.	7	≥ 1.5

I: Insecticide;
In: Industrial intermediate
H: Herbicide;
S: Solvent;
Im: Impregnation agent;

F: Field-test;
L: Laboratory test;
R: Successful test; reliable NOEC;
LR: Successful test; less reliable NOEC;
U: Test with error; unreliable NOEC;

D.: Specific gravity;
D.R.: Recovery of specific gravity;
Mort.: Mortality;
Biom: Biomass;

Taxonomic groups: See table 4.4.2 for abbrevations;
*: All taxonomic groups were sensitive except for Tr, Co, G;
**: No groups were sensitive;

Table 4.4.2 Classification of tested species in taxonomic groups /1/.

	Taxonomic group		Example		Taxonomic group		Example
1	Bacterophyta	Ba	bacteria	17	Branchipoda	B	brachiopods
2	Cyanophyta	Cy	blue-green algae	18	Ostracoda	O	ostracodes
3	Chrysophyta	Chr	diatoms	19	Copepoda	C	water fleas
4	Euglenophyta	Eu	eye algae	20	Malacostraca	M	malacostracas
5	Dinophyta	Din	dinoflagellatae	21	Chelicerata	Che	chelicerates
6	Cryptophyta	Cr	yellow algae	22	Ephemeroptera	Ep	May flies
7	Chlorophyta	Chl	green algae	23	Odonata	Od	dragon flies
8	Macrophyta	Ma	duckweed	24	Plecoptera	Pl	stone flies
9	Mycophyta	My	fungi	25	Heteroptera	He	bugs
10	Protozoa	Pr	protozoans	26	Coleoptera	Co	beetles
11	Coelentherata	Coe	jellyfish	27	Diptera	Di	flies
12	Rotaria	R	rotifers	28	Trichoptera	Tr	caddis flies
13	Nematoda	N	nematodes	29	Heminoptera	He	bees
14	Gastropoda	G	snails	30	Pesces	P	fish
15	Oligochaeta	Ol	oligochaetes/worms	31	Amphibia	Am	frogs
16	Hirundinea	H	leeches				

For the 15 substances for which it was possible to determine reliable NOEC values, a literature search was made for single species tests of chronic toxicity. The test results that were obtained were used to calculate the 95% protection level (HCp) at 50 and 95% probability respectively, and the ECL. Furthermore the ratio between NOEC and the extrapolated values was calculated. The results of these comparisons are shown in table 4.4.3.

It will be seen that the ratios vary between $8.8 \cdot 10^{-4}$ and $1.6 \cdot 10^{9}$. There may be several explanations for this enormous variation: the NOEC values may be wrong because of imprecise methods for determining NOEC; there is a shortage of data for single-species chronic toxicity; no sufficiently sensitive species have been tested in multi-species and single-species tests; or the extrapolation methods may not be appropriate for the substances in question. The latter explanation should be viewed in the light of the fact that most of the substances for which data was found are substances with specific toxic action - particularly biocides.

Thus there appears to be a marked lack of usable data, partly as a consequence of the fact that most of the studies which have been made on effects of substances in multi-species systems have not been designed in such a way, or at such a level of quality, as to permit the determination of a NOEC.

On the basis of this relatively limited material it may be concluded, however, that the protection concentrations that can be calculated using the available extrapolation methods are in most cases lower than the NOEC values produced by multi-species and ecosystem experiments. Therefore it seems to be an acceptable procedure to calculate concentrations below which no effects are expected by means of these extrapolation methods, if sufficient data is available on the toxicity of the substances in question towards sensitive species /3/.

Table 4.4.3 Experimentally determined NOEC values, extrapolated HCp values (from Aldenberg et al., 1990) and ECL values (from van de Meent et al., 1990) as well as ratios between these /3/.

Chemical	NOEC (µg/l)	HCp(95) (µg/l)	HCp(50) (µg/l)	ECL (µg/l)	Ratio[a] HCp(95)	Ratio[a] HCp(50)	Ratio[a] ECL
Azinphosmethyl	0.25[c]	0.014	0.090[d]	0.01	18	2.8	25
Parathion	0.1[c]	0.0000082	0.0023	0.002	12,000	43	50
Atrazine	5[c]	0.13	2.6	0.15	38	1.9	33
1,2,4-Trichlorbenzen	57[c]	2	47	19	29	1.2	3.0
3,4-Dichloranilin	12[c]	-	-	0.51	-	-	24
Diflubenzuron	0.1[c]	-	-	0.001	-	-	100
Methylparathion	0.1[c]	-	-	31	-	-	0.0032
Trichlorethylen	2.8	46	5400	3200	0.061	0.00052	0.00088
Pentachlorphenol	20	0.3	1.9	0.32	67	11	63
Permethrin	0.023	-	-	0.033	-	-	0.70
Trifluralin	10,000	-	-	0.2	-	-	50,000
Dichlorbenil[b]	5	-	-	7.8	-	-	0.64
Endosulfan	≥ 19	0.00011	0.066	0.04	170,000	290	470
Melathion	≥ 4500	$2.9 \cdot 10^{-6}$[d]	0.0011[d]	0.0008	$16 \cdot 10^5$	$41 \cdot 10^5$	$56 \cdot 10^5$
Toxaphene	≥ 1.5	0.000018	0.0024	0.0025	83,000	630	600

[a]: Ratio: Quotient between NOEC values and extrapolated value
[b]: NOEC value from field studies are used here
[c]: Reliable NOEC values
[d]: Extrapolation values for which the single species toxicity data do not follow a log logistic distribution
-: Insufficient data for using the extrapolation method and the calculation of ratio

4.4.3 Investigation of the effects of whole effluent on ecosystems

In the early 1980's the US Environmental Protection Agency (US-EPA) carried out a series of studies on the possibility of using toxicity tests for prediction of environmental effects in recipients as a result of effluent discharges. These studies were reported in 8 US-EPA reports and a series of scientific papers (see /2/ for references). It should be stressed that different methods were used to compare the laboratory results with the ecosystem effects in freshwater and marine recipients respectively. For this reason the results are not immediately comparable, but taken together they give valuable information. The main conclusions will briefly be presented here.

In the marine studies /5/, the toxicity of complex effluent was tested using 5 different species: *Champia parvula* (macroalga), *Arbacia punctulata* (sea urchin), *Mysidopsis bahia* (mysid shrimp), *Cyprinodon variegatus* (sheepshead minnow), and *Menidia beryllina* (inland silverside (fish)). For comparison, toxicity tests were also conducted on undiluted receiving water in which the wastewater concentrations were estimated by discharge of coloured tracer substances. Generally speaking, the receiving water was found to be toxic to the test species in those situations where the wastewater concentrations exceeded the effect concentrations that had been recorded in the wastewater toxicity tests in the laboratory. The results of the studies are shown in figure 4.4.2 /2/. The studies did not attempt to compare the laboratory tests with the actual impact found in the receiving waters, and even though US-EPA has utilized the results of the studies, they are not considered directly relevant to the further discussions in this section. They will therefore not be referred to any further.

In the freshwater studies /2/, the toxicity of complex effluent and receiving water (stream/river) samples were tested using *Pimephales promelas* (fathead minnow) and *Ceriodaphnia sp.*, measuring survival and early life stage growth (minnow), and survival and reproduction (daphnia), after 7 days. The general conclusion of the studies was that the receiving water samples for which toxic effects were recorded corresponded in their wastewater concentration to the effect concentrations determined in laboratory tests of wastewater samples, using the same test species. From these results, as with the results of the marine tests, one can conclude that in the time elapsing from the discharge of the wastewater into the recipient until it reaches the sampling station there does not appear to be any diminishing of toxicity as a consequence of any factors other than dilution. In other words, no significant adsorption, evaporation, or degradation seems to occur.

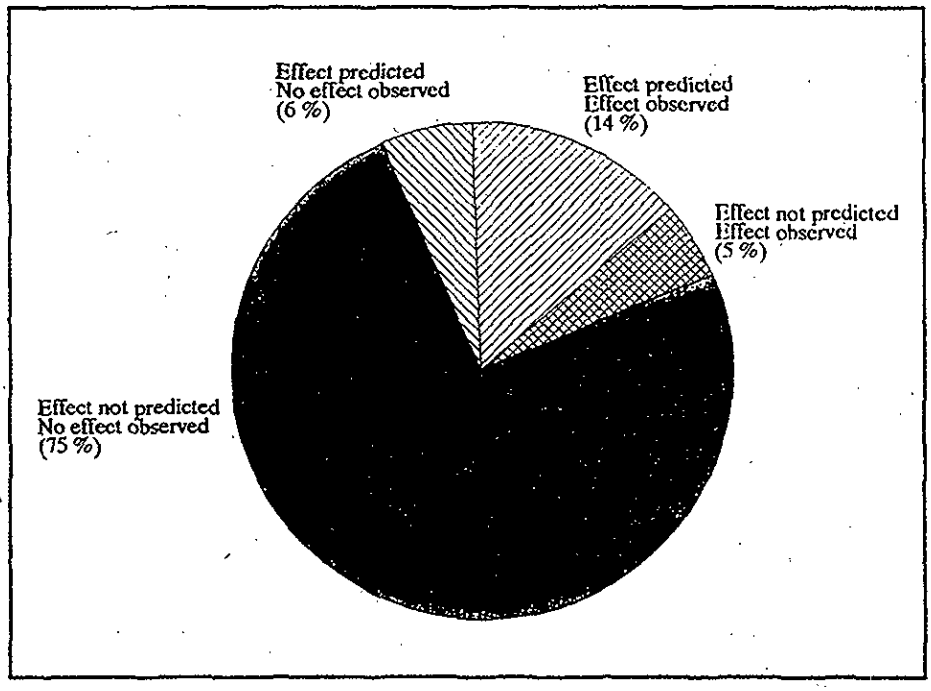

Figure 4.4.2 Results from comparison of predicted effects based on toxicity measurements with effects in recipient water (79 stations at 4 outlets in marine areas) in the USA /2/.

In addition to the above-mentioned studies, field investigations (monitoring) were performed, and the density and number of species of planktonic organisms, periphyton, benthic macroinvertebrates, and fish were recorded. This material was used to compare the results of the laboratory studies with the state and species composition of the receiving waters. At the stations where the recipient water was measured as being toxic to the most sensitive of the two test species, it was generally found that there was a reduction in number of species in at least one of the organism groups examined. The results of the studies are shown in figure 4.4.3 /2/.

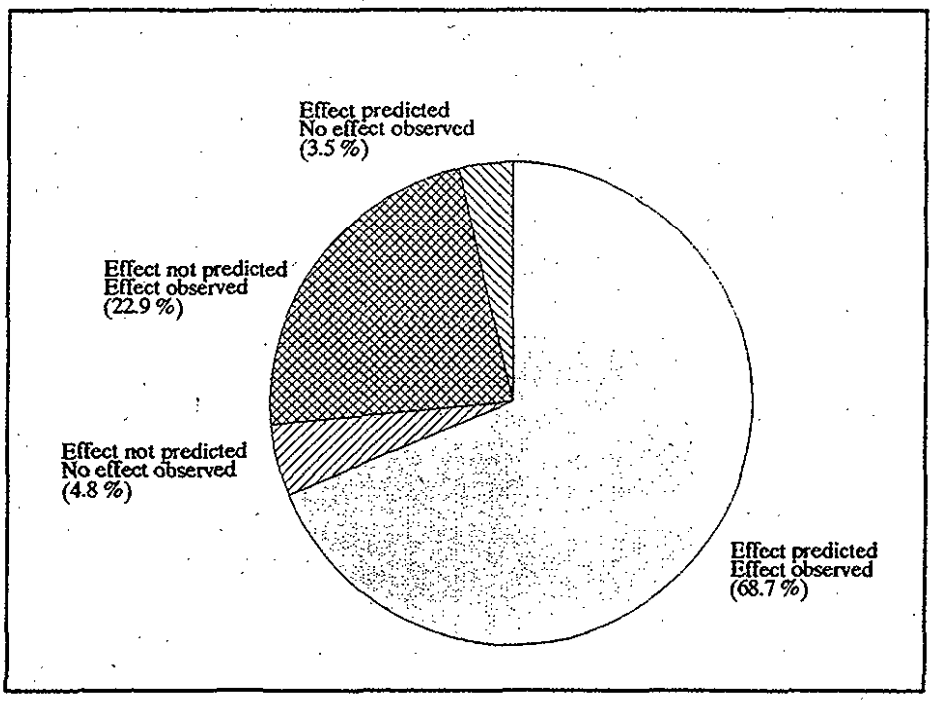

Figure 4.4.3 *Results from comparison of predicted effects based on toxicity measurements with effects in recipient water (83 stations in 8 freshwater outlets) in the USA /2/.*

It should be added that the monitoring carried out in the receiving waters corresponds closely to the monitoring carried out in Denmark by the regional authorities, particularly in marine areas.

In the US-EPA studies the number of species was used as a parameter for recipient quality. The reports state that one of the weaknesses of using species richness is that a species may be severely affected but still present, and this will not be reflected in the monitoring data. The method in itself is thus not particularly sensitive.

US-EPA concluded, however, that the number of species is the parameter most closely reflecting the toxic effect in the recipient, and that number of species (or in other words, a structural parameter) is the best parameter to use for comparisons with toxicological data.

Another weakness in the study was that the proportion of correct predictions depends on how the concept of effect is defined. In the studies cited here, 20% mortality in the laboratory tests is taken to represent the lowest observable effect concentration. Similarly, in the monitoring studies a 20% reduction in the number of species or number of organism groups was accepted as a positive effect. An alteration of these definitions of "effect" in the direction of greater mortality in the toxicity tests produces a fall in the proportion of results in which an effect was correctly predicted, whilst the proportion of results in which an effect was found, but was not predicted, increases correspondingly /2/.

Effects on species richness in recipients may not be due solely to effluent discharges, however, since leaching of chemicals from waste disposal sites and agricultural land, and direct physical

effects on ecosystems, can also influence the number of species, whilst there will be natural fluctuations in numbers too.

Finally, the positioning of the sampling stations is very important in determining how many of the predictions will be fulfilled, since a relatively large number of sampling stations close to the discharge point will make it easy to predict correctly that there will be an effect, whereas a large number of stations far from the discharge point will result in many correct predictions that there will be no effect. Bearing this in mind it can be seen in the reported results that the proportion of incorrect predictions in the freshwater studies was 26.5% (3.6 + 22.9).

Environmentally speaking the most problematic result would be an incorrect prediction that the discharge will have no effect (i.e. a false negative), since such predictions could result in no action being taken to limit discharges where this in fact was necessary. In the freshwater studies cited here (figure 4.4.3), effects were found in the recipient in 83% (= 22.9/(22.9 + 4.8)) of the cases where effects had not been predicted.

Partly on the basis of these studies, US-EPA recommends that a concentration that protects against acute effects in the environment be set at 30% of the LC50 for the most sensitive of three test species. The correction factor 0.3 is applied to extrapolate from LC50 to LC1, which in the studies in question was considered by US-EPA to represent the NOEC value. A concentration protecting against chronic effects in the environment should be set at the EC50 for chronic toxicity for the most sensitive of three test species /2/.

In addition to the above-mentioned US-EPA studies, a number of other comparisons have been made of the effects of complex effluent in laboratory tests and in the recipient. Pontasch *et al.* /4/ investigated the toxicity of a complex wastewater in acute and chronic laboratory tests, in various microcosm systems set up in the laboratory, and by means of investigations of species composition in the recipient. The most sensitive of three species in the acute toxicity tests was *Daphnia magna*, in which the LC50 was found to be a wastewater concentration of 188 ml/l. At a wastewater concentration of 30 ml/l, inhibition of reproduction was observed in laboratory tests using *Ceriodaphnia dubia*. In the microcosm experiments, using foam rubber mats colonized by protozoa, effects were found at wastewater concentrations of 10 ml/l, and in experiments with microcosms containing macroinvertebrates (insects) a reduction in the number of sensitive species was observed at 10 ml/l, while the number of individuals of less sensitive species increased at a concentration of only 1 ml/l. In investigations of the species composition on foam rubber mats placed in the recipient, effects were found at wastewater concentrations of about 140 ml/l, while the macroinvertebrate species composition in the recipient was affected at a concentration of 35 ml/l, and no significant changes could be detected at about 10 ml/l.

On the basis of the above-mentioned results, Pontasch *et al.* consider that application factors of 100 for the lowest measured acute toxicity and 10 for the lowest measured chronic toxicity are sufficient to protect the recipient against the effects that were observed in the microcosm and recipient investigations respectively.

4.4.4 Conclusion

The results presented above lead to the conclusion that at present there is insufficient knowledge about how toxic effects in ecosystems manifest themselves, and thus how they can be detected in practice. The effects that have been detected in various studies have in many cases been difficult to distinguish from natural variation and thus no clear cause-effect relationship has been obvious. Generally speaking, ecological monitoring will not reveal effects at the substance or effluent concentrations that have been established as effect concentrations in single-species tests. The conclusion thus appears to be that the use of laboratory tests on single species is an acceptable method for evaluating the environmental effects of discharges.

Another conclusion must be that the methods available today for extrapolating from laboratory tests to environmental NEC values have not been validated. Among the reasons for this situation are the lack of sufficiently precise determinations of NOEC for ecosystems, and the fact that there is no clear relationship between a calculated concentration which in theory will protect 95% of the species, and an actual description or demonstration of this protection level in the environment. A 95% protection concentration is in itself "merely" a concentration which has a certain probability of ensuring an adequate level of protection.

It must therefore be concluded that at present there are extrapolation techniques for calculating concentrations (NECs) of *individual substances* at which there is reasonable probability that unacceptable effects in the environment will not occur. These methods are based on knowledge and experience concerning the variation in the sensitivity of certain species towards specific toxic substances. Knowledge about this variation also forms the basis for determining the size of the application factor for calculating the ECL.

Similar knowledge and experience concerning the variation in the sensitivity of certain species towards *complex samples* is not available today. Thus the application factors recommended by US-EPA today are not very securely based. The Danish EPA therefore intends to initiate studies of the variation in sensitivity of test organisms towards complex samples, in order to improve the basis for determination of application factors and for developing extrapolation methods for calculating protection concentrations for complex mixtures and discharges.

References

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/2/ US-EPA (1990): Revised technical support document for water quality-based toxics control. Office of Water Enforcement and Permits & Office of Water Regulations and Standards, April 1990.

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/4/ Pontasch, K.W.; Niederlehner, B.R. & Cairns, J. Jr. (1989): Comparison of single-species, microcosm and field responses to a complex effluent. Environ.Toxicol.Chem. 8(1989):521-532.

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5. Strategy for environmental assessment

5.1 Principles for environmental assessment of chemicals

In connection with their work of evaluating and regulating chemicals which are hazardous to health or the environment, governments have developed a series of different methods and definitions. The principles involved will be described in this section, since they also have considerable relevance for the assessment of complex mixtures.

The concept "hazardous" may initially be defined as the potential of a substance or an effluent to cause damage to health or to the environment. The concept of risk is used in the evaluation of an actual scenario in which the probabilities and the size of the damage can be quantified in relation to the actual exposure (see figure 5.1.1). The evaluation may in fact consist of a stepwise process /1,2/ consisting of:

- hazard identification
- hazard assessment
- risk assessment

A *hazard identification* aims to identify those inherent properties which are of importance for the substance's potential for causing damage to the environment. This could for example be the biodegradability, bioaccumulative potential, toxicity, and physico-chemical characteristics. The hazard identification may result in a classification or ranking.

In a *hazard assessment* an evaluation is carried out of the inherent properties of the substance. The effect-related data are assessed in order to determine the dose-response relationships and from this the "No Observed Effect Concentration" (NOEC), which is the highest concentration at which no toxic effects could be detected in the tests that have been performed. The NOEC is determined for each species tested, and from these results a combined NOEC is derived covering all the tested species. If sufficient data is available on the toxic properties of the substance it will also be possible to establish environmental quality criteria.

Physico-chemical characteristics, bioaccumulative potential, and biodegradability are included as elements in the assessment of the distribution, spreading and fate of the substance in the environment. Finally, using the knowledge about potential sources, a calculation can be made of the expected or potential concentrations in the environment ("Predicted Environmental Concentrations" - PEC). On the basis of these results, a preliminary assessment may be made of potential effects on the environment if the substance is emitted.

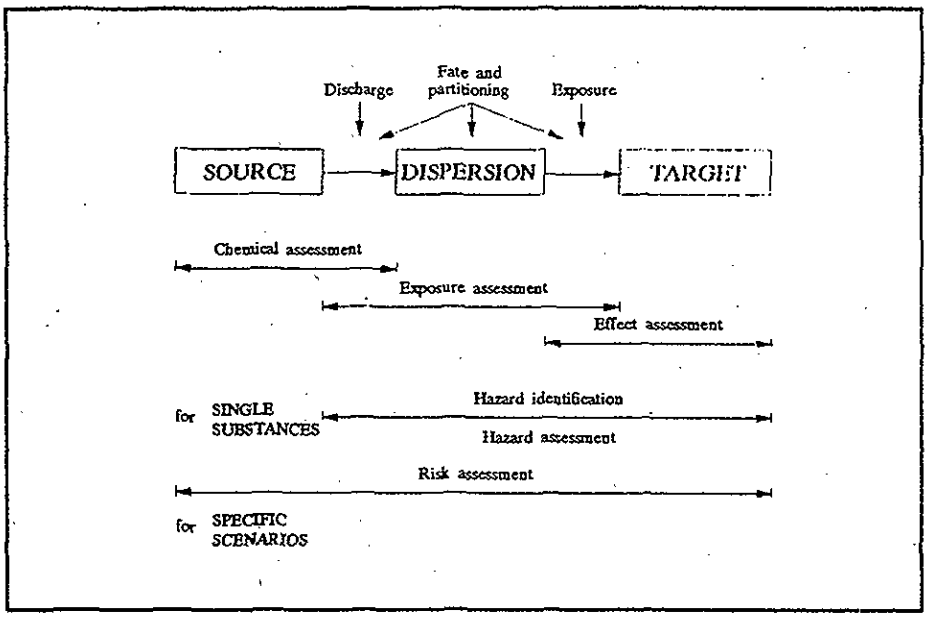

Figure 5.1.1 *Concepts and correlations in hazard and risk assessments /3/.*

A *risk assessment* is related to a specific scenario such as the usage pattern for a chemical, or actual discharges of the chemical, where the concentration (PEC) in the environment or in relevant compartments can be quantified (size, frequency, duration). Furthermore it is necessary to gather as much information as possible about effects so that an ecological "No Effect Concentration" (NEC) can be calculated; this is defined as the highest concentration at which there is a (defined) probability that no toxic effects affect a given percentage of the species of the ecosystem, or where there are no unacceptable effects in various parts of the environment or on the ecosystem as such. This concentration will normally be regarded as an acceptable concentration, and will therefore be used to derive discharge criteria or water quality criteria. In the risk assessment for a specific scenario it will often be possible to quantify the probability that damage will occur, and the size or extent of the likely damage.

A more detailed presentation of the elements and relationships between hazard assessments and risk assessments will be found in references /1,2,3/.

The purpose of carrying out environmental assessments of chemicals is usually to assess whether the substance can be expected to occur in the environment at sufficiently high concentrations so as to give a likelihood of unacceptable effects. In other words, whether the concentration in the environment (C) can be expected to be higher or lower than the concentration which just does not cause unacceptable effects (NEC).

If the concentration in the environment (C) is lower than the no-effect-level (NEC), then it is reasonably certain that no ecotoxicological effects will occur. Thus the following criterion may be estab-

lished for when a substance can be expected not to cause ecotoxicological effects in the environment:

$$\frac{NEC}{C} > 1$$

If NEC or C is determined on the basis of inadequate or insufficient knowledge, this can be compensated for by applying an uncertainty factor (UF). The size of the UF will depend on the amount of knowledge available, and the quality and relevance of the data. An increase in the amount of knowledge will therefore result in a reduction in the UF. The introduction of an UF into the calculation of NEC or C will therefore lead to the following modified criterion:

$$\frac{NEC}{C} = \frac{NOEC/UF_{NEC}}{PEC \cdot UF_C} > 1$$

or:

$$\frac{NOEC}{PEC} > 1 \cdot UF_{NEC} \cdot UF_C$$

This means that the greater the uncertainty in the calculations of NOEC and PEC respectively, the greater the difference must be between the two concentrations before there is a reasonable certainty that no ecotoxicological effects will occur in the environment.

5.2 Strategy for investigation and assessment of wastewater

In studies and assessments of industrial wastewater and other complex effluents, the same stepwise procedure as described above is often followed. The aim of an assessment is to determine whether there is any risk of damage to the recipient if the discharge is permitted, and if this is the case, to determine what sorts of effects may be expected. The assessment can focus either on individual, identified hazardous substances in the discharge, or on the complex effluent as a whole. The substances to be focused on may be priority substances (included on national priority lists, the EC List I, or the "North Sea Conference List" etc.) or substances which on the basis of their inherent characteristics must be defined as particularly hazardous to the environment (toxic, bioaccumulative, or persistent).

The steps described in the environmental assessment process can be combined into a stepwise strategy for investigation and assessment of industrial wastewater (figure 5.2.1). The basis of the strategy is that at each step an evaluation must be made as to whether the available data is sufficiently reliable to permit administrative decisions to be taken with regard to the discharge, or whether additional information is needed. The administrative decision will usual-

ly result in the approval of a discharge permit, in which there may be requirements concerning steps to reduce the discharge of particular substances or qualities (by means of substitution of chemicals, process modifications, effluent treatment, etc.) if on the basis of the available information it is regarded as likely that unacceptable damage will be caused in the recipient.

The following sections will discuss the individual steps in the investigation and assessment strategy.

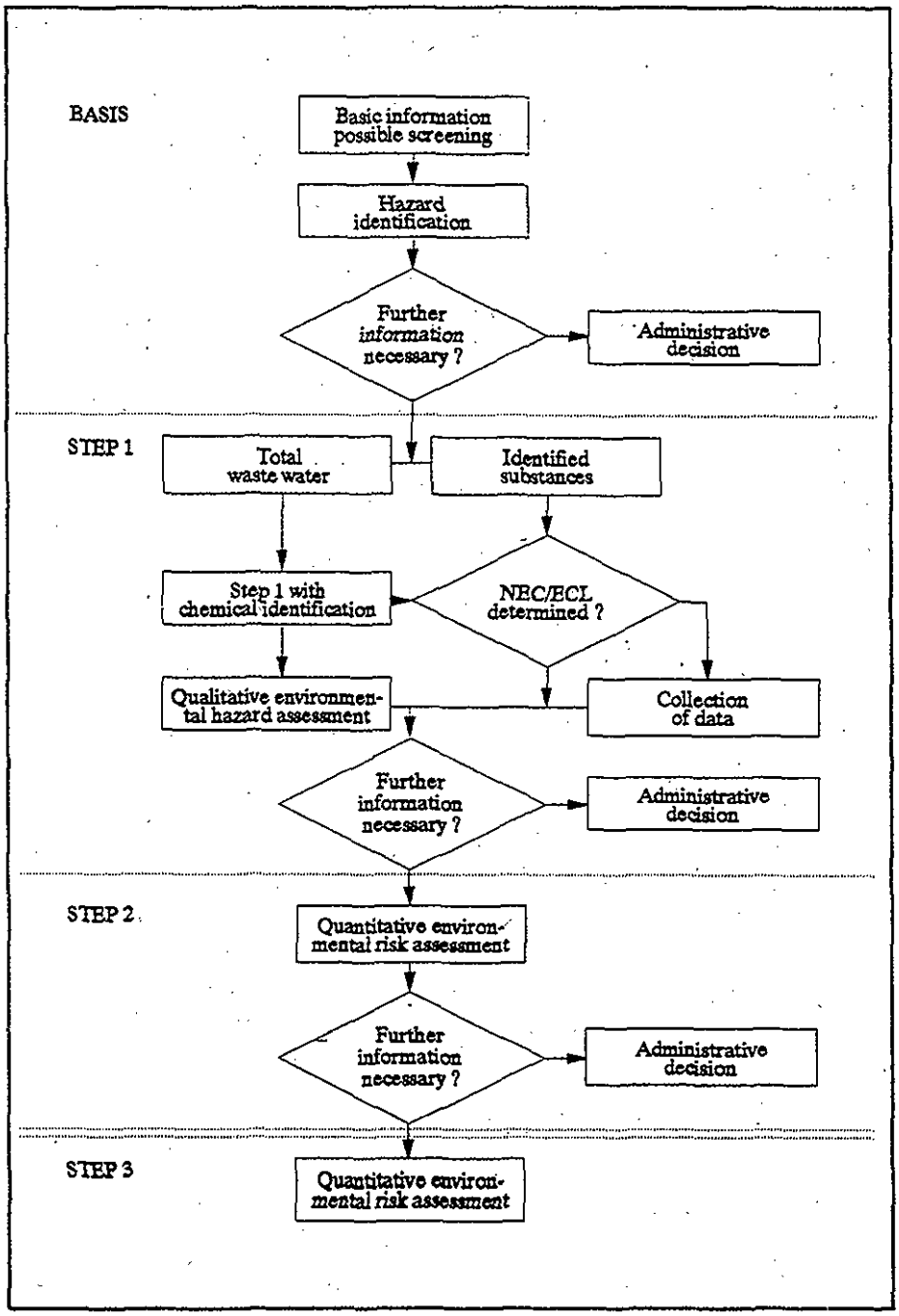

***Figure 5.2.1** Strategy proposal for testing and assessment of industrial wastewater.*

5.2.1 Wastewater hazard identification

The purpose of this step is to make an initial assessment, on a conservative but realistic basis, of the potential ecotoxicological properties of the industrial discharge, so that a decision can be taken as to whether the wastewater potentially could cause ecotoxicological effects in the recipient.

The initial hazard identification is made on the basis of all available information on the properties of the wastewater and its spreading in the receiving environment (basis information). As examples of relevant information can be mentioned:

- information on substances used or produced in the process, drawn up in a mass balance with the aim of determining which substances may be present in the effluent,
- information on measured quantities of substances in the effluent
- information on the physico-chemical properties of the substances
- information on the biological properties of the substances, for example biodegradability, bioaccumulative potential, toxicity and genotoxicity,
- information about the environmental hazard classification of the substances
- information about measurements of physico-chemical properties, degradability and persistence of the whole effluent
- information about currents and possible spreading patterns in the environment
- information about uncertainties and quality of data used in the above

In the initial assessment the use of simple screening tests could also be considered, such as sludge inhibition tests, or limit tests with undiluted effluent (see section 4.1), or measurements could be made of simple overall parameters if these results are necessary for permitting an administrative decision to be taken.

5.2.2 Wastewater hazard assessment

In the first step a preliminary investigation programme is carried out on the whole complex effluent in order to obtain a minimum data set for use in the qualitative environmental hazard assessment. The data must be sufficient both for determining dose/response relationships - thus providing data concerning the toxicity of the whole effluent - and for determining exposure-related parameters such as biodegradability, bioaccumulative potential, and a number of physico-chemical data.

The programme could include a small number of tests for acute toxicity on recipient-relevant species (fish, crustacea, algae). From these the NOEC is determined; as mentioned in section 5.1, this is not an indication of the NEC, but it is still an important item of information for an environmental hazard assessment.

In addition, in order to evaluate the spreading of the effluent in the recipient (PEC), it will be necessary to perform a number of physico-chemical investigations, investigations of biodegradability, and

an evaluation of the dilution patterns. If the effluent is not treated biologically before discharge to the recipient or to the sewerage system, it may also be appropriate to carry out an aerobic stabilization of the wastewater and to evaluate the effects on microorganisms-/treatment plant, and afterwards to test for "persistent toxicity".

In parallel with the investigations on the whole complex effluent, information must also be collected about the substances which have been identified in the effluent, either from information about raw materials and production processes, or from chemical analysis of the effluent. If any of these substances are classified as hazardous, discharge criteria (limit values) or water quality criteria (NEC) may have been established by national authorities, EC (List I substances) or US-EPA (Water Quality Criteria). For substances for which no discharge criteria have been set, as many data as possible must be collected concerning the inherent characteristics.

The results of these investigations will make it possible to carry out a detailed evaluation of the potential of the effluent to cause ecotoxicological effects in the recipient.

Both in the hazard identification and the hazard assessment processes, the focus is mainly on the situation in the industrial enterprise itself: production and wastewater characteristics etc., but if it is possible to establish NEC and C from this information, then it may be possible to quantify possible environmental effects.

If NEC and C can not be established using the information available, preliminary and less precise parameters may be used instead. For assessing the whole effluent, the NOEC divided by an application factor may be used as a rough indication of the NEC. For assessing individual substances the calculated "Environmental Concern Level" (ECL) may be used (see section 4.2) - this parameter is only defined for individual substances. Instead of C, the concentration of the substances after initial mixing may be used.

At this point the decision can be taken to initiate steps to reduce the discharge of certain problematic substances or to reduce some of the overall parameters of the wastewater, or to reduce the size of the discharge as a whole. Examples of this type of initiative are process modifications, substitution by less hazardous substances, or treatment steps. Amongst the methods used to identify where such initiatives need to be taken are source-tracing and toxicity identification /10/.

5.2.3 Wastewater risk assessment

In this step (step 2), an ecotoxicological test programme must be carried out in sufficient detail so as to permit the calculation of NEC and C with so large a degree of certainty that a quantitative environmental risk assessment can be carried out. This is then used to establish discharge criteria for the whole effluent or for hazardous substances in the effluent.

Step 2 can include tests for acute toxicity using a greater number of species, or it can include tests of longer duration, which can permit an assessment of sublethal effects caused by longer exposure (particularly relevant if the effluent is known to contain bioaccumulating or persistent substances) (see section 3.3). The tests may

be made on the whole effluent or on relevant components, or on identified substances for which insufficient data is available for establishing the NEC.

In addition, supplementary tests and analyses of the effluent will be necessary to determine the fate of the effluent after discharge. The objective is in fact to collect so much information and data that the effluent concentration in the recipient can be quantified on a statistical basis.

If necessary, a third step may be added, consisting of additional tests which are chosen and adjusted to suit the actual effluent-/recipient situation. They may for example focus on specific effects of individual problematical substances in the effluent mixture, or tests may be made on the turnover and effects in specific environmental compartments (sediment for example), or studies might be made of effects on the interaction between species (multi-species tests). In addition, recipient studies and monitoring activities could also be included in step 3.

5.2.4 Environmental assessment of effluent

The objective of the individual steps in the environmental assessment procedure will always be to calculate both the actual concentration (C) of the wastewater or of specific chemical substances in the recipient (exposure assessment), and the concentration (NEC) at which no unacceptable ecotoxicological effects are expected in the recipient (effect assessment). A comparison of C with NEC will then give a measure of the expected harmful effect in the recipient.

The actual concentration in the recipient (C) may be calculated at various distances from the discharge point, or in several different areas of the recipient water body. The effluent concentration may for example be calculated on the basis of the initial mixing or in relation to the establishment of an allocated impact zone. The concentration which it is relevant to calculate must be decided on the basis of the official environmental quality objective for the recipient in question.

Calculation of effluent concentrations at specific points in the recipient can be done either on the basis of conservative dispersion calculations, in which only the hydraulic dispersion is taken into account, or corrections may also be made for physico-chemical properties and degradability.

For assessing the likelihood of acutely toxic concentrations occurring at a specific point in the recipient, the first step is to calculate the maximal concentration of effluent which can occur as an average over 1 hour or 1 day (for example). Using an appropriate uncertainty factor, C may be calculated from PEC. Correspondingly, a concentration which will not give unacceptable acute-toxic effects (NEC_a) may be calculated from the results of acute toxicity tests. This environmental assessment procedure is shown in figure 5.2.2.

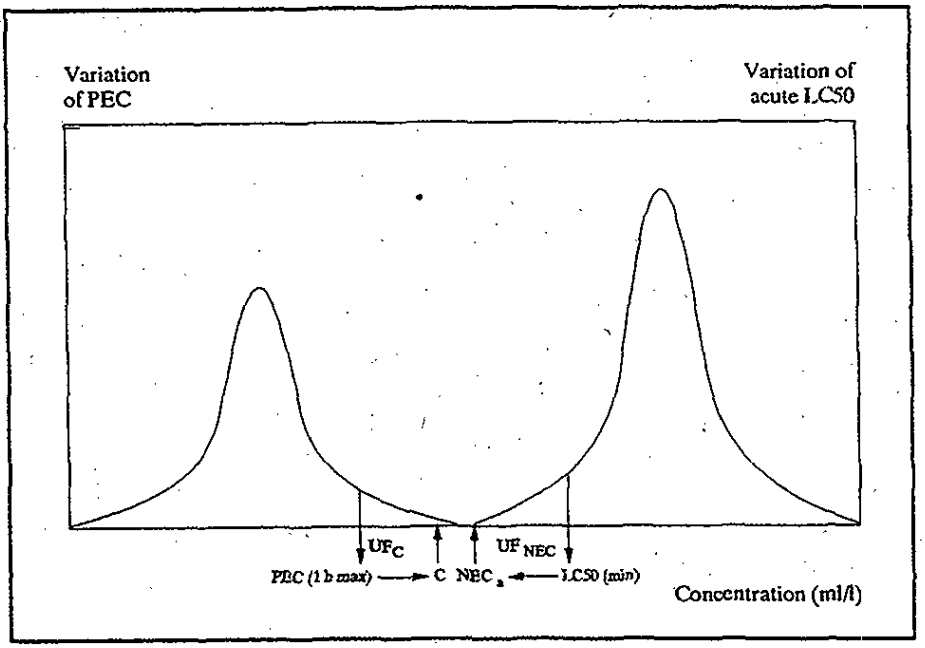

Figure 5.2.2 Assessment of potential acute toxic concentrations at one specific point in the recipient.

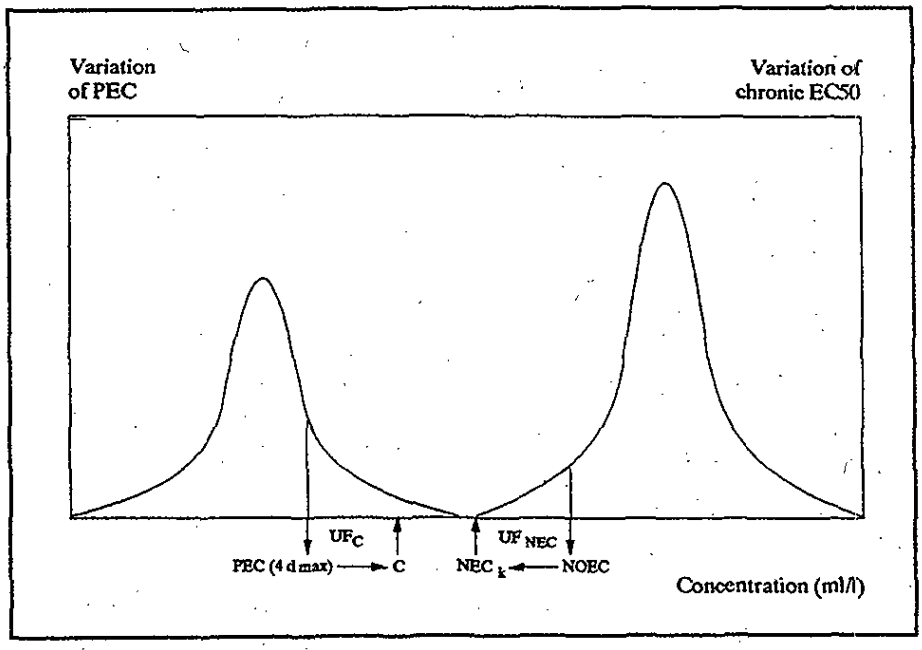

Figure 5.2.3 Assessment of potential cronic toxic concentrations at one specific point in the recipient.

For assessing the likelihood of chronically toxic concentrations occurring at a specific point in the recipient, the starting point is the maximum effluent concentration which will occur over a period of 4 days. This concentration must be compared with a con-

centration which will not cause unacceptable chronic-toxic effects on the ecosystem and its species (NEC_c). This is shown in figure 5.2.3.

As already mentioned, assessments of this type may be made for various areas within the recipient. The areas selected for assessment will depend on the environmental quality objectives for the areas, but often the need will be for criteria for the situation *after initial mixing* and at the *impact zone boundary*.

5.3 A proposed stepwise investigation programme

In order to carry out the environmental assessments at the various stages explained above, it is necessary to collect information and data concerning either the individual substances or the whole effluent. To illustrate what data is needed for these assessments a proposal has been drawn up for a stepwise investigation programme (table 5.3.1). This section describes how the results can be employed to carry out environmental assessments of individual substances, fractions of the whole effluent, or the whole effluent itself. Since in many cases it will be impossible to obtain information on individual substances from the literature, it will often be practical to concentrate the effort on investigating the whole effluent.

It is not the purpose of this proposal to stipulate investigations both of the individual substances and of the whole effluent. In many cases the problems of toxic effects, for example, may be dealt with satisfactorily using investigations of the whole effluent, whereas specially hazardous substances, identified from production data etc., can be dealt with separately.

Generally speaking it is not possible to formulate a fixed investigation programme which can be used for all types of wastewater discharge. Therefore it will always be necessary to carry out a specific expert review of the information needed in order to establish the criteria to be applied to the discharge in question.

5.3.1 Assessment of individual substances

On the basis of the inherent properties of chemical substances and information about the quantities discharged it is possible, if the amount of data is sufficient, to assess the exposure-related aspects such as dispersion in the water phase, adsorption to particles and tendency to settle, bioaccumulation and biodegradation. Toxicity tests provide information on the possible effects of the substance. Some of these parameters, however, will be less suitable for assessment of individual substances in whole effluent, since they are affected by the presence of other substances in the complex mixture. Toxicity is the most obvious example, but properties such as adsorption and biodegradation may also change depending on the presence of other substances in the whole effluent.

The *composition and variability* of the wastewater with regard to the individual substances it contains can be assessed at the preliminary stage using information about mass balances and production processes. Additional information is obtained in stage I from a chemical analysis programme and supplementary analyses at stage II can provide information about the variability of the content of chemical

substances in the wastewater. Finally in stage III more precise information can be obtained about the variability by means of continuous recording of selected indicator parameters through a period of time.

The *biological degradation* of the substances may be assessed at the preliminary (basis) stage on the basis of handbook literature and data on chemical classification. At stage I a more detailed literature search may be made if necessary, to which may be added tests for ready or potential degradability. At stage II and III simulation tests may be carried out which reflect more closely the situation in the recipient in question.

Initial assessment of *bioaccumulation* of chemical substances in wastewater discharges may also be made using handbook material and classification data. At stage I more detailed searches of the primary literature may be made supplemented with HPLC or TLC screening. At stage II the use of bioaccumulation tests is recommended, using fish for example, and at stage III more specific bioaccumulation tests or monitoring studies in the recipient may be carried out if necessary.

Finally it is proposed that the *toxicity* of the chemical substances is determined in the preliminary stage on the basis of handbooks, classification data, existing environmental quality criteria or lists of environmentally hazardous substances. At stage I the primary literature may be searched for additional information, QSAR-evaluations may be undertaken, and acute toxicity tests on up to three different species (planktonic algae, crustacea, fish) could be carried out. On the basis of this information, NOEC values can be established, and if there is sufficient data in the literature, environmental quality criteria can also be defined. At stage II an additional 2 tests for acute toxicity could be added so that enough data is obtained to permit calculation of the NEC and the probability of toxic effects. Alternatively, chronic toxicity tests after more lengthy exposure could be made if the substances in question are not readily degradable. If further investigations are necessary then tests for specific effects on individual species or on multi-species systems can be carried out under stage III.

5.3.2 Assessment of complex wastewater mixtures

Investigation of complex effluent will primarily produce data on the overall effects. On the other hand it is more difficult to draw conclusions about the dispersion and distribution of the effluent mixture in the external environment, since biodegradation and bioaccumulation in particular are qualities which primarily derive from the individual substances or fractions in the effluent mixture. These questions will be discussed further below.

As already mentioned, in the preliminary stage an initial assessment could be made of the *composition and variability* of the effluent based on a knowledge of the production process and mass balances. Already available results from earlier studies can also provide important information. If it is judged necessary to carry out investigations during stage I it is proposed that a number of physico-chemical parameters should be measured (for example: temperature,

		BASIS	I	II	III
WASTE-WATER COMPOSITION AND VARIABILITY	INDIVIDUAL SUBSTANCES	* HISTORICAL DATA	* CHEMICAL IDENTIFICATION: SUBSTANCES HAZARDOUS TO ENVIRON.	ASSESSMENT OF VARIABILITY (PEAKS): SPECIFIC MEASURING PARAMETERS (24H-TESTS/BATCH)	CONTINUOUS REGISTRATION OF INDICATOR PARAMETERS
	WASTEWATER	* PROCESS RELATIONS * MASS BALANCES	* PHYSICAL-CHEMICAL (& ECOTOXICOLOGICAL) SUM PARAMETERS * DISCHARGED AMOUNT (24H/WEEK TESTS)		
DISPERSION AND DISTRIBUTION	INDIVIDUAL SUBSTANCES	QUALITATIVE ASSESSMENT * SIMPLE MASS BALANCES	QUANTITATIVE ASSESSMENT * MASS BALANCES * SIMPLE DISPERSION MODELS	FATE & DISPERSION MODELS, NEAR AND DISTANT ENVIRONMENT	FATE & DISPERSION MODELS, ENVIRONMENTAL COMPARTMENTS
	WASTEWATER				
BIODEGRADATION	INDIVIDUAL SUBSTANCES	* HANDBOOK DATA * CLASSIFICATIONS DATA	* LITERATURE DATA * TESTS (IF ANY): READY/POTENTIAL BIODEG.	SIMULATION OF ENVIRONMENT BATCH-TESTS	SIMULATION OF ENVIRONMENTAL COMPARTMENT (F.INST SEDIMENT) DYNAMIC TESTS (FIELD/LAB)
	WASTEWATER	* HISTORICAL DATA * BOD_5/COD	* STABILIZATION: TREATMENT SIMULATION		
BIOACCUMULATION	INDIVIDUAL SUBSTANCES	* HANDBOOK DATA * CLASSIFICATION DATA	* LITERATURE DATA * HPLC/TLC-SCREENING	BIOACCUMULATION TESTS (FISH)	* MONITORING (EELS, MUSSELS) * ENVIRONMENTAL COMPARTMENT, BIOACCUMULATION TESTS (FIELD/LAB)
	WASTEWATER	* HISTORICAL DATA	* HPLC/TLC-SCREENING	BIOACCUMULATION TESTS, TOTALLY WASTEWATER	

Table 5.3.1 A Suggestion to test activities at various stages.

		BASIS	I	II	III
EFFECT ASSESSMENT	INDIVIDUAL SUBSTANCES	* HANDBOOK DATA * CLASS. OF SUBSTANCES HAZARD. TO THE ENV. * LISTS OF HAZARDOUS SUBSTANCES * ENV.QUALITY CRITERIA AND LIMIT VALUES (DANISH EPA, EEC, EPA)	* LITERATURE DATA/QSAR * ENVIRONMENTAL HAZARD CLASSIFICATION * LIMIT VALUE AND CRITERIA ASSESSMENT * 3 ACUTE TOX. TESTS ON - PLANKTON ALGAE - CRUSTACEANS - FISH	FURTHER 2 ACUTE TOX. TESTS ON SPECIES FROM OTHER TROFIC LEVELS, F.INST.: - BAKTERIA - AQUATIC PLANT - INSECT - MOLLUSC CHRONIC TEST(S) ON SENSITIVE SPECIES	TESTS FOR SPECIFIC EFFECTS ON - SINGLE SPECIES - MULTI SPECIES TEST SYSTEMS
	COMPLEX WASTEWATER	* EXISTING TEST DATA * TOKSICITY ADDITION FROM KNOWLEDGE OF PARTICLES OF SUBSTANCE	* 3 ACUTE TOX. TESTS ON - PLANKTON ALGAE - CRUSTACEANS - FISH		TESTS FOR SPECIFIC EFFECTS ON - SINGLE SPECIES - MULTI SPECIES TEST SYSTEMS
ENVIRONMENTAL ASSESSMENTTAL		HAZARD IDENTIFICATION	QUALITATIVE HAZARD ASSESSMENT	QUANTITATIVE RISK ASSESSMENT, LOCAL AND DISTANT ENVIRONMENTS	QUANTITATIVE RISK ASSESSMENT, ENVIRONMENTAL COMPARTMENTS

Table 5.3.1 B Suggestion to test activities at various stages.

pH, density, suspended solids, DOC, AOX etc.). Additional investigations at later stages of the assessment can then focus on the variation of these parameters with time, and the possible dependence of this on production, or on recording of specific indicator parameters.

The *dispersion* of the effluent in the recipient may be assessed on the basis of the physico-chemical data using simple or more advanced spreading models (see section 3.2). For evaluating the distribution in the external environment, detailed knowledge about which substances are present will of course give the best prospects, but in the great majority of cases extensive analytical work will be needed to get this far. Other types of assessment may be carried out based on the results of biodegradation and bioaccumulation tests.

Assessment of *biodegradation* of the complex effluent mixture can most easily be carried out by measuring BOD and COD. In addition to this, stage I could include (aerobic) stabilization studies or simulation of wastewater treatment plants. In the following stages degradation studies may be carried out, simulating the conditions in the recipient or parts of it. If substances or fractions of the effluent are found not to be readily degradable they may persist in the environment and spread more widely, and they should of course be assessed more carefully. The natural variation in the degradability test results will probably be so large, however, that on the basis of the biodegradation parameters alone it is almost impossible to rule out the possibility that persistent substances or fractions may be present in small concentrations in the wastewater. Some progress can be made by investigating the persistence of some of these parameters (such as bioaccumulation or toxicity) following stabilization of the complex effluent (cf. section 3.3).

Assessment of the *bioaccumulative* fractions or substances can be made in stage I using HPLC- or TLC-screening. Any bioaccumulative fractions can then be identified more specifically by chemical analysis. Additional investigations of the content of bioaccumulative substances can be made by means of test organisms such as fish, or by collecting organisms such as mussels from the recipient and subjecting them to chemical analysis. Bioaccumulative substances which also are persistent or toxic are of particular interest, and investigations of remanence after biodegradation tests in order to reveal any bioaccumulative substances or fractions will give valuable information.

The *effect* of the whole effluent can be assessed at the preliminary stage on the basis of existing test data or from a knowledge of the substances present in the effluent, assuming addition of toxicity. At stage I, as with assessment of individual substances, acute toxicity tests using three species can be made in order to establish a NOEC value. At stage II it is recommended either that a further 2 species be tested so that the probability of ecotoxicological effects can be quantified, or that tests for chronic toxicity after longer exposure time be performed. The latter will be particularly relevant if there are persistent (remanence in stabilization tests) or bioaccumulative fractions present in the effluent. Finally, at stage III, tests may be made for specific effects on individual species or on multi-species systems.

5.3.3 Conclusion

This section has presented a proposal for a stepwise industrial effluent investigation programme, in which at each step a consistent environmental assessment may be made if the required amount of data is available. It should be underlined that the proposal has been developed with available methods and existing data in mind, and that new methods and new knowledge may lead to changes in the programme.

5.4 Strategy for establishing criteria and monitoring compliance

Corresponding to the proposed strategy for investigation and assessment of effluent (section 5.2), it is possible at each stage to make an administrative decision on which an effluent discharge permit could be based. The permit will normally contain a series of requirements or criteria for the composition and characteristics of the effluent, and a description of the conditions to be fulfilled for the permit to be valid. The effluent criteria are established in such a way as to ensure that the effluent discharge will not make it impossible to fulfil the recipient quality objectives established by the regional authorities. According to current Danish law it is the responsibility of the discharger (the enterprise, or the local authority in the case of a municipal treatment plant) to provide documentation showing that the criteria have been met; this must be done via a monitoring programme which is specified in the discharge permit.

The effluent criteria can be of the following types:

- criteria based on water quality, setting an upper limit for the toxicity of the effluent based on the diversity and functioning of the ecological community in the recipient. These criteria may be monitored by means of biotests;
- criteria based on clean technology principles, setting an upper limit for the content of particularly harmful substances (persistent, bioaccumulative, and/or highly toxic substances). These criteria may be monitored by means of chemical analysis.

In cases where the toxicity of the effluent can be ascribed to relatively few substances in the effluent, the water-quality-based criteria may be replaced in whole or in part by criteria concerning the content of individual substances.

The following section outlines an expanded strategy for establishing criteria for toxicity of individual substances and whole effluent respectively.

5.4.1 Establishing criteria and monitoring compliance: individual substances

In the Danish EPA Guidelines for recipient water quality planning in coastal waters /5/, a series of guidelines are laid down for establishing criteria for xenobiotic substances. According to the guidelines, no acute toxicity is permitted in the receiving water within the impact zone of the effluent discharge, in which the less stringent quality

objective applies (this may, however, exclude the immediate mixing zone around the outlet). Any chronic effects are only tolerated within a zone which should be as small as possible, and must not occur outside the impact zone boundary. The discharge of substances displaying both chronic toxicity *and* either persistence or bioaccumulation must be reduced as much as possible ("clean technology").

Protection of the recipient with regard to the acute toxicity of individual substances should be related to the maximum criteria which apply after the initial mixing; i.e. concentrations which must not be exceeded in any samples. The criteria may be set on the basis of the NEC for acute toxicity (NEC_a) and the lowest expected initial dilution (for example at turn of tide in marine recipients or at lowest flow in rivers). The values should be set both as a concentration in the wastewater (mg/l) and as a total discharged quantity (kg/d).

With respect to the potential chronic effects of individual substances (including bioaccumulative and persistent substances), continuous average criteria should be set based on NEC for chronic toxicity (NEC_c), the critical dilution in the impact zone (impact zone boundary, 95% fractile) and an assessment of the variability of the effluent (statistical calculation of mean and variance). Continuous average criteria must be complied with, using an averaging period on a rolling basis, i.e. after each new sample the compliance is assessed on the basis of a constant number of samples from the elapsed averaging period.

The criteria (and an acceptable variance) must be compared with the mean and variance for the data from each period using the following relationship:

$$C = M + k_n \cdot S$$

where
- C: the stipulated value to be achieved
- M: mean of measurements during the monitoring period
- S: variance ("spread") of the mean
- k_n: a constant determined by the normal distribution function, which depends on the acceptance probability and number of samples (Normally set at 0,95; i.e. 95% probability that the criterion is fulfilled if for example the 80%-fractile is lower than C).

The above method for establishing and monitoring continuous criteria is in agreement with standard practice as applied today to virtually all municipal and a considerable number of industrial wastewater discharges in Denmark. The method has been described in detail in a Danish Engineering Federation (DIF) recommendation of 1981 /9/.

5.4.2 Establishing criteria and monitoring compliance: whole effluent

Criteria for the overall properties of effluent have typically been established on the basis of the individual substances detected in the effluent and on available knowledge of the fate and effect (and existing criteria, if any) of the substances in the environment.

In recent years, the parallel use of overall chemical parameters, such as NVOC, and biotests have revealed a number of weaknesses in this traditional practice, especially with respect to assessment of the *combined toxicity* of the wastewater /10/:

1) Experience in general shows that even after a considerable effort only a small proportion of the total carbon content of the effluent (measured as NVOC) can be identified by chemical analysis. Apart from the contribution from humic acids, etc., even advanced techniques such as GC/MS are unable, according to /10/, to detect about 80% of the more than 50,000 synthetic chemicals which are commercially available today. To these must be added a potential content of known and unknown degradation products.

   Even for detectable chemicals the analysis of complex samples can be problematical due to the high detection levels and poor resolution of the GC/MS chromatogram, with a relatively high analytical uncertainty in consequence. It should be noted that substance concentrations below the chemical detection level are not necessarily below the "toxicological detection level". This situation has been observed in several Danish examples, especially where highly soluble organic compounds are involved.

2) The variability in the combined toxicity of the wastewater can not be expected to coincide with the variability in the individual substances giving rise to the toxicity, unless the toxicity is related to an individual substance or to substances with identical distribution functions. Thus, establishing protection levels for individual substances (in accordance with the DIF norm /9/) will not give the same level of protection for the combined toxicity.

3) Effect-based water quality criteria are only available internationally for less than 150 chemicals, as mentioned in section 2.3. For the great majority of the remaining substances the documentation of environmentally relevant characteristics is sketchy or non-existent. A detailed assessment of the NEC-level for individual substances will therefore only be possible for a relatively limited number of substances. It should be pointed out, however, that calculation of an "Environmental Concern Level" (ECL) using application factors can be made using relatively small amounts of data (cf. section 4.2).

The above-mentioned comments relate specifically to the possibility of assessing complex effluents. For effluents in which the toxicity derives from a relatively small number of well-documented substances, it will in most cases be most cost-effective to relate the criteria and compliance monitoring programmes to individual substances in the effluent.

5.4.3 Establishing discharge criteria on the basis of recipient quality objectives

Criteria for discharge of industrial effluent can also be based on objectives for the environmental quality of the recipient. For example, the objective could be that the effluent must not be acutely toxic after the initial mixing /12/, or that it must not give rise to chronic effects after mixing or within the impact zone.

For the critical point(s) in the recipient the objective can be formulated as $C < NEC$, where C = effluent concentration at the critical point(s). Depending on whether the criterion focuses on acute or chronic toxicity C can be formulated as $C_{(1\ d\ max.)}$ and $C_{(4\ d\ max.)}$, and NEC can be defined as NEC_a and NEC_c respectively (cf. section 5.2.4).

For complying with the requirements concerning toxicity in the recipient ($C < NEC$) it is possible to use the same extrapolation methods as used in the environmental assessment (section 5.2.4) and calculate backwards to a criterion for the toxicity of the wastewater. If the recipient quality objective is based on acute toxicity, the requirements to toxicity will be based on a maximum value which never - or only at a certain frequency - may be exceeded. If the recipient quality objective is based on chronic effects, it will be most useful to define the effluent toxicity criteria as an average (maximum) toxicity which must be complied with over a specified control period.

In connection with the establishment of discharge criteria it will also be necessary to specify precisely the sampling methods, the frequency of compliance monitoring, and the procedures to be used in biotests. The nature of these specifications will depend on the criteria being used. For example, the sampling duration (such as whether it should be 1-day or 4-day flow-proportional composite samples) will depend on whether the discharge criteria are based on maximum (acute) toxicity or average (chronic) toxicity.

Similarly, the compliance monitoring frequency will be correlated to the way in which the discharge criteria are formulated, since more frequent compliance monitoring will give a more detailed picture of the variation in the wastewater, permitting more precise criteria to be established. Less frequent compliance monitoring will of course give a less detailed picture of the variation, and therefore the requirements to the types of compliance testing to be performed must be stricter if the same degree of certainty is to be achieved that the discharge criteria are being respected.

Finally, the biotests used for compliance monitoring must be chosen as the most "cost-effective" on the basis of the characterization of the effluent using a variety of test methods. Cost-effectiveness is here understood to include the following factors:

- the effluent is toxic to the chosen organism(s) at such a level that the "toxicity" can be measured ("low level of detection")
- the method has a high degree of reproducibility (low "analytical uncertainty")
- the cost of performing the test is as low as possible.

Ideally, biotests used for monitoring of compliance should be chosen on the basis of knowledge of the variation in the effects caused by the effluent on at least three trophic levels of organism (such as algae, crustacea and fish).

If the distribution of the results is relatively uniform, i.e. the tests that are used give similar predictions as to the relative level of toxicity, then the most cost-effective method should be used (cf. figure 5.4.1 A).

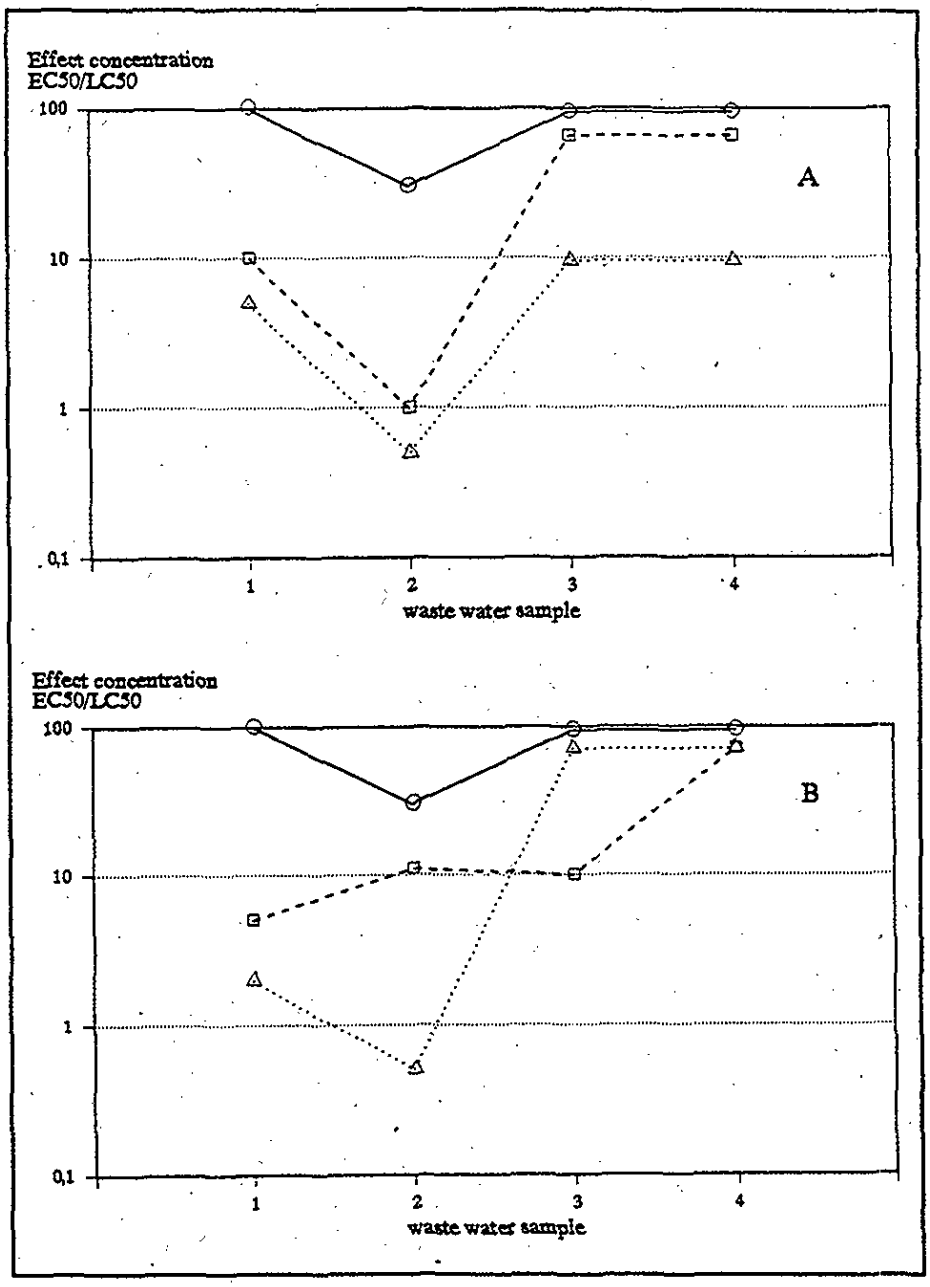

***Figure 5.4.1** Theoretical example on A) homogeneous distribution function and on B) different distribution function of results from three different biotests.*

If a highly variable distribution function is found (cf. figure 5.4.1 B), i.e. the effect concentrations of the methods used can be related to different substances or substance groups in the wastewater, each with a different distribution function, it may be necessary to include several biotests in the compliance monitoring procedure, or to identify the reason for the variation by means of chemical analysis.

In addition to "recipient realistic" tests, characterization studies may also include methods chosen solely because of cheapness (for example the Microtox test). The aim of these tests can be to examine how closely the results produced mirror the results of the "proper" characterization methods, so that - if results are favourable - the compliance monitoring programme can employ Microtox tests, etc. as an indicator variable for the combined toxicity of the effluent.

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Resumé:
Udenlandske og danske undersøgelser af industrispildevand gennemgås. Metoder til spildevandskarakterisering og miljøvurdering til grundlag for udlederkrav beskrives. Der gennemgås en række økotoksikologiske testmetoder, og der er forslag til strategi for miljømæssig vurdering og kontrol af industriudledninger. Principper for miljøvurderinger af kemiske stoffer og komplekse blandinger gennemgås, og der introduceres et trinopdelt undersøgelsesprogram, hvor der fra hvert trin kan foretages miljøvurdering.

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Ecotoxicological Evaluation of Industrial Wastewater

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Abstract:
This technical report describes the present Danish approach to the analysis, characterization and evaluation of the ecotoxicological properties of industrial wastewater. This is based on Danish and international experiences. Existing principles for environmental hazard and risk assessment of chemical substances and complex mixtures are described. A concept is proposed for a step-wise assessment programme, with an integrated evaluation after each step.

Terms:
testmethods; analyses; hazardous substances; hazard risk assessment; limit values; ecotoxicology; industrial wastewater

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Ecotoxicological Evaluation of Industrial Wastewater

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