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Instructions for the Implementation of Chemical-Biological Monitoring Programs for Plant Protection Products in Agricultural Landscape Surface Waters

Anleitung zur Durchführung eines chemisch-biologischen Monitoring von Pflanzenschutzmitteln in Gewässern der Agrarlandschaft

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Instructions for the Implementation of Chemical-Biological Monitoring Programs for Plant Protection Products in Agricultural Landscape Surface Waters

Contents

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1 Introduction and Objectives

Agriculture, unlike the transportation and other industries, is a branch of commerce in which substances, especially fertilizers and plant protection products (PPP), are deliberately and purposefully introduced into the agricultural landscape on a large scale, namely in order to secure or enhance yields and quality of produce. These substances are applied to specific target areas, but eventually may reach **non-target areas**, such as surface waters, via a number of entry routes such as drift, run-off or drainage, but also through improper handling (CARTER, 2000; HURLE, 1992; MÜLLER-WEGENER, 1994; SEEL et al., 1995). It is imperative that we strive to improve our understanding of and to minimize the negative effects these substances may have on aquatic biocoenoses in order to achieve careful and sustainable land use practices.

There are tools for assessing environmental stress and hazards caused by PPPs and for introducing risk minimizing measures and ensuring a safe use of PPPs. Significant among these tools are the procedures for PPP assessment during the registration process as well as various models (e.g., SYNOPS, DRIPS) for predicting environmental risk (ANONYMOUS, 1992 and 1998a; GUTSCHE & ROSSBERG, 1997; RÖPKE et al., 2004; BACH & FREDE, 2003).

A plant protection product will gain regulatory approval pursuant to article 15, paragraph (1) nos. 3d and 3e of the German Plant Protection Act (Pflanzenschutzgesetz, ANONYMOUS, 1998b/2003) if and only if an examination of the plant protection product shows that, in the light of current scientific findings and technology, given its intended and proper application or as a result of such application, this product does **not have any harmful effects** on animal health nor on groundwater and does not have any other unacceptable effects, particularly on the natural balance as well as on the hormonal balance of man and animals. The hazard to be expected to aquatic organisms when PPPs are applied near water bodies is determined based on the toxicity studies (mainly laboratory studies in planktonic algae, daphnias and fishes) and exposure assessments filed with the application for regulatory authorization. Where necessary, appropriate limitations of application (e.g., buffer zone requirements) are to ensure that surface water concentrations **do not exceed ecotoxicologically derived target values**.

However, values in excess of these target values have been measured in surface waters over and over again (e.g., BISCHOFF et al., 2003a and c; KREUGER, 1998; LUNDBERGH et al., 1995; SÜß et al., 2004a and b). These findings may be the result of improper or non-compliant application of PPPs, or of point source contamination stemming from farm run-off or improper disposal. On the other hand, a possible failure to correctly assess possible

hazards for waters during the registration process cannot be completely ruled out, especially if no further data are available.

Whenever potential **burdens and hazards** for the health of humans and animals or for the natural balance caused by approved PPPs are observed, pursuant to article 15 paragraph 7 of the German Plant Protection Act, the regulatory authorities may require that such findings be further investigated by "**post-registration monitoring**" in order to obtain further insight into the use of the particular plant protection product, and that the results be reported to the authorities[1]. When **authorization** is granted based on article 18, paragraph 1(4) of the German Plant Protection Act, such investigations may be requested as well. Furthermore, the designation of **special areas** for PPP applications by the federal states (e.g., the "Altes Land" fruit-growing area) may be tied to the requirement of monitoring the chemical or biological state of the water bodies. Determining the condition of surface waters is also required in order to attain the set goal of the EU Water Framework Directive (EC, 2000), which states that anthropogenic effects, e.g., from PPP entries, are to be reduced in such a manner that, in the medium term, a "good" condition of the water bodies is achieved. Similar data are needed for reviewing and validating the effects of the **program for the reduction of plant protection** (BACKHAUS et al., 2005) or the processes and **models** used in exposure and hazard assessments during the PPP registration process, respectively.

All of these aspects form the basis for a need to measure the true PPP loads in surface waters and to investigate their actual effects on aquatic biocoenoses under application conditions common in agricultural practice. This investigation ought to be implemented by **monitoring** loads and effects on a regular and scheduled basis.

The German Federal Biological Research Centre for Agriculture and Forestry contributes to a sounder approach to this effort by providing these **practice-oriented instructions** for the planning and implementation of PPP monitoring procedures in the water bodies of the agricultural landscape. One of the goals in doing so is, following the request by the German Advisory Council on the Environment (Deutscher Bundestag DS 15/3600, 7/2/2004), to support the federal states' eco-political capacity to act and to facilitate their fulfilling the monitoring duties that fall into their jurisdiction.

In addition to the individually cited literature, experience gained from the authors' own studies was considered in compiling these instructions (e.g., REESE-STÄHLER & PESTEMER, 1999; STÄHLER & REESE-STÄHLER, 1999; SÜß et al., 2000; BUHR et al., 2001; BISCHOFF et al., 2003a and b; MUELLER et al., 2003; PESTEMER et al., 2003; STÄHLER & PESTEMER, 2003; BISCHOFF et al., 2004; SÜß et al., 2004a and b) .

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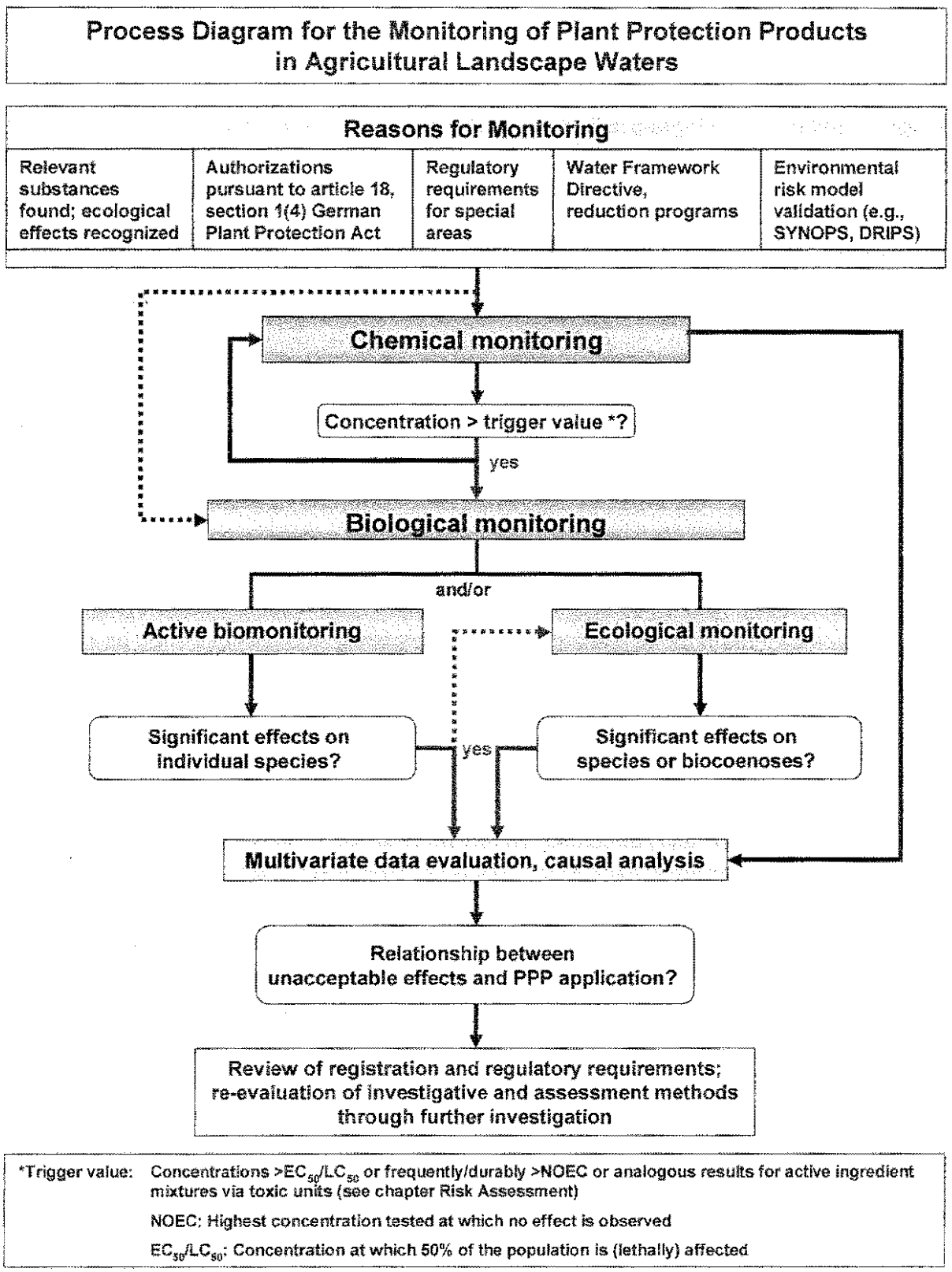
[1] Article 15, paragraph 7(2) PflSchG (Plant Protection Act). Approval may be modified based on this. If that is impossible, in extreme cases approval may be revoked pursuant to article 16a, paragraph 2, PflSchG.

2 The Monitoring Concept

The backbone for the instructions presented here is the following process diagram for chemical-biological monitoring procedures.



As discussed in Chapter 1 and noted in the process diagram, there are different **reasons** for which monitoring may become necessary. Consequently, monitoring may involve different questions that require a differentiated approach.

In the case of relevant (i.e., frequent, valid, and ecotoxicologically alarming) observed concentrations of PPP active ingredients in surface water, or of other findings regarding a potential water hazard posed by certain approved PPPs, and as part of PPP authorization proceedings pursuant to article 18 paragraph 1(4) of the German Plant Protection Act, **single-substance or single-product monitoring** usually is what is called for. This means that a given PPP with one or more active ingredients is to be investigated following one-time or multiple application(s).

Monitoring for the purpose of ensuring the protection of surface waters may also be made part of the requirements accompanying the creation of special plant protection areas (e.g., the "Altes Land" fruit-growing area) and of other regulatory exemptions. Monitoring also constitutes a conceivable measure for assessing the achievements of reduction programs. In those cases, the **application regimes of different PPPs** need to be investigated, taking into consideration the range of products specific to the crop species, their individual application rates, application methods as well as other parameters of the **plant protection procedure** being investigated, including buffer zone requirements. In investigations in monocultures or permanent croppings, the effect of the plant protection procedure is captured against the backdrop of an often pre-determined crop growing procedure, with annually changing weather and pest occurrences causing different patterns of PPP application. If a crop rotation is investigated, type and intensity of plant protection measures, as well as agricultural measures such as sowing, mowing, harvesting and tilling, change as crops are rotated. Agricultural measures are related to PPP entries because they have an effect on the run-off hazard. Even wider networks have to be considered when PPPs are to be monitored in a **catchment area**. In those cases inputs not only from directly adjacent areas and their effects are observed, but also the effects from remote areas, e.g., through drainage, as well as transfer or dilution effects. When larger water sections are monitored, the spatial changes in the morphological and structural water parameters also need to be taken into consideration. The implementation of the EU Water Framework Directive requires just such a complex **environmental monitoring** process, for, in this instance, the deviation of the water condition from its natural state, i.e., the impact of all anthropogenic influences, including PPP inputs, is to be recorded. Residue data from catchment areas as well may be used to calculate active ingredient output amounts (loads).

When PPPs are monitored in surface waters, the main question is that of the intensity of the parameter "PPP," and thus a chemical monitoring approach is required in which tests for contamination with PPP active ingredients are scheduled on a regular basis for waters in the agricultural landscape. This may, in theory, involve all active ingredients applied in the area, or only selected active ingredients.

The active ingredient concentrations found have to be evaluated by comparing them to certain limits and trigger values (target values and other ecotoxicological parameters such as NOEC or EC_{50}), depending on the task at hand. If such a **hazard assessment** finds

concentrations exceeding the trigger values, opportunities for reducing the input and the hazard, respectively, have to be sought. Afterwards, the compliance with target values has to be verified again. If no reduction is possible, and the concentrations observed suggest a possible impairment of the aquatic ecosystem, chemical monitoring is to be complemented by **biological monitoring** procedures conducted in parallel. The aim of biological monitoring processes is to determine whether the observed PPP concentrations actually cause direct or indirect effects on aquatic organisms or biocoenoses, respectively.

A basic distinction has to be made between ecological (passive) and active biomonitoring (DFG, 1994). Usually, in active biomonitoring approaches, individual organisms or several organisms of the same species, often laboratory raised, are exposed to the contaminated surface water. In contrast, in ecological monitoring, the biocoenoses (or parts thereof) present in the ecosystem are observed or monitored, respectively.

Biomonitoring often is used to detect existing burdens, and may serve to replace long-term, costly, and difficult measurements of the disruptive element PPP. In the concept presented here, organisms in ecological biomonitoring approaches are primarily studied as independent target objects.

As can be seen from the process diagram, in certain cases it may be feasible to conduct joint chemical-biological monitoring right from the start, or even solely biological monitoring throughout.

In order to be able to evaluate the results of such an investigation, entries and fate of PPP active ingredients as well as changes in the investigated aquatic organisms and/or biocoenoses have to be monitored against the complex background of the entirety of abiotic and biotic site factors. The **causal relationship** between biotic changes and the measured or estimated PPP loads as well as the **acceptability** of the observed effects have to be determined.

Details on selecting and characterizing study sites as well as on planning, executing, and evaluating monitoring projects will be given in the following chapters.

3 Study Sites

The term "site" refers to the water bodies and their immediately adjacent agriculturally used environment that is directly connected to the water.

Study site selection needs to be **representative** with respect to the task at hand, especially in terms of the region to be studied, climatic and geographic conditions, soil texture, crop culture or crop rotation, agricultural and plant protection procedures, and type and morphology of the water body. Depending on the specific monitoring goal, site selection can be done either with the aim of capturing a "**realistic worst case**" or an average situation regarding exposure and PPP application effects. Special aspects, such as the main entry routes to be investigated, have to be taken into consideration as well. The crop cultures grown, and thus the type, frequency and timing of PPP applications, the locally practiced application procedures and tilling practices, all are critical factors for the PPP concentration in water bodies. Also important are site parameters such as soil textures, slope, drainage,

amounts and distribution of precipitation, length of the treated area bordering on the water, as well as any buffer zones, design of buffer strips and banks/shores, bank-/shoreside vegetation, water depth and width, and flow rate.

Unless monitoring is to be limited to chemical monitoring only, in choosing a site for chemical monitoring the following aspects of a possible biomonitoring approach should also be taken into consideration.

It usually will be necessary to study **reference sites** in addition to the water bodies that were exposed to PPPs. Reference sites should not be generally undisturbed, natural sites, but water bodies without PPP burdens. Sites should be selected so that exposed sites and reference sites are as similar to each other as possible with respect to all of the significant abiotic and biotic characteristics other than the investigated factor of plant protection, so that similar biocoenoses would be expected in the absence of PPPs. All of the important site parameters, especially water morphology, water regime and flow rate, substrate and soil texture, nutrient and oxygen contents, salinity, water temperature, shading, vegetation and fouling, neighboring crops, date of last dredging and seral stage, have to be taken into consideration (see also Chapters 4 and 6).

In reality, selecting a suitable reference site usually will be difficult. For several of the water parameters, more or less extensive observations, experience, information gathering or measurements are required prior to the start of monitoring. In flowing waters, an upstream segment (i.e., one closer to the spring) can be used for comparison with an exposed downstream segment, provided that the habitats in both segments are sufficiently similar. It also may be feasible to study several sites for which the intensity of the parameter studied (intensity of plant protection measures) is very different or tiered. The less similar the habitats are, the more sites will have to be included in the monitoring procedure. In order to obtain meaningful results, at least three "exposed" and "not/less exposed" sites each have to be studied in case of parallel chemical and biological monitoring.

Especially in cases of single-substance monitoring, it is advantageous to select water bodies for the study that previously have not been significantly burdened by PPP because a possible impairment of the biocoenosis by the investigated active ingredient will become much more readily apparent in such a water than in waters that are regularly exposed to PPPs and thus home only to PPP tolerant species (BLANCK, 2002). It is also advantageous if no PPPs besides the target active ingredient are applied.

In general, if many additional influencing parameters are present, it will be difficult to recognize the effects of the studied PPPs on the biocoenosis. Thus, sites should be selected so that the expression of the biotic study parameters is influenced as little as possible by the accompanying environmental factors. For this reason, only continuously (and as consistently as possible) water-bearing waters should be selected unless temporary waters are explicitly targeted.

The table in the appendix lists all of the required **site and accompanying parameters** as well as all additional data that have to be recorded for chemical and biological monitoring procedures, either once or throughout the entire study period, and that are necessary for interpreting the results.

4 Chemical Monitoring

4.1 General Considerations

Chemical monitoring serves to test selected waters for contamination with PPP active ingredients. Its purpose on the one hand is to regularly record active ingredient concentrations, often including peak concentrations, and on the other hand to calculate the PPP loads in the affected water bodies (e.g. ALTMAYER et al., 2003; KREUGER, 1998; REESE-STÄHLER et al., 2001; SEEL et al., 1994).

Single-substance (single-product) monitoring constitutes a special case in which, after a one-time or repeated application of a certain product, investigation is made as to whether the proper application (BURTH & FREIER, 1999) under defined conditions results in an entry from treated areas into water directly bordering on the treated areas. This also may serve to identify the importance of certain entry routes for surface waters.

Monitoring programs for **plant protection or agricultural procedures**, and also programs in **catchment areas**, serve to determine or monitor the input into surface waters caused by application regimes of several PPPs under conditions common to the agricultural practice. These studies record both diffuse and point inputs, e.g., via surface run-off, drainage, interflow, drift, atmospheric deposition, and farm run-off (e.g., FRAHM & GEBEL, 1996; AUGUSTIN et al., 2002).

A prerequisite for conducting targeted monitoring studies is the collection of all data relating to **PPP applications** at the beginning of the study, and then to continuously update these data throughout the study period. The data should include all information on application rate, type, time, and frequency on a per-field basis (see Appendix).

Additionally, data on the **physico-chemical properties** of the active ingredients applied are required. These include water solubility, adsorption and volatilization tendencies, degradation rates (DT_{50}) in soil and water, photo stability, mobility (K_{OC} value), but also ecotoxicological preventive (target) and threshold values, such as NOEC, EC_{50}, LC_{50}. These data are necessary in order to develop a monitoring program suitable for the specific situation at the site, and to continuously adapt it to the changing conditions of agricultural practice (e.g., changes in the products used).

Efficiency with regard to the information sought should be a high priority in planning residue analytical laboratory work, so that the expense is reasonable with respect to the obtained results. One way of limiting analytical expense is to **select** a defined number of frequently used or ecotoxicologically relevant active ingredients and test water samples for their presence.

When selecting PPP active ingredients to monitor, it is important to keep in mind that, especially in single-substance monitoring, all ecotoxicologically relevant active ingredients that might reach the water and modify its biological condition have to be captured so that any biological monitoring conducted in parallel can be evaluated.

The experimental design should in any case be discussed with the regulatory authorities.

4.2 Application Verification

In single-substance monitoring programs it may be important to determine the initial amounts of active ingredients applied to the treated areas in order to verify that for all applications the intended application rates were achieved. Various different approaches for verification are possible, including soil sampling, setting up Petri dishes or carriers containing an adsorbent in the experimental area (see Fig. 3). Samples need to be processed and analyzed with appropriate methods.

4.3 Sampling

Sampling methods and extent will be determined by the specific goals and the manpower available for a given investigation.

Water sampling can be done regularly or can be based on input events. Input events that trigger a sampling event might be scheduled PPP applications or certain amounts of precipitation in areas with run-off hazards. In both cases, water samples may be drawn either by hand or automatically with the help of sampling devices (Fig. 1). Individual samples and pooled samples (either for a given time period or a given amount) are distinguished as well. Details on water sampling can be found in the respective documentation on standardized water testing procedures (DIN 38402-12, 1985; DIN 38402-15, 1986; DIN EN 25667-2, 1993; DIN EN ISO 5667-3, 1995).

4.3.1 Sampling at Regular Intervals

When a monitoring study is conducted at a site at which PPP may reach waters in various ways (run-off, drainage, drift, etc.), there are special sampling requirements because entry events cannot be predicted in a time- or space-related manner. In flowing waters the situation is complicated by the fact that active ingredient entries are continuously diluted, making the recording of peak concentrations especially difficult.

If water samples are drawn at scheduled times (e.g., weekly, monthly or quarterly) from selected waters, only a snapshot of the PPP burden to the water at the time of sampling is obtained.

If water samples are to be taken from **flowing waters**, the use of automatic (electronically controlled) sampling devices is preferable. Various devices are commercially available from different manufacturers, but it is also possible to specifically design sophisticated proprietary solutions for the problems at hand (FISCHER, 1996; LIESS et al, 2001). In some devices samples are kept cool during the collection period or can even be extracted within the device.

Automatic samplers facilitate regular time- or flow-paced sampling as well as event-related sampling (both modes can be used in parallel). They can also be programmed to draw a

specified number of samples at scheduled times, with the sample volume determined by the user.

Event-related sampling can be done in different ways depending on the type and features of the device used. Sampling may be triggered by either the water level or the conductivity exceeding set threshold values. See section 4.3.2 for more details.

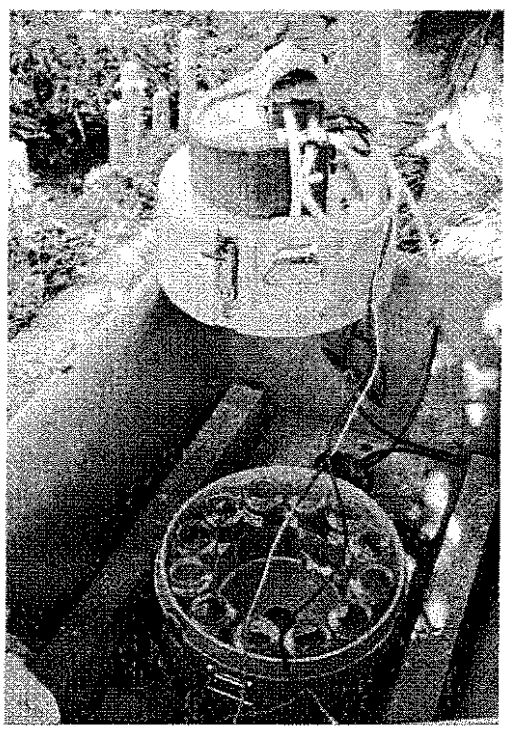


Fig. 1:
Automatic sampler
(ISCO 6700)

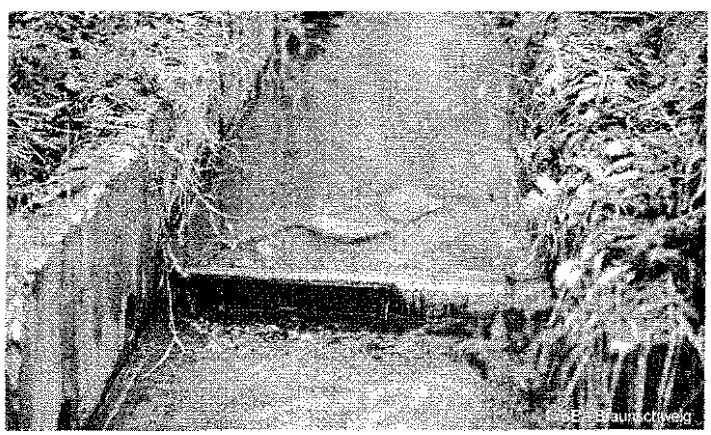


Fig. 2: Horizontal weir

Integrated data loggers enable automatic samplers (Fig. 1) to continuously record and save a range of parameters (precipitation, temperature, water level, conductivity) at user-defined intervals over extended periods of time. The data can be downloaded to a computer either on-site or in the laboratory, and then can be processed with commercially available software.

When **water catchment areas** are to be sampled, the automatic sampler must be installed at the outflow point of the area in question so that the treated areas are situated upstream of the sampling site. In those cases a weir (Fig. 2) may be installed in the water in order to measure flow.

When flowing waters alongside **individual treated study areas** are monitored, automatic sampling should take place in the influent (upstream) and effluent (downstream) of the area. Electronically connecting both samplers allows for triggering a pre-programmed sampling series in both samplers by a sampling-triggering event. Figure 3 illustrates a possible design of such a field trial as part of a single-substance monitoring study. The samples drawn from the influent are needed especially for determining the target substance loads, if any, stemming from areas upstream of the investigative site.

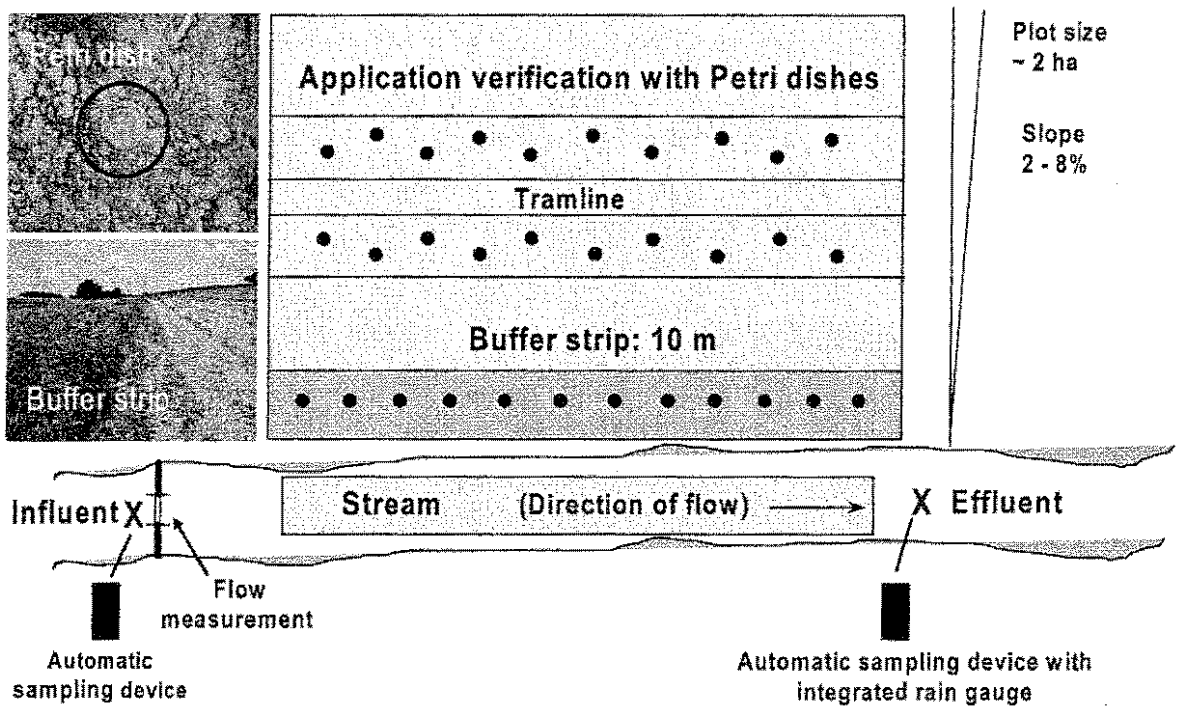


Fig. 3: Design of a field trial (BISCHOFF et al. 2003b)

In **standing waters** or waters with a very low flow rate, the sampling method of choice also will be determined by the specific task at hand. Here, too, automatic sampling devices may be used. However, it has been shown that in standing waters the parts of the device that are in contact with water (intake tube, sensors) will get dirty more quickly and thus may require more maintenance.

Correct placement of devices alongside the water is an important factor and has to be determined on a case-by-case basis depending on the situation at the site and the questions that the monitoring seeks to answer.

If automatic samplers are used, daily pooled samples need to be analyzed during the initial period in order to verify that active ingredient inputs can actually be detected and recorded over the course of time. In order to reduce the **number of samples**, weekly pooled samples can be created by taking defined aliquots from daily pooled samples and pooling them. This will lower the concentration of the targeted active ingredients, so back-up samples from the daily pooled samples need to be retained in a cool place for later analysis in the event that the weekly pooled samples test positive for a given substance. This procedure is useful in long-term studies during the months of the year in which, although no PPPs are applied, continuous sampling of the selected waters is desired in order to monitor any entry events, especially during the precipitation rich months of fall and winter.

How frequently samples will need to be collected from the sampling sites (e.g., weekly) depends on the stability of the active ingredients in question and the holding capacity of the sampler.

There are a number of PPP active ingredients and classes of ingredients that will need to be analyzed immediately after a potential entry event has occurred, without further storage of the water samples. These include on the one hand substances that have low hydrolytic

and/or photo stability (e.g., the fungicides dithianon and captan), and on the other hand compounds that tend to "disappear" from the water system rather quickly because they are bound to particles and/or sediment due to their adsorptive properties (e.g., the pyrethroid insecticides). If a study focuses on substances of this nature, not only the sampling procedures, but especially transport, storage, and residue analytical procedures have to be adapted to their specific properties.

The filled bottles in the sampler are removed and replaced by empty, clean bottles so **sampling can continue without interruption**. Weekly sample pick-up has proven useful in many cases because during the sampling site visits not only do the samples need to be picked up, but the samplers have to be inspected, maintained and possibly repaired as well.

4.3.2 Event-triggered Sampling

In monitoring programs of plant protection and agricultural measures or in catchment areas, the combination of regularly scheduled sampling with event-oriented sampling may be beneficial. As stated above, event-related sampling processes are triggered when certain threshold values (precipitation intensity, water level, conductivity) are exceeded.

Measuring water levels with submerged sensors (hydrostatic pressure) allows for the recording of changes in the water level so they can be considered when samples are taken, and on the other hand can be used in calculating the effluent water volumes. In order to determine effluent volumes, weirs with a defined cross-section (e.g., horizontal weir, 90°-V-weir) have to be installed in flowing waters (Fig. 2). The effluent water volumes can be used to calculate the loads from potential PPP inputs.

Hand-drawing samples is useful when the event triggering the sampling is a known, scheduled PPP application (e.g., in areas with special regulations) and the main monitoring interest lies in determining PPP peak concentrations that may occur in the water, caused by factors including, but not limited to, drift.

Sampling is relatively easy when the active ingredient concentration to be determined will change only very slowly, for instance in **standing waters** (ponds, tarns, lakes) or in flowing waters with an extremely low flow rate (ditches, brooks). Usually a sampling stretch will be selected according to local conditions and will be marked for future sampling. For practical reasons the sampling stretch should be about 100 meters long. If the area of interest bordering on the water is significantly longer than 100 meters, the sampling stretch is to be selected so that it contains those topographical sections that might encourage PPP entries (e.g., lack of vegetation, no earth banks, short distance between field and water).

Following a PPP application, if possible immediately, a pre-defined number of samples is drawn from the waters bordering on the affected areas, in regular intervals along the sampling stretch. A practical example constitutes 5 samples of 0.5 liters water each that are used to fill a 2.5 liter glass bottle to the rim. If copper is one of the target substances, an aliquot of each of the samples drawn is to be transferred into a PE bottle to form a pooled sample. Prior to sampling, both sampling device and bottle need to be rinsed with water from the water body to be investigated. When selecting appropriate sample containers, care has

to be taken to avoid containers and lids that might contaminate the samples and/or adsorb the target substances. Opaque containers and bottles made from brown glass may minimize photosensitive processes (DIN EN ISO 5667-3, 1996).

A proven device for hand-drawing samples is a tumbler (volume about 1 liter) attached to a telescopic handle. From the bank, and at the same distance for every sample, the sampling tumbler or bottle is immersed into the water in a slow, regular motion down to a given depth and then lifted from the water body. Deeper water bodies will require the use of specially constructed sampling devices. In order to prevent contamination or other disturbances of the water, the water should not be stepped into by the sampling staff during sampling.

Depending on the objectives of the investigation, the individual hand-drawn samples may be **pooled** before analysis or may be analyzed individually.

Additionally, whenever possible, samples should be taken **prior to applications** or from the water upstream of the treated areas. This is especially necessary when dealing with connected water bodies, samples from which might be contaminated with PPPs from other sources or previous applications rather than only from the current application.

The pre-application and upstream samples can be used not only to determine the blank values in analyses, but also for additional accompanying experiments for validating methods for the target substances (see Chapter 4.4).

Estimating the time of the expected peak of any input is harder in **flowing waters** than in standing waters because any active ingredients entering the water body are continuously diluted. In such cases sampling should be staggered parallel to the application process. For example, in a fruit-growing area sampling might begin when the possible drift after the first run of the plant protection application device has reached the sampling point. From the 1st to the 5th run, 5 water samples of 0.5 liters each would be drawn and transferred into a 2.5 liter glass bottle.

A general prerequisite for detecting PPP peak concentrations is the timely notification of the person in charge of sampling of the scheduled application of the target substance(s). This holds especially true in case of active ingredients that, after application and possible entry into surface waters, "disappear" rather quickly from the system due to their physico-chemical properties and various instantaneous processes (photolysis, hydrolysis, adsorption, etc.). The drawing of additional staggered samples allows for the monitoring of the changes in concentration of the target substance(s) over time.

Immediately after a sample has been drawn it has to be labeled in waterproof writing. The label needs to contain data on the site, date, time, and occasion of sampling (i.e., sampling before or after application) so that every sample is clearly identified. Additional data should be entered into an accompanying form (see Appendix).

4.3.3 Sample Transport and Storage

How samples are transported and stored has to be decided based on the properties of the active ingredients and the distances to be covered.

Water samples are to be transported in a refrigerated container (or, if the stability of the target substances allows, without refrigeration) to the analytical laboratory. If immediate analysis is not possible, they should be stored frozen whenever possible.

If freezing of the water samples is not possible, they have to be stored in a cool and dark place at about 4°C until they can be analyzed. Storage times should be as brief as possible. With respect to the stability of the active ingredients, storing extracts or cartridges after solid phase extraction in a freezer (about –18°C) is preferable over long-term storage of water samples at about 4°C and without inhibiting biological activities.

4.4 Residue Analysis

Detecting PPP residues in samples from surface waters is costly, difficult, and associated with different challenges depending on the objectives of the monitoring effort. All factors influencing the outcome need to be considered. These include, in addition to representative sampling, sample transport and storage, as well as the continuous verification (validation) of the analytical methods used. The accuracy and comparability of the data obtained form the necessary basis for assessing the state of an ecosystem as part of a monitoring program. Thus, the following section will focus mainly on quality assurance measures for analysis and less on the analytical details of sample processing and measuring.

The objective generally is to establish equally good and reproducible recoveries for all active ingredients of interest at or near 0.05 µg/l (50% of the limit for drinking water). For substances for which toxicity-related effects are expected below this concentration, an appropriately lower value has to be met. However, it is not always possible to achieve limits of quantification (LOQ) below the targets set by the German Working Group of the Federal States on Water Issues (LAWA) for certain PPP active ingredients.

The properties of PPP active ingredients may differ greatly so that methods have to be used that are adapted to the group of substances in question. For single-substance monitoring programs this is a given.

If a larger number of active ingredients has to be detected in water, multi-methods are commonly used that are based on solid-phase or liquid-liquid extraction. Many methods of this kind have been described in the literature. Figure 4 shows the simplified diagram of a multi-method used at the German Federal Biological Research Centre.

The limits (limit of detection and limit of quantification) and the certainty (recovery) of a method for detecting the target substances in water are established when a method is validated. This validation usually is done at the beginning of a study and repeatedly throughout the period of investigation, and can be done in parallel to sample analysis. In order to establish recovery, surface water from the study sites is spiked with various concentrations (e.g., 0.05 µg/l, 0.10 µg/l and 5.0 µg/l) of the active ingredients of interest. The concentrations are selected in a manner that ensures that a wide range of concentrations, as well as the required limit of quantification, is covered.

The **limit of detection (LOD) and the limit of quantification (LOQ)** (also referred to as the limit of determination) of a given analytical procedure depend on the processing and detection methods used, the analytical parameters, and above all on the matrix properties of the sample to be analyzed. The LOD is the lowest concentration of a given substance that can be detected in a given sample. It allows solely for a qualitative determination of whether or not the substance is present. Chromatographical practice primarily accepts the threefold of the analytical "noise" as LOD.

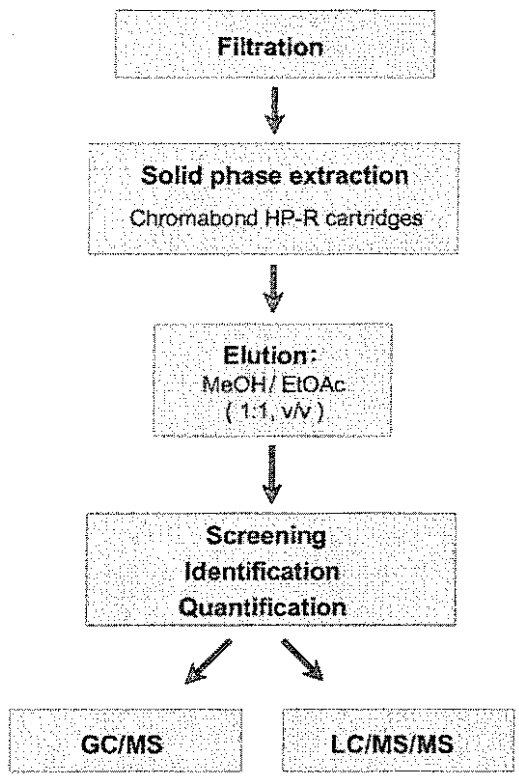


Fig. 4: Example of a residue analytical multi-method for water samples (BISCHOFF et al., 2004)

Quantitative results can be reported with statistical certainty once the values are above the limit of quantification (LOQ). Below the LOQ no numerical results should be reported. Active ingredients which are found at concentrations between the LOD and the LOQ thus are considered detected but not quantifiable (HUBER, 1994).

Commission Directive 96/46/EC lists requirements regarding LOQ (limits of determination in the language of the Directive) and recovery (see also: HÄNEL & SIEBERS, 1998). The mean recovery for each spiked concentration and substrate, according to the Directive, should fall within the range of 70% and 110% with a relative standard deviation of less than 20%. The LOQ is the lowest validated concentration for which these requirements have to be met.

LOD, LOQ, recovery, and variance demonstrate the performance capabilities of an analytical method. They determine the conditions under which samples can be analyzed. However, recovery may significantly differ depending on the nature of the surface water (e.g., DOC

content), the class and concentration of the active ingredient, and the stability of the active ingredients in different waters.

When evaluating residue data one needs to keep in mind that, due to the filtration step, any particle bound amounts of active ingredients will be neglected and that active ingredients adsorbed to soluble carbon compounds (DOC) will be detected in the analysis.

In order to improve and ensure the analytical quality of the daily routine examination of samples of different matrix contents, **internal standards** and **surrogates** are used. The internal standard must be a substance that is not expected to show up in the samples and is added to the sample extracts after processing in order to verify the chromatographic analysis. A surrogate is a standard that is added to each sample prior to processing. The entire analytical process then can be verified based on the surrogate recovery. A surrogate standard has to meet certain requirements: It must not be expected to be found in the samples; it should be quantitatively recovered under the given methodological constraints (within the limits stated above); and it should be structurally as similar to the investigated substances as possible. This last requirement is very hard to meet when a number of structurally very different target substances are studied. In such cases, one structure should be selected on which the surrogate is to be modeled, for it is better to verify the analytical process by including at least one surrogate than not to have any information on the analytical quality whatsoever. Samples for which the results obtained for the surrogate do not meet the required criteria cannot be considered in the monitoring assessment, or only in a limited manner.

For these additional experiments, surface water may be used that was taken from the investigative site, e.g., prior to an application or from a sampling point upstream of the area of interest. In that case, the changes, if any, in recovery over the entire sampling period can be determined.

If for standing waters or waters with extremely low flow rates methods cannot be validated in parallel to the sample analysis during the application periods because the blank values are too high (background contamination), the required verification of the analytical methods is carried out with water samples drawn prior to the start of the application period.

Storage stability testing is conducted in order to determine whether the monitored substances remain sufficiently stable under the transport, storage, and processing conditions chosen. These tests need to take into consideration the storage conditions inside the sampling device, e.g., the type of sampling container used (glass or plastic), maximum and average temperatures, and the time for which samples remain in the device.

For the testing of storage stability, surface water may be used that was taken from the investigative site, e.g. prior to application or from a sampling point upstream of the area of interest. The spiked concentrations should be roughly that of the expected residue concentrations, but at least should be the 10-fold of the LOQ in order to detect potential degradation (BEUTEL et al., 1992).

Storage stability for a given period of time is considered established if the recovery in the stored sample is at least 70% of the amount in the freshly spiked sample (EC, 1997).

Additionally, the **identity** of the PPPs found in the extracts has to be **confirmed** by coupling gas chromatography (GC) or liquid chromatography (LC) with mass spectrometric methods (MS, see Fig. 4).

The **report** on the residue analysis results from a chemical monitoring program also should include any information on the validity of the method that is required for a sound evaluation of the results. Which parameters must be included depends on the study conditions.

Information that always should be listed includes all details of the analytical method, the physico-chemical properties of the active ingredients, their recovery from surface water at different spiked concentrations with the associated coefficients of variation, and the LOD and LOQ. Optional information may include data on the current recoveries of the target substances over the course of the sampling period, data on the surrogate recoveries in the analytical samples, and data on the storage stabilities of the target substances under the study conditions.

5 Risk Assessment

The primary goal of the chemical monitoring of active ingredients is the assessment of the risk they pose to aquatic organisms. To that end, the measured concentrations are compared to the relevant ecotoxicological parameters from standardized toxicity testing (laboratory tests and mesocosm studies). The outcome of the risk assessment can serve as background for triggering a biological monitoring program or for deciding on other steps, such as input reducing measures.

The protection of the aquatic biocoenoses is considered ensured when the measured concentrations for the individual active ingredients to not exceed the applicable **target value**. The target value is a prevention value, calculated from ecotoxicological parameters (especially from laboratory experiments with algae, daphnias, fish, and occasionally chironomids) and safety factors. It is either 1/10 of the lowest NOEC (for explanations see process diagram, p. 5), or 1/100 of the EC_{50}, or the ecologically acceptable concentration derived from realistic mesocosm studies. If registered (approved) plant protection products are properly applied, the target values theoretically should not be exceeded, and the biocoenoses thus not endangered.

If the prevention value or the NOEC for one of the test species is exceeded, no hazard to the aquatic biocoenoses need be automatically assumed because the NOEC and EC_{50} for different active ingredients may differ by various extents, and also may differ greatly for the various species. In addition to the lethal and other direct effects of a single active ingredient that is examined during the registration (authorization) process, however, **other possible effects** in the form of behavioral changes, anomalies of development, lessened fitness and competitive ability, or emigration, as well as the possible presence of **more sensitive animal species,** need to be taken into consideration. The effects of individual active

ingredients may also be increased by combination effects of other stressors or synergies when multiple substances are involved.

The monitored waters, especially in the case of procedure-related monitoring, normally will contain not only individual PPP active ingredients, but **mixtures of active ingredients** resulting from the combination of residues of simultaneously or sequentially applied PPP. For the ecotoxicological evaluation of such mixtures, the total hazard should be calculated using toxic units (LIESS et al., 2001, SÜß et al., 2004a). Assuming a purely additive effect, these are calculated according to the following equation, in which n is the number of active ingredients:

$$\textbf{Total hazard} = \sum_{i=1}^{n} \frac{\text{active ingredient concentration}}{LC_{50} \text{ or } EC_{50} \text{ of the a.i.}}$$

The total hazard computed on the basis of the LC_{50} or an appropriate effective concentration (EC_{50}) indicates that the substance mixture may be toxic at values >1. In the same way the total hazard may be calculated based on the NOEC, whereby values <1 indicate that toxic effects of the substance mixture are not to be expected. For each substance, the values of LC_{50}, and EC_{50} and NOEC for the most sensitive organism, individually, should be used.

Biological monitoring should be initiated if a measurable effect of the active ingredients at the observed concentrations on the relevant organisms cannot be ruled out.

We suggest the following **trigger values** for a biological monitoring program:

- Concentration of any single active ingredient >LC_{50} or EC_{50}
- Concentration of any single active ingredient frequently or longer lasting >NOEC
- Total hazard >1 toxic unit (based on the values for LC_{50} or EC_{50}, respectively)
- Total hazard frequently or longer lasting >1 toxic unit (based on the values for NOEC)

In addition to an assessment of a given PPP's active ingredient's toxicity at its initial concentration, the evaluation needs to take into consideration the fate and degradability of the active ingredient and/or its dilution in flowing waters. Also to be considered is the fact that the residue amounts detected are not always identical with the bioavailable amounts.

A serious impediment to independently evaluating the significance of any residues found is the lack of publicly available lists of the main ecotoxicological parameters. As of this writing, lists are available that were issued by the German Working Group of the Federal States on Water Issues (LAWA), the EC or the German Federal Office of Consumer Protection and Food Safety (BVL) that contain quality targets and target values, but do not include all active ingredients (e.g., LAWA, 1998; UBA, 2005; STRELOKE, pers. comm., 2004).

There is a clear need for current and accessible lists of target values (or ecotoxicologically acceptable concentrations) as well as NOEC and EC_{50} values for the main representatives of aquatic organisms for all registered PPP active ingredients. Such lists would have to have the approval of all relevant authorities.

A prerequisite for estimating the total hazard to aquatic organisms and for a subsequent causal analyses is the complete recording of all relevant active ingredients, especially of those that are highly toxic. If no residue data are available, active ingredient concentrations can be estimated with the help of models, e.g., using drift benchmark values (www.bba.de/inst/ap/publ/d8.pdf).

6 Biological Monitoring

If there is any evidence of a possible hazard to the biocoenosis, especially if the active ingredient concentrations determined by chemical monitoring are above the trigger values listed in Chapter 5, and if reducing inputs, e.g., by means of special regulatory requirements, is not possible or not acceptable, the effect of the PPPs on aquatic organisms should be investigated by biological monitoring (see process diagram, p. 5). This includes the assessment of whether the demonstrated – mostly in laboratory experiments – ecotoxic potential of an active ingredient is actually realized in an environmental compartment and under more realistic or natural conditions. A review of monitoring projects conducted in Germany (HOMMEN et al., 2004) found that in several studies active biomonitoring could not confirm effects that had been expected based on the results of standard tests.

Active biomonitoring is a type of biological monitoring that is suggested as a first step. In active biomonitoring, the potentially contaminated water is tested on single species that usually are raised in the laboratory. In the process diagram presented on page 5, active biomonitoring can play the role of an independent or complementary testing method, or it can be used as an interim step that will trigger ecological (passive) monitoring only if effects are found during the active monitoring effort.

Ecological monitoring is directly oriented towards monitoring the biocoenoses present in an ecosystem and facilitates an assessment of the actual biological condition of the water bodies. Unlike active biomonitoring, this procedure is limited to observation only, and therefore is also called passive biomonitoring. Ecological monitoring is rather complex in terms of the amount of work involved both in collecting and interpreting the data, so it should be triggered only if chemical monitoring, including the subsequent ecotoxicological evaluation or active biomonitoring, has yielded any evidence of impairment to be expected to aquatic organisms, or if there is other evidence of possible damage to the biocoenoses by PPP residues (see Chapters 2, 4, 5 and 6). Ecological monitoring is aimed especially at detecting and recording subchronic and chronic effects.

The presence of disturbing factors such as PPP loads may in some instances only be detected when changes in the biocoenoses are observed. This may be the case for active ingredients such as pyrethroids that are hard to detect and are only briefly present in the system. A review by the German Working Group of the Federal States on Water Issues (LAWA 2000) reports on potential uses of biomonitoring activities for the observation of long-term effects of toxic substances in water bodies, especially in rivers.

6.1 Active Biomonitoring

Active monitoring allows for the determination of the toxicity of active ingredient mixtures in natural waters *in situ* or, after water samples have been taken, in the laboratory.

An advantage of the active biomonitoring approach is the option of working with organisms for which the concentration-effect relationship is known. Other advantages are the short period of time in which screening with acute tests can be conducted to obtain yes/no decisions, the simplicity and reproducibility of the tests, as well as the comparability of the data.

Methods

The test systems listed in Table 1 are examples of tests that fulfill the selection criteria for toxicity tests to be used in active monitoring. The respective ecotoxicological test procedures are described in detail in the guidelines cited in the table and have been summarized by HEGER et al. (1998). They can be employed as individual tests or as a combination of several tests, and can be used in a substance-specific way for herbicides (green algae) and insecticides (water fleas). A major prerequisite for conducting such tests with, e.g., algae and daphnias, is the availability of laboratory raised test organisms. Other possible organisms are rotifers (for this test resting eggs of *Brachionus calcyflorus*, commercially available as Rotoxkits, may be used; PERSOONE et al., 1992) and chironimid larvae (bloodworms) of the genus *Chironomus* (BLÜBAUM-GRONAU, 2004).

Table 1: Organisms for Active Biomonitoring in the Laboratory

Organisms	Ecological role	Test parameter (test)
Green algae *Scenedesmus subspicatus* or *Selenastrum capricornutum*	primary producers	Growth inhibition at 72 h (according to OECD Guideline 201, OECD, 1984a or DIN 38412-9, 1991)
Wasserfleas *Daphnia magna*	primary consumers	Immobilization at 24h and 48h (according to OECD Guideline 202, OECD, 1984b or DIN 38412-11, 1991)

When the toxicity of the environmental sample is to be assessed **in the laboratory**, the "contaminated" water samples should be drawn immediately after PPP applications or other entry events such as run-off, because the acute tests only respond to effective concentrations. Event-related samples can be drawn manually or by automatic samplers. A simultaneous use of water samples for residue analysis and active biomonitoring is best (STÄHLER und PESTEMER, 2003) because that way dose-response relationships and thus causal relationships can be determined. Control samples that are free of contaminants have to be tested in comparison with the water to be investigated. Preferred controls are samples from the study water that were, e.g., drawn prior to the first PPP application, or samples from

a reference water (e.g., uncontaminated upstream regions of flowing waters). Alternatively, the artificial media named in the respective methods may be used. However, in this case negative effects, if any, of other components besides PPPs present in the study water cannot be detected. The test organisms are placed into the water sample to be tested as well as into uncontaminated control water. A sufficient number of replicates for statistical analysis are to be used. A comparison of the test parameters (endpoints) in the "contaminated" and the control samples allows the determination of any effects.

If a contamination above the EC_{50} is suspected, serial dilutions should be prepared by diluting the surface water to be tested with water from the controls. Serial dilutions are to be set up with concentration levels that allow the determination of EC_{50} or EC_{10} values if an effect of more than 50% is observed. The observed toxicity will always be the mixture toxicity from several substances contained in the environmental sample.

Another option in active biomonitoring is the determination of the toxicity *in situ* in the water body by means of "biosensors" or "biomonitors," e.g., when biological-electronic test procedures are used. The largest body of experience with the use of *in-situ* methods can be found in the areas of wastewater treatment and the monitoring of large rivers such as the Rhine river. Mussel monitors are a well-known example. With these, the **continuous** recording of shell movement allows the detection of pollutants in the water. In the same manner, the swimming activity of water fleas can be continuously recorded in a test chamber through which river water is flowing (FENT, 2003).

In-situ methods that have been used in PPP monitoring have involved exposing organisms from the macrozoobenthos (e.g., gammarus and caddisworm) for a certain period of time in cages or microcosms through which water was flowing in contaminated and uncontaminated segments of a water body. At certain points in time, i.e., **discontinuously**, mortality and other parameters were compared (e.g., SCHULZ & LIESS, 1999; SÜß und SCHMIDT 2002). This approach allows for the detection of acute as well as chronic effects.

Generally speaking, active monitoring indicates the presence of biologically active substances or mixtures of substances at effective concentrations through the use of sensitive (surrogate) organisms. The assessment of the effects, if any, on the biocoenoses of the ecosystem that is possible this way is limited.

6.2 Ecological (Passive) Monitoring

6.2.1 General Considerations

The role ecological monitoring plays within the concept presented here is to answer the questions of whether and to what extent PPPs actually cause **changes in the biocoenoses**. We will describe evaluation approaches that may answer the question whether these changes are **acceptable** from a scientific point of view.

The aquatic biocoenosis at any given study site never is static, not even when no xenobiotics are present. Instead, it is characterized by the seasonal developments of the populations and by the impact changing site parameters of any kind have on them. It has to be taken into consideration that in waters with steady prior PPP loads it may be difficult to capture the

effects of individual PPP applications, because a selection of PPP tolerant species may already have occurred, or because the biocoenosis as such may have become tolerant (BLANCK, 2002). In order to be able to recognize a certain condition at a certain point in time or changes in a biocoenosis over time as the effect of PPP entries, it generally is desirable to monitor the condition or changes in populations in PPP exposed waters and **reference waters** without PPP burdens (see Chapter 3) in parallel. For evaluation reasons, only steadily water-bearing waters should be selected. The use of a reference water constitutes a necessity, especially when multiple applications rather than one single application are to be studied.

Sampling methods should be selected according to the different **groups of organisms** (see Chapter 6.2.3) and types of waters. Traditionally, ecological water studies have focused on the organisms of the macrozoobenthos. Zooplankton studies are relevant only in standing, dammed up, or extremely slow flowing waters. When determining the effects of PPPs on aquatic organisms, studies of vertebrates, water plants and algae may be of interest as well, but these investigations will not be described in these instructions.

6.2.2 Sampling Times

The composition of a biocoenosis in a water body exposed to PPPs constitutes an integrating parameter that may indicate entry events in the past. Thus, sampling does not necessarily have to be tied to individual entry events.

Especially in single-substance monitoring, but also in monitoring programs studying novel plant protection and agricultural procedures, effects should be determined by recording the biocoenosis in the exposed and the non-exposed water at several times prior to and after any exposure or prior to and after the switch to the new procedure. If no suitable reference waters are available, effects can be determined only by observing chronological series of data that are generated either through frequent sampling within one year or over a period of many years. This approach, however, will require, on the one hand, comprehensive prior knowledge of the water studied, e.g., the annual course of the population development. On the other hand, very distinctive changes in the coenosis have to occur in order for a causal relationship with any measured PPP contamination to be established.

In single-substance monitoring the sampling should continue until a possible recovery occurs, otherwise until the end of the vegetation period.

If the ecological monitoring is intended to determine the potential effects of entire **plant protection procedures** (e.g., special areas, reduction programs), it seems that a period of at least three years - with at least four sampling times per year - would be suitable to assess the situation following the change in procedures. Ideally, the investigation prior to the change in procedures would be carried out over a similar period of time. However, in most cases this is not possible. On average, three sampling times per year have been recommended for tracking the macrozoobenthos development, with the number of sampling times and the duration of sampling periods varying depending on the studied species (e.g., five sampling times for stoneflies, two times for water beetles, PEISSNER, 1992). In order to determine

what, if any, recovery mechanisms are present during the contamination-free season, additional sampling times should be selected annually prior to the first and some time after the last application of PPPs. Depending on the crop culture, sampling times in late March/early April, late May/early June, early August, or late September/early October may be suitable. This generally should allow for capturing any seasonal aspects. Short-term effects usually cannot be detected this way. However, short-term effects generally are considered acceptable as long as the population recovers within the same season. If stronger temporary effects are suspected, sampling should be conducted more frequently.

When zooplankton is sampled, the natural fluctuation in this group of organisms has to be taken into consideration. Even under natural conditions, large populations may collapse within a few days. Thus, if species abundance is to be compared, rather frequent samplings will be necessary.

6.2.3 Study Objects and Sampling Methods

In order to determine the biological condition, the occurring species or higher taxa and their abundance (density of individuals) and/or biomass have to be quantified. The sampling of the study waters has to be carried out in a **uniform** and **representative** manner. It is especially important to ensure that the sampling of the PPP exposed waters and the reference waters are done in the same manner and to the same extent. In the agricultural landscape, the surface waters to be investigated primarily are **small water bodies**, such as streams, ditches, tarns, and ponds. These usually are characterized by anthropogenic influences such as straightening, water maintenance measures, more uniform habitat structures, lower and fluctuating water depths, stronger temperature fluctuation, and higher nutrient contents than in undisturbed waters. The following methods, that have been adapted to selected compartments of flowing and standing waters as well as to different groups of organisms, have been developed (based on: SCHWOERBEL, 1986; KLEE, 1993; TÜMPLING & FRIEDRICH, 1999; AQEM consortium, 2002a; HERING et al., 2003; DIN 38410-1, 2004).

Suitable and representative sampling stretches (e.g., 100 meters) have to be selected at the investigative sites. It should be ensured that the selected sites will allow for both chemical and biological monitoring.

Samples with an acceptable number of replicates have to be drawn from the **compartments** that are primarily populated in the respective water types (water including water plants, substrate, sediment). The more uniformly a given habitat is structured or populated, the fewer **replicates** (individual samplings) are required. In order to achieve a representative study on the one hand and statistically evaluable results on the other hand, the water segments selected for sampling should be rather homogeneous and typical both for the PPP contaminated water and the reference water. If clearly differentiated microhabitats can be observed within the selected sampling segments, and if they need to be taken into consideration, additional replicates have to be drawn. The number of replicates in individual microhabitats (e.g., areas with gravel, alluvial soil, or detritus cover) depends on the percentage of the investigative area covered by them. For tracking the organisms of the macrozoobenthos in relatively homogeneous habitats, 5 to 20 replicates (samples) for each

compartment and/or each sampling method appear to be sufficient. Zooplankton sampling should be done in 20 replicates.

Macrozoobenthos

The macrozoobenthos is the society of animals, especially invertebrates, living at the bottom of the water that are visible to the naked eye. According to PEISSNER (1992) the macrozoobenthos is especially suited for studies of aquatic biocoenoses because it

- is present in virtually all types of waters with a sufficient number of species,
- is composed of a large number of species with very different requirements (e.g., regarding food, development, distribution, colonization structures),
- includes biological indicators and characteristic species for numerous qualities, and
- provides sufficiently long generation times for long-term studies.

In **flowing waters,** sampling is preferably done using so-called SURBER samplers. Inside the base frame (e.g., 30 cm x 30 cm) that is placed on the stream bottom, sediment is stirred up with a stick or similar device up to a depth of about 10 cm so that the current can wash the bottom organisms into the attached net. For these samplers, mesh widths of 0.5 mm to 1 mm are suitable. Sampling begins at the lowest point and is conducted in an upstream direction.

In **standing waters, sediment samples** can be collected with small bottom grab samplers designed specifically for this purpose (BIRGE-EKMAN dredges) that allow the drawing of a defined sample volume. In more shallow waters, a stable net attached to a strong handle-bar with reinforced frontal edge (shovel sampler according to MACAN, scratcher) is suitable for scraping off the top sediment layer (e.g., 5cm of an area of 0.1m^2). Animals located in the **water body** are caught by dragging a dip net alongside a pre-determined length of the water (e.g., for 3 meters per replicate, with a defined net and mesh size).

It is also possible to collect animals from rocks, dead wood, and plant roots. In that case, the sample size has to be pre-determined as well. Also, the distance to the bank at which samples are to be taken is to be determined. If the number of (spatial) replicates is identical for all methods, the numbers of animals found per replicate can be summarized for the statistical evaluation. This will enable a methodically reproducible sampling with respect to area or volume. The increasing plant and algae growth at the bottom and in the water as the year progresses may cause a problem, especially in standing waters, because it may hinder sediment sampling and the dragging of nets.

The following sampling methods may be used to answer specific questions, but they will not be discussed in detail:

- Exposing substrate bags (e.g., net bags filled with nylon coils) or basket samplers in order to measure colonization

- Using drift traps (long, tube-like nets) in flowing waters to assess organism drift as caused by PPP contamination
- Setting emergence traps for the area-related registration of insects hatching from the water

For **processing**, sediment samples should be rinsed in sieves with a set mesh width (e.g., 0.5 mm) in order to remove the fine sediment. If larger amounts of plant material are caught in the nets, they need to be thoroughly rinsed on-site in a large tub so that any attached animals are washed off. The rinsing water then is to be concentrated appropriately by means of filtration. Living samples are to be transported as soon as possible and in coolers. They should be stored at about 4°C. Extended storage times may cause losses due to mortality and predator activity. If large sample volumes cannot be sorted immediately, it is helpful to fix the completely sieved sample material prior to sorting (pour out water, top off with at least 80% ethanol, possibly replace once). However, sorting the living samples makes it easier to find all animals and allows for the assessment of their condition. Generally, sorting by hand, especially of sediment samples, is very time consuming, even if sieve fractionation is applied. Species identification, just like sorting, usually should be done in the laboratory. Sorting and identifying fauna on-site as part of methods that involve collecting animals over a definite period of time is not recommended, because sites with a high density of species or individuals may be underestimated.

Zooplankton

The zooplankton includes all animals floating in the water body that exhibit only little spontaneous movement, especially protozoa, rotifers, crustaceans and the larvae of other groups of animals. They can be retrieved from the water using tumblers, bottles, nets and other devices with a mesh width upwards of 10 µm.

For quantitative work, defined sampling using **water sampling bottles** has proven useful. Different types of water bottles can be used depending on the water body and the task at hand. In small and shallow standing waters, using 3 liter water bottles attached to a telescopic handle is the optimum method. When regularly sampling a site it is important to ensure that the sampling bottle is moved slowly and uniformly to a certain depth and, after a short calming period (3 seconds), is lifted carefully to the water surface, opening facing up. This procedure has to be maintained even as the macrophytic vegetation increases. Deeper waters can be sampled with special column samplers that allow the "cutting out" of a defined water column.

Once drawn, the sample is filtrated with a **plankton sieve** and subsequently partially fixed in 70% ethanol for a few seconds. Using a wash bottle filled with a 2% formalin solution, the organisms contained in the filtration residue can be transferred to sample vials. By selecting specific mesh widths for the plankton sieves, the desired groups of organisms can be selectively filtered from the sample. Mesh widths between 100 µm and 150 µm will hold back most individuals of all species and developmental stages of water fleas, copepods, ostracods, and planktonic insect larvae, while protozoa, rotifers, algae, and detritus will mostly pass through the net. At a mesh width of 50 µm rotifers will be held back as well, but at the same time detritus will interfere with the evaluation and samples will have to be sorted.

In processing the zooplankton as well as the macrozoobenthos, the material can be **subsampled** according to a pre-determined scheme if very large numbers of individuals are present.

Identifying the species and higher taxonomic groups (e.g., genera, families) and determining the number of individuals is done with the help of a stereomicroscope, if necessary also a microscope, and requires extensive expertise and experience. The AQEM consortium (2002b), for instance, lists 90 volumes essential for species identification in Germany. Another extensive list is contained in MAUCH et al. (2003).

Ecological monitoring results are presented as **lists** of species or higher taxonomic groups of the macrozoobenthos and zooplankton, including the densities of individuals per studied unit and possibly developmental stages (e.g., larval or adult stages of insects) per replicate, compartment, study method, and sampling date.

6.2.4 Recording of Accompanying Parameters

For evaluating the biological results, further water and environmental parameters in addition to the general site parameters listed in Chapter 3 are important. These may change over time and should be recorded at the biomonitoring sampling times or continuously (possibly automatically). These include, but are not limited to:

- **Hydrogeological parameters** (such as substrate, water depth, flow rate, water withdrawal or damming)
- **Physico-chemical water parameters** (such as temperature, pH, conductivity, salinity, oxygen content, DOC, nutrient content), and
- **Biotic parameters** (such as macrophytes, density of algae, detritus cover).

This will allow recording natural as well as anthropogenic influences (e.g., nutrient input via fertilizers, defoliation, and mowing). The degree of coverage for macrophytes has to be evaluated onsite for the water body and the water surface separately. The density of algae that play a role as food can be assessed via pigment measurements. The presence of other important species not considered in biomonitoring, such as predatory fish species, should be observed. A summary of the parameters that are to be recorded is listed in the Appendix.

6.2.5 Evaluation, Causal Analysis, and Assessment

In evaluating the data generated by ecological monitoring, the goal is to determine to what extent the populations and biocoenoses differ spatially (between exposed and non-exposed sites) and/or temporally (prior to and following exposure). Against the complex background of all abiotic and biotic factors, judgment should be made as to whether any differences or changes detected in the aquatic biocoenoses were caused by PPP exposure. The active ingredient burden can be determined via chemical monitoring (see Chapter 4) conducted in parallel to the biological monitoring, or alternatively can be estimated using models.

From appropriately summarized biomonitoring data, suitable **biological parameters and indices** may be calculated. These serve to characterize the biocoenoses, to indicate similarities between biocoenoses of different sites or from different time periods, and they can be used to assess the condition and any disturbances of biocoenoses caused by various stressors. The parameters and indices traditionally used are: number of species, abundance, dominance structure, indices of diversity and similarity, evenness, distributions of habitat types and feeding, and species deficiency (see, e.g., MÜHLENBERG, 1993; BOHN et al., 2003). In addition, several multi-metric procedures have been developed and tested for the assessment of primarily the coenoses of flowing waters (e.g., ROLAUFFS et al., 2003; BOHN et al., 2003; BÖHMER et al., 2004; OFENBÖCK et al., 2004).

If possible PPP effects are to be determined, it is helpful to use parameters that take into account the percentage of **PPP endangered species**. These are the taxa that on the one hand are very sensitive to toxic substances (including PPP) and on the other hand are distinguished by low reproductive and recolonization potentials (WOGRAM & LIESS, 2001; OHE & LIESS, 2004). Evaluation approaches based on these specific "species at risk" have been described in the literature (LIESS & OHE, 2005), but the accompanying data pool is not freely accessible. However, it still is possible to determine the percentage of sensitive taxa, e.g., according to WOGRAM & LIESS (2001).

While the absence of individual species as compared to the reference site (species deficiency) cannot necessarily be attributed to the use of PPPs, the presence of taxa at risk indicates that in spite of the – often only temporary – PPP burden, survival or recolonization has been possible.

If the data collected are sufficiently abundant, they should be evaluated using multivariate statistical methods. Cluster analysis and principal component analysis (PCA) are examples of such methods.

A great deal of expertise and experience is required to correctly identify the effects of PPP burdens and to demonstrate a causal relationship between measured and estimated PPP contamination and changes or differences in aquatic biocoenoses. A detailed account of this procedure is beyond the scope of these instructions.

If PPP effects have been demonstrated, the question is whether these effects are **acceptable**. To answer this question one has to take into consideration the extent, time, and duration of the observed effect. Council Directive 91/414/EEC in its Appendix VI does not establish any evaluation criteria. A criterion for acceptability, inspired by the objectives of the EU Water Framework Directive (EC, 2000), would be the requirement that a water body should be in "good" condition, even when exposed to PPPs. This means that the abundance, composition and diversity of the taxa present, as well as the percentage of sensitive taxa, deviate only "slightly" from that found in non-exposed reference waters. ROLAUFFS et al. (2003) suggested a maximum deviation of 25% compared to the reference state as a criterion for the (saprobial) quality standard of "good."

Changes in aquatic coenoses that are reversed within a maximum of one vegetation period, either through recovery of the population, immigration from non-exposed water segments or other areas, are considered acceptable. ROTHERT (1992) deemed any effect acceptable from

which the affected populations were able to recover before the next PPP exposure. He also suggested, as is done for terrestrial coenoeses, not only to assess the species composition, but also the maintenance of the function of the aquatic biocoenosis as a criterion for acceptability.

Maintaining a "steady size" of populations occurring in a study water cannot be considered a suitable criterion in monitoring. On the one hand, due to developmental cycles only few of the aquatic species at the site will be "steadily" present, and on the other hand, extreme sampling expenditures are associated with proving the steady presence of species of low abundance.

7 Concluding Remarks

The instructions presented here are intended to provide a basis for a better understanding of the requirements and issues involved in chemical and biological monitoring in small water bodies in the agricultural landscape. From this basis specific monitoring concepts have to be developed, taking into consideration the specific questions and conditions of a given project. The Institute for Ecotoxicology and Ecochemistry in Plant Protection of the German Federal Biological Research Centre for Agriculture and Forestry can provide assistance with that.

The monitoring described here registers the condition or tracks changes in the condition of waters with respect to their PPP loads under realistic conditions and to the expression of their aquatic coenoses. Chemical monitoring yields concrete residue values that then are ecotoxicologically evaluated. A possible pitfall of this approach is that residue analysis may detect active ingredients that are not bioavailable *in situ*, which in turn may lead to overestimating the present risk based on toxicity values derived with standardized water. A second problem is that results that are mainly obtained in laboratory studies cannot be directly applied to biocoenoses of ecosystems due to the different degrees of PPP sensitivity of different species and the occurrence of indirect effects.

The resulting assessment gaps can be closed only by biomonitoring. Active biomonitoring may present a cost-efficient approach to directly assessing the effects of PPP active ingredients in field waters. However, only representative (surrogate) organisms and mostly direct, acute effects will be assessed. To avoid false-negative estimates of the environmental risks it is necessary to use sensitive test organisms, to investigate chronic toxicity, and to consider sublethal parameters as well.

Ecological (i.e., active biological) monitoring is far more costly and also hard to evaluate, but it is directly targeted at the entity to be protected, namely the "aquatic biocoenosis." Due to the large number of environmental impacts on the biocoenoses it is difficult to selectively investigate PPP effects. Optimum monitoring design is a prerequisite for success. But even under less than ideal conditions, e.g., if an unfavorable site were selected or only limited studies were possible, ecological monitoring will document a given condition at a given time that possibly can be evaluated in the long-term in year-to-year comparisons.

Generally it will be necessary to find a compromise between as comprehensive as possible data collection and appropriate and acceptable expenditures.

As a consequence of the monitoring effort, it should be assessed whether the use of PPPs in the studied plant protection and agricultural procedures or in the establishment of special areas will have an acceptable or unacceptable environmental impact. In addition, it should be possible to verify the success of reduction programs, changes in agricultural practice, or efficiency of consulting activities.

If unacceptable PPP effects are demonstrated and confirmed, additional studies (e.g., analysis of dose-response relationships with mesocosm experiments) should be conducted and risk reduction measures sought out.

The creation of a central database of the collection of all the data obtained in chemical and biological monitoring should be considered.

Monitoring data can be used to answer **other questions** besides the original study objectives. For example, values obtained from chemical monitoring can be used to verify the exposure estimates from the registration (authorization) process and appropriate models by comparing the PPP loads measured under realistic conditions with the calculated amounts. Results from chemical and biological monitoring studies that were conducted in parallel can be used to validate risk assessment results. This is especially true for active biomonitoring, if the same test species and endpoints are used that are required by the relevant authorization guidelines. In general, a broader database on which risk management decisions could be based would be established. Results could also be used to determine whether PPP usage limitations are appropriate, unnecessarily restrictive, or insufficient (HOMMEN et al., 2004). In addition, data obtained from a well-characterized (representative) area could be used to develop regionally specific scenarios that may serve to improve the design of higher tier studies (see SETAC, 2003).

When planning a monitoring study, the potential secondary uses of the results should be taken into consideration, so that all necessary accompanying parameters will be recorded as well.

8 Summary

There are various reasons that may make it necessary to conduct scheduled and regular investigations of the environmental burden caused by plant protection products (PPPs) and their actual effects on aquatic organisms or biocoenoses under application conditions common to agricultural practice. The reasons for such monitoring efforts include studies done as part of the PPP registration process, the establishment of a special area, or the conduct of programs for the reduction of plant protection.

Practice-oriented instructions for the planning and execution of chemical and/or biological monitoring programs for PPPs in small water bodies in the agricultural landscape were developed to establish a general basis for a well-founded approach to the issues involved.

In most cases, **chemical monitoring** will be the initial step. Depending on the goal of the monitoring program, representative or more strongly exposed study sites have to be

selected, as well as methods for targeted sampling, taking into consideration the main entry route and the range of active ingredients to be monitored.

The active ingredient concentrations found have to be evaluated by comparing them to certain limits and trigger values (target values and other ecotoxicological parameters such as NOEC or EC_{50}), depending on the task at hand. Appropriate methods for the **assessment of the hazard** posed by individual active ingredients and mixtures of active ingredients are presented. If chemical monitoring indicates a potential hazard to aquatic biocoenoses and PPP entries cannot be reduced, biological monitoring is the logical next step.

In **active biomonitoring,** organisms of a single species, usually laboratory raised, are exposed to the environmental compartment to be tested, e.g., the potentially contaminated surface water. This can be done either *in situ* or in the laboratory. In doing so, the effects of the individual substances or of mixtures of substances are determined, mostly with acute tests, either continuously or discontinuously.

Ecological monitoring, a passive form of biomonitoring, is directly oriented towards monitoring the biocoenoses present in the ecosystem and facilitates an assessment of the actual biological condition of the water bodies. The comparison of coenoses at PPP exposed sites and non-exposed reference sites is of central importance. Methods for the sampling of macrozoobenthos and zooplankton organisms in various compartments of standing and flowing waters are described, and methods for the assessment of the current condition and for detecting disturbances based on various biological indices are recommended. Tracking the percentage of PPP endangered species is especially suitable for the detection of potential PPP effects. The **causal relationship** between changes in the condition of a given biocoenosis or of individual organisms and PPP loads, as well as the **acceptability** of the detected effects, have to be evaluated against the backdrop of all abiotic and biotic site factors.

The **use of monitoring data** for the verification of exposure and risk assessment models and for risk management in general is discussed.

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10 Appendix

Table 1: List of site, usage, PPP application, water property, and method parameters to be recorded in chemical-biological monitoring studies

Parameter to be recorded	When to record
General site information	
City, zip code/postal code	once
Exact location*	once
Size of catchment area or relevant agriculturally used area (ha)	once
Soil class, texture	once
Mean slope towards water (%)	once
Presence of drainage facilities	once
Other area-specific characteristics	once and whenever changes occur
General information on agricultural use	
Name, address and phone no. of farmer(s)	annually
Crops grown in neighboring fields, orchards (left and right)**	annually/when changed
Row spacing in orchards, hops (cm)	once
Width of unused buffer strip (cm) left/right**	annually
Type of plant community in the buffer strip, left/right**	annually
Type and time of work done in the buffer strip, left/right**	at every occurrence
Type, amount (kg/ha) and time of fertilizer application	at every occurrence
Time and type of soil cultivation/tillage	at every occurrence
Information on plant protection product application	
Date of application	at each application
Time of application (start/end)	at each application
Plant protection product(s) – exact name!	at each application
Plant protection product – active ingredient(s)	at each application
Application rate (kg/ha or l/ha)	at each application
Water application rate (l/ha)	at each application
Treated area (ha) left/right**	at each application
Application device (year of calibration)	at each application
Spraying width (cm)	at each application
Hight of spray boom (cm)	at each application
Number of nozzles, nozzle type, drift reduction (%)	at each application
Driving speed (km/h)	at each application
Spraying pressure (bar)	at each application
Other settings/devices	at each application
How many rows were sprayed facing away from the water?	at each application
Crop height (cm)	at each application
Crown height, for trees (cm)	at each application
Crop growth stage according to BBCH	at each application

Parameter to be recorded	When to record
Soil condition	at each application
Wind speed (m/sec) (anemometer)	at each application
Wind direction at time of application	at each application
Air temperature (°C)	at each application
Humidity	at each application
Cloudiness	at each application
Daily precipitation (mm) post-application	for each application
Height of vegetation in buffer strip (cm) left/right**	at each application
Density of vegetation in buffer strip (cm) left/right**	at each application
Height of bank/shore vegetation (cm) left/right**	at each application
Time (h) between application an subsequent precipitation (mm)	at each application
Information on the water body	
Type of water	once
Distance between upper edge of bank and field border (cm) left/right**	annually
Type of plant community of the bankside/littoral vegetation, left/right**	annually
Direction of flow (degree)	once
Straight running or winding water?	once
Hydraulic-engineering characteristics (weirs, bank reinforcement...)	once
Date of the last prior weed removal or complete dredging	once/when conducted
Water body width, upper edge to upper edge (cm)	once
Water width (cm)	for biological and chemical monitoring
Water depth (cm)	for biological and chemical monitoring
Flow rate (m/sec)	for biological and chemical monitoring
Date of damming or withdrawal of water	continuously
Physico-chemical and biotic water parameters	
Temperature (°C)	for biomonitoring
pH	for biological and chemical monitoring
conductivity (mS)	for biomonitoring
Oxygen content (mg/l)	for biomonitoring
BSB_5 (mg/l)	for biomonitoring
Nitrate content (mg/l)	for biomonitoring
Nitrite content (mg/l)	for biomonitoring
Orthophosphate content (mg/l)	for biomonitoring
Ammonium content (mg/l)	for biomonitoring
Iron content (mg/l)	for biomonitoring
Chloride content (mg/l)	for biomonitoring
Total water hardness (°)	for biomonitoring
DOC (mg/l)	for biological and chemical monitoring
Pollutants/xenobiotics (other than PSM)	for biomonitoring
Turbidity	for biomonitoring

Parameter to be recorded	When to record
Odor	for biomonitoring
Type and percentage (%) of water bottom substrates	for biomonitoring
Type and height (cm) of the organic substrate cover (detritus)	for biomonitoring
Degree of coverage by macrophytes in water (%)	for biomonitoring
Degree of coverage by macrophytes at the water surface (%)	for biomonitoring
Presence of algal bloom	for biomonitoring
Type of plant community of the bankside/littoral vegetation, left/right**	annually
Monitoring information	
Position of sampling sites for chemical monitoring*	for chemical monitoring
Date and time (start/end) of sampling(s)	for chemical monitoring
Positions of Petri dishes for application verification	for chemical monitoring
Position of exposure sites for active biomonitoring*	for active biomonitoring
Start and end (date and time) of exposure	for active biomonitoring
Position of sampling sites for ecological monitoring*	for passive biomonitoring
Date and time of sampling(s)	for passive biomonitoring
Exact description of method used	for all monitoring measures
Record keeper (name, signature)	for all protocols, logs

* Area map with field designations, agricultural use information, direction of flow, sampling sites, compass points, and scale

** left and right as viewed in direction of flow

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