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Aquatic dissipation studies for product registration

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INTRODUCTION

The registration of an aquatic herbicide is a lengthy and complex process. Approximately 140 distinct studies are required by the U.S. Environmental Protection Agency (EPA) to meet the registration requirements. These studies generate data on efficacy, ecological effects, human health, residue chemistry, and environmental fate processes. Also, unique to aquatic registrations, are requirements for fish and shellfish tolerances, tolerances for irrigated crops, risk to swimmers, and setback distances for potable water intakes. The requirements are based on the maximum contaminant level (MCL) determined by the EPA for the compound in question. All of this research must be conducted under Good Laboratory Practices (GLP), established by the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) in 1989.

GOOD LABORATORY PRACTICES

Issued in 1989, the EPA GLP guidelines provide guidance and requirements for research facilities pertaining to study design, maintaining data, record keeping, personnel training and duties, reporting, and archiving of information for pesticide research. The guidelines are based upon those issued by the U.S. Food and Drug Administration for the development of pharmaceuticals. Facilities conducting research under GLP are certified by the EPA, and undergo periodic inspection of both facility and research projects. There are fines and potential criminal charges for violations, based upon severity and intent.

With all these requirements, it's not surprising that the cost of registration usually limits registration of new aquatic herbicides to existing product lines, where the majority of the research has been conducted in support of major uses for crops or forage, and the toxicity profile and a market potential are favorable to an aquatic use registration.

When an aquatic registration is pursued, one of the last studies to be conducted is a field aquatic dissipation study. This study investigates the degradation of the final-use product under natural conditions, and confirms and bridges data generated from laboratory studies to real-world conditions. In addition, it provides input into such things as risk assessments which determine final exposure limitation and use restrictions for the water.

All studies conducted under GLP have EPA-issued guidelines that indicate how the study should be conducted, what data should be collected, and how the information should be reported. Unfortunately for aquatic dissipation, the guidelines have been based upon sediment/effluent data collection guidelines, and as such do not completely cover the scientific needs for registration.

Under GLP, an individual is designated the Study Director. This is defined in the regulations as a scientist who has the expertise and experience for responsibility of the study. This includes design, conduct, data analysis, data interpretation, reporting of results, and the single point of study control.

An additional and important component of GLP studies is the Quality Assurance Officer, usually referred to as the QA. The QA officer works independently to determine that all aspects of the study are conducted according to the GLP regulations and that the derived data is accurate. This is conducted by ongoing audits beginning with the protocol, observation of selected events in the field and laboratory, and finally a full audit of the final report. In this audit, the QA must be able to trace each factual statement or point of datum back to its source through the paperwork trail.

To do this, through the course of the study, a "study file" is created and maintained, that keeps each original document that has been produced. The requirements are so rigid that if the wind speed was recorded on a scrap of paper, that original scrap must be present in the study file, along with the appropriate document identification, signature, and date of the person recording those data.

Each facility or organization involved in the study maintain their own study file, which is later compiled into the final version. Additionally, multiple QA officers can work on various aspects of the study.

All personnel that participate in a GLP study must be GLP-trained and have the education and/or experience to perform their role. Their records must be documented in the study file, including their biographical information, job description, training records, and any license or certification they may hold.

STUDY DESIGN AND SITE REQUIREMENTS

A GLP study begins with the study protocol. This is a document that precisely outlines the goals and conduct of the study. It defines how the study is to be performed, the methodology for each task, and how data will be interpreted and reported. Failure to comply with the study protocol can result in the study being rejected by the EPA, and possible fines or criminal charges if it is determined there was malicious intent. Methods exist to amend the protocol, or note actions that deviated from the protocol guidance due to circumstances or error.

Field dissipation studies, whether aquatic or terrestrial, are unusual in that they require a worst-case scenario, not a mimic of direct actual use, as one would see in efficacy studies. For an aquatic study, ponds are preferred over lakes to meet these requirements. Ponds are preferred for

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conducting aquatic dissipation studies for the simple fact it removes dilution from the equation. Depending on anticipated use patterns, however, additional investigations might be conducted in canals or rivers to address flowing water uses. Constructed ponds are ideal because they usually have regular dimensions, which make measurements and calculations more precise.

The worst-case scenario means that the maximum anticipated label rate needs to be applied, and for aquatics, this means treating to obtain the maximum water concentration in the entire pond. In practice, the entire pond is treated, not just a portion as the label will usually indicate. Typically, the treatment is usually calculated to obtain 110 to 120% of the maximum rate when possible, in order to assure the maximum rate is represented. Failure to achieve the maximum labeled rate will often result in rejection of the study.

Another component of worst-case is that there should be no target weeds in the pond, and in fact little or no vegetation is preferred. Again, this is to obtain the maximum water concentration without the possibility of plant uptake or intercept. To relate to terrestrial dissipation again, those studies are conducted on bare soil plots.

In selecting a site to conduct the study, the guideline criteria require that one chooses at least two locations representative of the geographic areas in which the registered product will be used. Within the confines of the extent of the weed population, sites are selected to represent different climate conditions, possible use patterns, and other variables that differentiate the sites. Sometimes there are state regulatory requirements. For example, California requires an in-state site for any pesticide to be registered there.

Another factor in site selection is the requirement that the active ingredient cannot have been used in or near the site for 3 yr prior to the study. For aquatics, one needs to consider the potential for runoff from terrestrial use. This is especially important for aquatic dissipation because, as stated before, often the active ingredient under investigation has been previously registered for a terrestrial use.

SITE CHARACTERIZATION AND SETUP

A variety of information is collected prior to use of the pond. Samples of sediment and water are collected for physiochemical characterization. A 3-yr history of the pond and adjacent land is collected, paying particular attention to any pesticide use that might bias the study or complicate the laboratory analysis of the samples collected. The pond dimensions are measured, including multiple depth transects, in order to calculate the volume as accurately as possible. The ponds distance and direction from permanent landmarks is also recorded. Any water overflow or spillway is sealed to prevent loss of water from rainfall, and the pond level is adjusted to achieve the maximum possible volume while still allowing for some fill from rain without overflowing the pond. Fixed sampling locations are determined within the pond, usually based on pond dimensions and depth, so as to best represent the pond in three dimensions. Usually a minimum of three locations are chosen to assist in statistical analysis of the data, allowing for comparison of average concentrations across level and depth, and to provide a means of identifying outliers in the results. More sampling locations might be needed if the pond is very large or of unusual shape or irregular depth. These locations are marked with stakes or anchored floats.

A variety of instrumentation and markers are placed in or near the pond prior to application. The data collected from this instrumentation, as well as other readings of environmental data, are used to demonstrate the health of the pond through the course of the study, track seasonal variations, and capture unusual conditions that might impact the natural degradative processes. The data are also vital as inputs into computer models for simulation of other scenarios. These instruments can include an automated weather station that measures at a minimum air temperature and rainfall, but also usually solar radiation, humidity and wind speed and direction. Within the pond, a site depth gauge is installed. Because the pond might not be under constant observation during the course of the study, automated instruments are installed too. These can include recording depth sensors and temperature recorders at multiple depths, usually at the depths of sampling. These automated sensors are set to record on short intervals, usually every hour to 6 h. Although automated water quality monitoring is useful, these units require maintenance every few days to avoid sensor fouling during the intervals between sampling. Instead a portable "dip and read" monitor can be used to make spot measurements during sampling. At each sampling event, additional environmental data can be collected, which could include spot readings of water quality at various depths, and the depth of extinction of visible and ultraviolet light.

All this instrumentation again falls under the GLP regulations, which require a historical maintenance log, written procedures for their operation and data collection, and traceable calibration records.

APPLICATION OF THE TEST MATERIAL

Application of the test material should be by a technique that will be recommended by the final product label. If multiple sites are in use, different application methods listed on the label can be used, but the primary method should be utilized. The product (test material) used is usually a final product formulation. The test material is maintained under a chain of custody that can trace it from the applicator's hands back to the point of manufacture. Side studies related to the test material can include storage stability, tank mix stability, and purity analysis of the production lot.

Generally, application in a GLP study must be made by someone trained and operating under the GLP regulations. Given that aquatic applications require specialized equipment and skills, the EPA has accepted it be performed by a licensed aquatic applicator operating under the supervision of GLP-trained personnel.

The application equipment must undergo calibration testing prior to use. Calibration testing involves multiple collections of water from each output nozzle to determine

the output rate, and that each nozzle is operating with little variation of all others in the case of multiple outputs. Test runs utilizing water are made to determine speed, pump settings, and other information that are recorded and will be used for the actual application. In most cases the application should be planned to cover as much of the pond surface area as possible to provide for an even application. Following application, the pump system is purged with clean water and discharged into the pond to ensure that no tank mix remains in the tank, pump, and/or plumbing of the application system.

SAMPLING PROCEDURES

The collection of water and sediment samples begins 24 h prior to application, and continues for 12 to 18 mo, or until the degradation of the active ingredient is well-demonstrated, and the occurrence and decline of the major degradation products is defined. A major degradation product is any compound resulting from the breakdown of the parent that equals 10% or more of the applied parent compound.

Samples for residue analysis typically range from 20 to 100 ml for water, and 100 to 250 mg for sediment, depending on the requirements of the analytical method. Because there is often no untreated control pond associated with the study, bulk samples are collected prior to application to meet all the needs of the analytical laboratory for creation of spike and control samples. These samples can consist of several liters of water and 2 to 5 kg of sediment, because it is necessary to err on the side of caution.

The sample schedule is weighted to best represent the expected decline curve of the parent, so as to obtain the best-calculated half-life. The expected rate of degradation is usually obtained from laboratory and field efficacy studies. This means that sampling is heavily weighted at the start of the study. Often samples are collected within hours of the application, and daily or multiple times a day for the first few days. This is to capture both the early occurrence of degradation products, as well as to determine that the required in-water concentration has been achieved. Depending on the expected degradation rate of the test material, a typical sample schedule might be: 1 d (preapplication) and then following application at 3 and 6 h, 1, 3, 7, and 14 d, and then monthly to 1 yr postapplication.

At each scheduled sampling event, samples of water and sediment are collected and chilled or frozen as soon as possible following collection. Freezing is the most common method of storage unless analysis is going to be conducted very soon after sample collection, or if freezing might compromise the integrity of the sample in some manner. The samples are usually stored in coolers on ice or ice packs until refrigeration or freezing. The type of container to be utilized is usually determined during the development of the analytical method for determination of residues. Glass is the most common material, because it provides less potential for any material to bind to the container, which can occur with plastic.

Water is collected utilizing a discrete grab sampler or peristaltic pump, to obtain a sample from a given depth. Water is usually sampled at multiple depths at each location, such as one-fourth, one-half, and three-fourths of the depth of the water column at each location, measured from the bottom of the pond. In deeper systems, such as lakes, depths will be chosen based on factors such as water movement and temperature gradients. If the water samples require any initial preparation, such as acidification to stabilize the compounds of interest, it is performed in the field as part of the sampling process. It is preferable to collect multiple replicates in small containers versus a single large container, so that the integrity of each replicate is maintained during storage and laboratory analysis as well as provide a backup in case of breakage during storage or transport.

Sediment is collected utilizing a coring device, grab sampler, or scoop sampler. It is usually collected to a total depth of 2 to 6 cm, and might be collected in discrete depth intervals if deemed necessary by the environmental fate characteristics of the test material.

The sediment samples are usually drained of excess water in the field before being sealed in containers for freezing. With the additional burden of record keeping required, preprinted data forms and sample labels are usually utilized.

All equipment used for sampling should be rinsed with clean water between each sample, and cleaned and decontaminated between each sampling event.

Following each event, all samples are stored in temperature monitored freezers until transfer to the analytical laboratory. The transfer is usually accomplished by overnight shipping with dry ice, or via commercial frozen transport services catering to GLP research.

An additional operation that takes place at least once in the course of the study is the creation of field spikes. At one or more determined sample events, multiple measured aliquots of untreated water and sediment will be spiked with either the parent or one of the degradation products, to create a sample with a known volume. Alternatively, travel spikes can be created at the analytical laboratory, frozen, and shipped to the field site personnel. The travel spikes will be thawed on the specified sampling event and taken to the pond. The field or travel spikes are then treated as the other samples, frozen, and shipped to the analytical laboratory.

SAMPLE ANALYSIS

The laboratory that analyzes the water and sediment samples might be the sponsoring company, or a contract laboratory. The samples are analyzed by a method that has previously undergone development, testing, and validation under GLP regulations.

When receiving samples, the laboratory logs them into their storage system, comparing each sample to the accompanying transfer paperwork. Sediment samples will eventually be prepared by running the frozen sample through a soil grinder in the presence of dry ice, to maintain it in a frozen state. This results in frozen powder or pellets.

The water and sediment samples are analyzed, utilizing residue methods developed and tested for both water and sediment. These methods are more accurate and can determine much lower residue levels than the enforcement

methods that are also required for compliant sample analyses. The methods specify step-by-step instructions for preparing and analyzing the sample, and include laboratory blanks and spiked samples created from untreated sample material to provide quality control. It is common for the Study Director to specify a certain percentage of samples to undergo multiple analyses to demonstrate that the results are repeatable.

The field or travel spikes created earlier will be analyzed with or as near as possible with the field samples collected on the day the spikes were utilized. With a predetermined concentration, the purpose of these samples is to prove that the field samples did not experience any loss or transformation of the compounds of interest during shipping or storage. Due to the importance of this, it's becoming more common to utilize travel spikes created by the laboratory. The laboratory conditions allow for more precise preparation, and an additional replicate of each sample can be analyzed immediately so that the exact baseline concentration is established.

FROZEN STORAGE STUDIES

An additional study that is often run concurrent with the field dissipation is a frozen storage stability study (FSS). In this study, clean water and sediment collected from the study sites is spiked to create known concentrations of the parent and degradate compounds. The FFS samples are analyzed routinely, sometimes up to 2 yr postcreation. The purpose of this study is to determine the stability of each compound in water and sediment during extended frozen storage times. The FSS study must be conducted at least as long as the longest time any sample from the dissipation study is in storage until final analysis. Because later analysis of the residue data might result in samples being reanalyzed, this time period can be greater than the length of the field study. The FSS data is often partially presented in the report of the field study, and later be issued in its own report.

REPORTING AND RECORD KEEPING

The final report for the study is a detailed documentation of methods, results, and interpretation of the results along with conclusions. It can include appended reports from the field investigators and analytical laboratory, and is not uncommon to run several hundred pages. It is essentially a legal document with a specified format, information that must be included, and has undergone audit to determine its accuracy.

The final report is audited by the QA officer, with each factual statement traced back to the original documentation in the study file. For large data sets, such as analytical data or electronically captured data such as temperatures, usually 10 to 20% of the data points are traced at random. The QA officer issues an audit report listing the findings, and each must be addressed and documented before the final report can be issued.

After submission of the report to the EPA, the study file must be indexed and placed into permanent archives. The archives are usually maintained by the sponsoring company or a data storage company they have contracted. Again, a chain of custody must be maintained to track the transfer of the study file. In addition, the remaining portions of all samples generated in the course of the study must be maintained until the study director approves of their disposal. This can happen any time from immediately after issuing the final report, to after the EPA has accepted and approved of the study report.

Finally, at any time. the study can be audited by EPA. This is usually part of their GLP inspection process, where an EPA Inspector will visit the testing facility. The EPA inspector will identify a completed study they wish to audit prior to the on-site inspection, and ask that the study file be transferred to them shortly after notice.

During this audit they will determine that the facility and required records meet GLP requirements, including equipment logs, personnel records, labeling of chemicals, written procedures, facility maps, yearly project lists, and numerous other records. They will also randomly select an in-progress study and review all documentation and data currently available.

Aquatic dissipation studies are a major investment in money to register a new aquatic herbicide, and can take an average of 2 to 3 yr to complete once planning has begun. The requirements imposed by GLP regulations generally double the time and cost involved for the field portion and can triple the analytical laboratory costs. It's a large investment into a small market, which is why most often it's an already-registered herbicide that has proven unique properties that is promoted for aquatic uses.

LITERATURE CITED

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