Plant Metabolism Studies:

Options for Plant Cultivation

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Contents

Introduction

Regulators across the world are concerned with ensuring that any residues left in or on a crop after application of a plant protection product (PPP) present minimal risk to the health of humans and animals.

To achieve this, regulators need information on the identity of the residues and the levels of residues remaining in or on a crop in order to assess dietary risk and set maximum residue levels (MRLs). The testing approaches used are harmonized across most countries worldwide, focusing on the Organisation for Economic Co-operation and Development (OECD) Test Guidelines (TGs) for pesticide residue chemistry,[1] which are listed in Table 1.

Table 1: OECD Section 5, TGs for Pesticide Residue Chemistry[1]

TG	Title
501	Metabolism in Crops
502*	Metabolism in Rotational Crops
503	Metabolism in Livestock
504*	Residues in Rotational Crops Limited Field Studies)
505	Residues in Livestock
506	Stability of Pesticide Residues in Stored Commodities
507	Nature of the Pesticide Residue in Processed Commodities – High Temperature Hydrolysis
508	Magnitude of the Pesticide Residues in Processed Commodities
509	Crop Field Trial

*Additional draft guidance has been produced to provide further detail on the conduct of these studies.[2]

This eBook focuses on the laboratory-based plant cultivation methods that underline the success of OECD crop metabolism studies, namely, TGs 501 and 502.

Types of Crop Metabolism Studies

It is important to assess the metabolism of an active substance within a crop, in order to establish its transformation pathway and to identify and characterize the resulting metabolites and degradation products. Knowledge of the levels of these residues, combined with data on their toxicity, informs risk assessment and the MRLs set.

There are two types of laboratory-based crop metabolism studies:

- Metabolism in Crops (TG 501)[3]
- Metabolism in Rotational Crops (TG 502)[4]

Both studies require the use of a radiolabeled active ingredient to allow the quantification and identification of radioactive residues. Given the use of a radioactive substance, studies are usually carried out in confined environments—for example, large pots or containers—to eliminate the risk of environmental contamination.

Metabolism in Crops

The goal of these studies is to identify and characterize the residues of the active ingredient of a PPP remaining in each raw agricultural commodity (RAC) of the treated crop.

These studies use a radiolabeled version of the active ingredient applied directly or indirectly to the crop. The aim is to identify or characterize at least 90% of the total radioactive residue (TRR) in each RAC.

These studies enable:

Measurement of the TRR in each RAC after crop treatment, which allows the distribution of residues within the crop to be determined

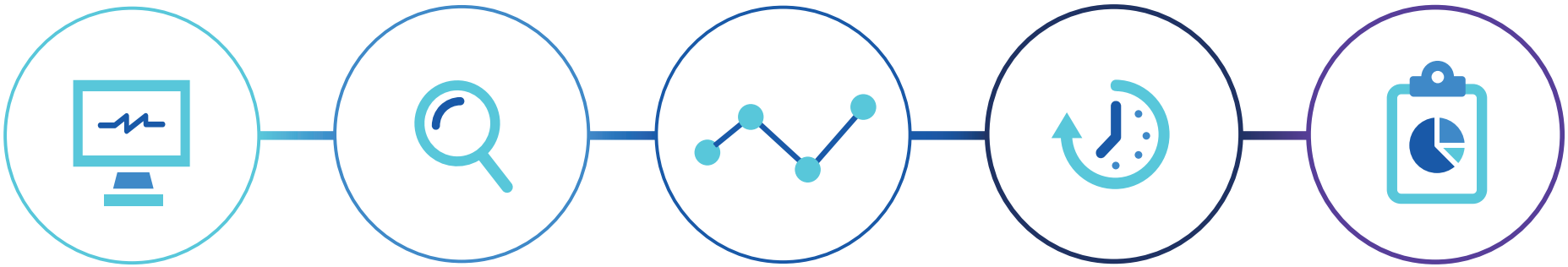
Identification of the major metabolites in each RAC: As these are the metabolites that could pose a dietary risk, their identification drives the subsequent magnitude of the residue studies (TG 509), leading to the creation of residue definitions for both risk assessment and enforcement

Elucidation of the transformation pathway of the active ingredient

Metabolism in Rotational Crops

Residues from active ingredients applied to one crop can persist in the soil and may accumulate in the subsequent crops grown in that soil—namely, rotational crops—via uptake from the soil.

These studies enable:



Estimation of the TRR in each RAC via uptake from the soil

Identification of the major metabolic components in each RAC

Elucidation of the transformation pathway of the active ingredient in rotated crops

Determination of appropriate rotational intervals (the time from original pesticide application to the time when crops can be rotated) and/or rotational crop restrictions, based on residue

Provision of information to help determine the need for limited field trials for rotational crops which are outlined in TG 504

Studies of metabolism in rotational crops aim to characterize the nature and amount of pesticide residue(s) that may accumulate through uptake from the soil. These studies are triggered by the persistence of the active ingredient in soil, as measured by its DT_{50}, or degradation half-life.

For confined rotational studies, the radiolabeled active ingredient is applied to the soil in a confined system—usually a large pot or container—to enable the effective characterization of accumulation and uptake in subsequent crops. The interval between successive crops can impact the amount of soil degradation and therefore residue uptake; thus, in general, three different rotational intervals need to be assessed:

- Resowing after crop failure
- A typical rotation after harvest
- A typical rotation in the following year

Rotational studies for permanent or semipermanent crops are not usually required.

Radiolabeling of the Active Ingredient

It is important to radiolabel the active ingredient to allow the tracking of all the major metabolites and degradation products as far down the transformation pathway as possible. The usual radioisotope used is carbon-14 (^{14}C).

If the active ingredient contains multiple rings or significant chemical groups, it may be necessary to radiolabel each of these, unless a justification can be provided that the molecule will not be cleaved. This is to ensure that each transformation pathway can be followed. The number of different radiolabeled forms of the test item will influence the size of your study.

Specific Activity of the Test Item

It is necessary to ensure that the specific activity of the radiolabeled active ingredient is sufficient to allow you to complete the data requirements defined in the TGs. Quantification of TRR values of 0.01 mg/kg in crop matrices is required.[5] If the TRR in a crop part is ≤0.01 mg/kg, it is not necessary to differentiate the radioactive residues, unless there is a reason to be concerned about their toxicological potential.

Optimizing Crop Metabolism Studies

The design of a crop metabolism study is driven by the Good Agricultural Practice (GAP) for the PPP. To achieve the best results, it is important to consider some key elements influencing the study.

Selection of Crops for Metabolism Studies

Crop metabolism studies use five main crop groups or categories: root vegetables, leafy crops, fruits, pulses and oilseeds, and cereals. Testing needs to cover each group for which use is proposed. If studies are available for crops from three of the five categories and the metabolism in these is comparable, these studies are sufficient to cover all crop groupings.

For confined rotational studies, three crop groups are normally selected: root vegetables (carrots, radishes), leafy vegetables (lettuce, spinach) and cereals (wheat).

Selection of the study crop or crops is determined by the target for the PPP. Once the crop has been selected, there are additional considerations that impact the study design, including the type and timing of the application, whether to use seeds or small plants and how to manage studies on permanent or semipermanent crops such as oranges or grapes.

Treatment Considerations

It is essential to mimic the type of application (seed, soil or foliar), formulation, application rate, number of applications and the timing of applications outlined in the GAP. You need to reflect any suggested minimal intervals between applications and between the final application and harvest, that is, the preharvest interval. If crop metabolism studies are conducted prior to finalization of the GAP, the risk is that residues at harvest may be under- or overestimated. The worst case would be that studies would need to be repeated.

Formulating the Test Item

The radiolabeled test item must be delivered in a formulation that mimics the formulation that would be used in practice in the field. This is usually achieved by combining the blank formulation (prepared without the active ingredient) with the radiolabeled test item. The procedure should follow the manufacturing process as closely as possible but is performed on a very much smaller scale. Preparation of the formulation can, therefore, be challenging, especially for some formulation types. It is useful to evaluate the formulation procedure, stability and homogeneity before commencing the study.

Applying the Formulation

PPPs may be applied to seeds (seed treatment) or the soil (pre-emergence herbicide) in which the seeds will be grown, or be applied directly to the grown plants (foliar application). Foliar applications are usually applied as sprays. Different sprayer options are available, from automatic spray systems to simple hand-held sprayers; the goal of all approaches is to get an even application of the formulation over the entire plant.

If an assessment of translocation is required, then a section of a larger plant (grapevine, fruit tree, tomato plant) may be covered during spray application. This section would be harvested and analyzed separately from the remainder of the plant.

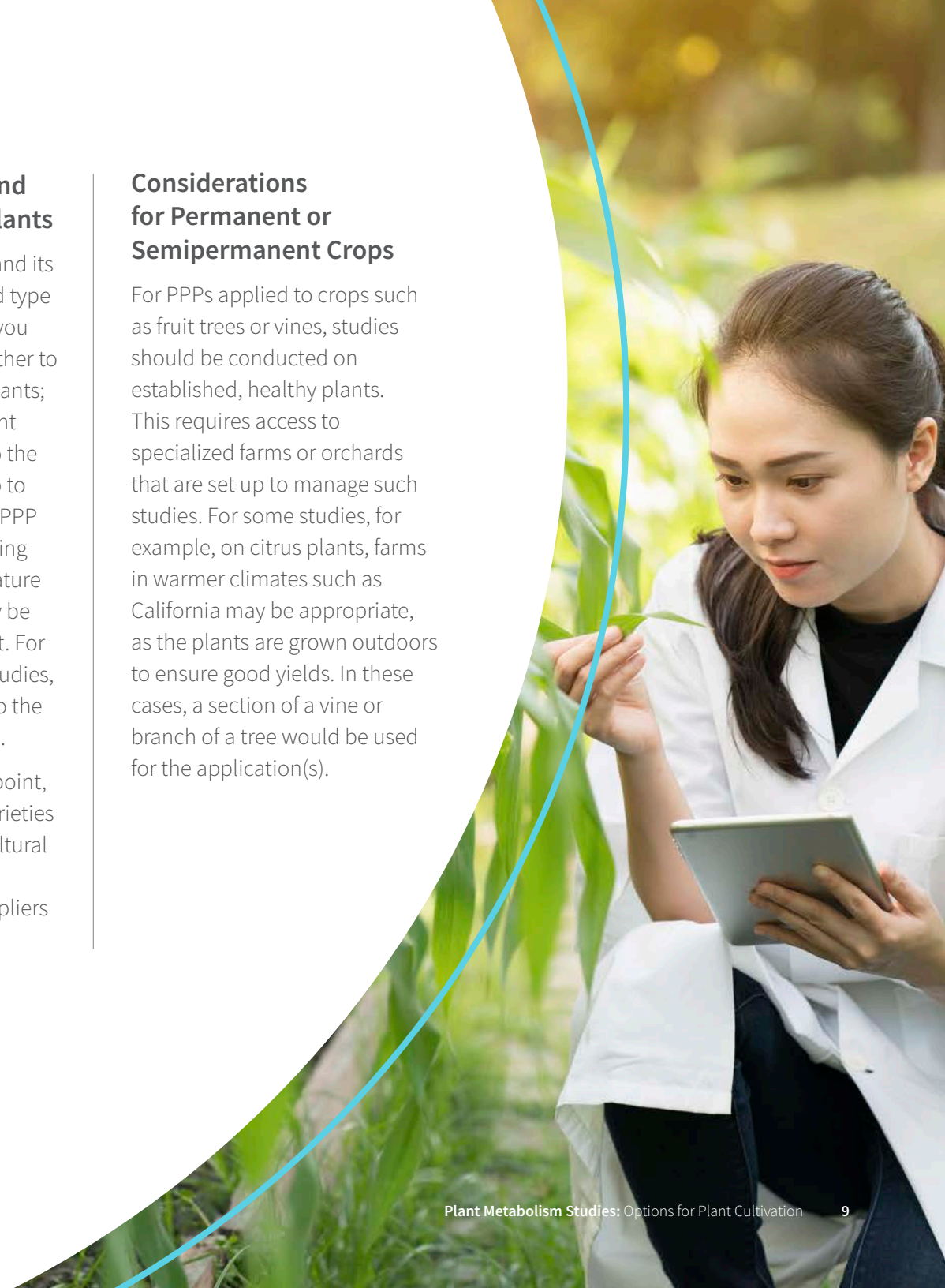
When To Use Seeds and When To Use Small Plants

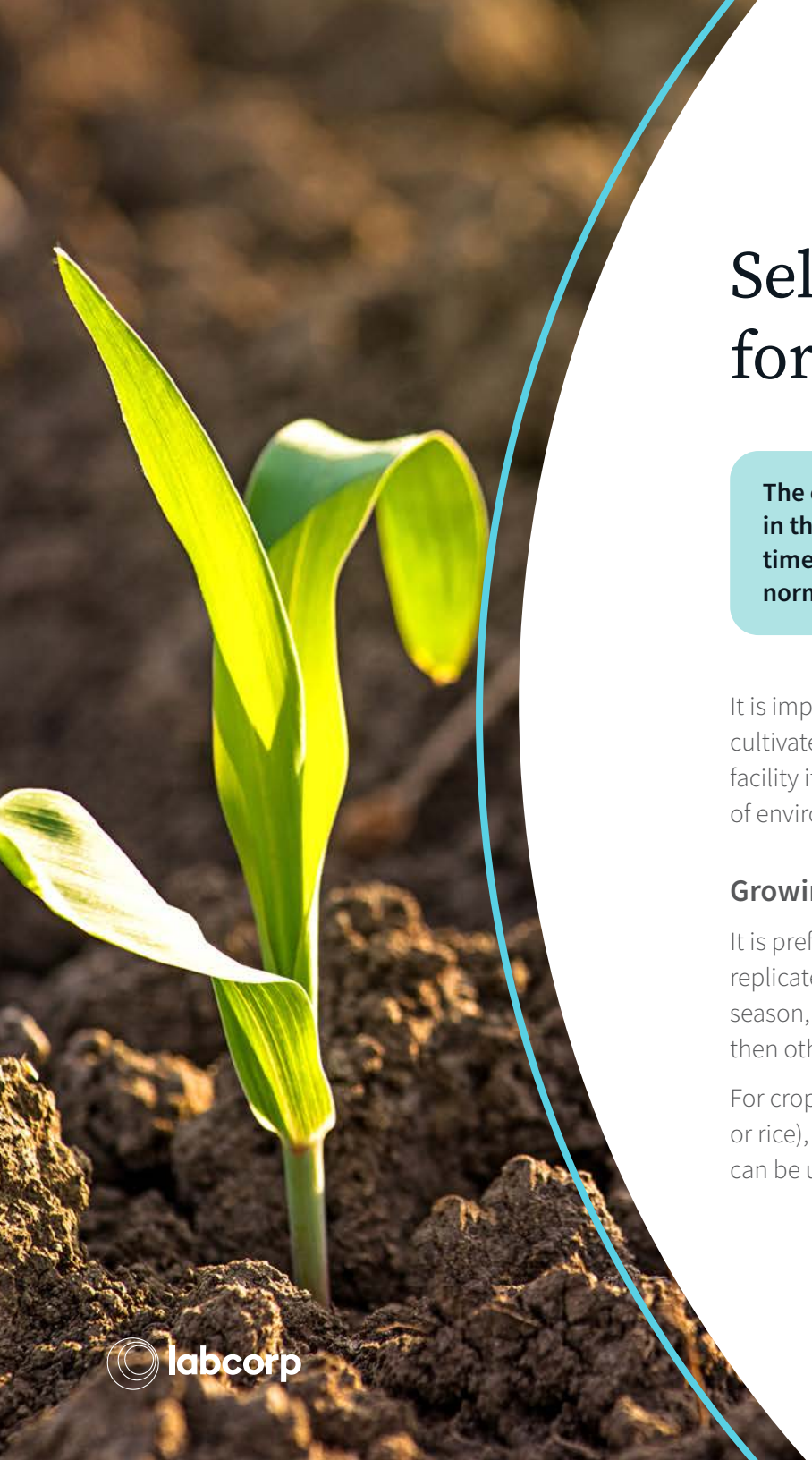
Based on the type of PPP and its use, and once the crop and type of application are known, you also need to consider whether to start with seeds or small plants; for example, a pre-emergent herbicide that is applied to the soil would require the crop to be grown from seed. If the PPP is applied later in the growing season, then smaller immature or even mature plants may be the optimum starting point. For confined crop rotational studies, where the PPP is applied to the soil, seeds are always used.

Regardless of the starting point, it is important to obtain varieties that are in common agricultural use, where possible, from reputable commercial suppliers or seed merchants.

Considerations for Permanent or Semipermanent Crops

For PPPs applied to crops such as fruit trees or vines, studies should be conducted on established, healthy plants. This requires access to specialized farms or orchards that are set up to manage such studies. For some studies, for example, on citrus plants, farms in warmer climates such as California may be appropriate, as the plants are grown outdoors to ensure good yields. In these cases, a section of a vine or branch of a tree would be used for the application(s).





Selecting a Test Facility for a Metabolism Study

The optimum conditions for growing test crops are either under cover or outdoors in the normal growing season under commercial practice; however, regulatory timelines or the study design may dictate that crops are grown outside their normal growing season.

It is important to document the environmental conditions under which the crops were cultivated (e.g., the temperature, lighting, etc.) and provide descriptions of the facility itself (e.g., outdoors, growth room or greenhouse, with or without the use of environmental control systems, etc.).

Growing Outdoors

It is preferable to grow your test plants outdoors if that crop is usually grown outside, as this replicates normal growing conditions more closely; however, most crops have a specific growing season, and if the timing of the study does not coincide with the growing season for the crop, then other options (greenhouse or indoor room) can be explored.

For crops that require a warmer climate than is present in the U.K. (melons, citrus plants, olives or rice), alternative facilities (e.g., a greenhouse or indoor room) or locations (e.g., California) can be used depending on the crop.

Growing Under Glass

Some crops such as melons, aubergines or tomatoes are often grown in greenhouses; therefore, test crops are also grown under glass. With heated greenhouses, which are kept warm in the winter, it is also possible to grow crops out of season. Supplementary LED lighting allows the effective day length to be extended where required for optimal growth.

One consideration though is that while greenhouses are easy to warm, they cannot be effectively cooled in summer, so certain crops may not be grown successfully under these conditions.

Growing Indoors in Plant Growth Chambers

When growing crops indoors under LED light, it is possible to have greater control over the temperature of the growing conditions, as growth rooms can be heated or cooled. The ability to cool the indoor rooms is important when growing crops such as wheat out of season, as seed germination requires cooler temperatures than can be achieved outside or under glass during the summer months.

This is especially important for confined crop rotational studies, which require plants to be cultivated out of the normal season. Rice requires warmer temperatures than those outdoors in the U.K.; however, indoor rooms can provide the right conditions to successfully cultivate rice.

Crop Cultivation

In order to achieve the objectives of plant metabolism studies, it is important to cultivate healthy plants that produce a good yield. In addition to maintaining suitable environmental conditions, plants need to be watered regularly, particularly if they are grown indoors. Depending on the crop type (e.g., tomatoes and other fruits), plants may also need regular applications of a suitable fertilizer.

If plants become affected by diseases or pests, it may be necessary to apply appropriate pesticides. These pesticides should be of a different chemical structure to the test item. Advice from a trained agronomist can be useful to ensure swift corrective action is taken.

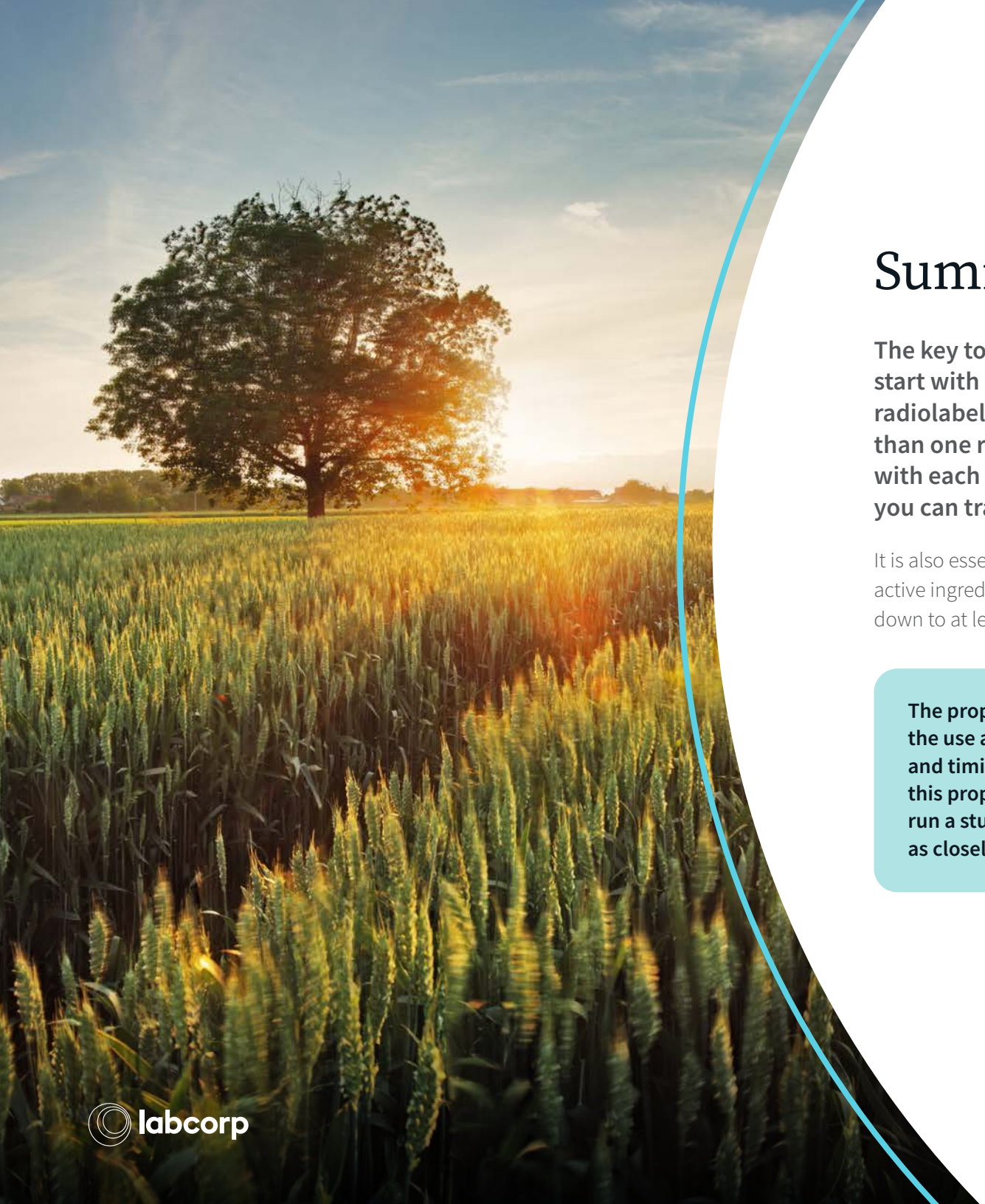
Crop Sampling

Sampling will be dictated by the type of crop and the number of RACs that each crop yields. For potatoes, for example, the RAC is the potato, so sampling is conducted at harvest. Wheat, however, has multiple RACs, with sampling of forage and hay, as well as straw and grain, required.

Considerations for Analysis

The first step in analysis of plant samples from a metabolism study is to homogenize the RAC and measure the TRR. For crops that would normally be washed (such as grapes, lettuce or apples), a solvent rinse can be performed, which allows a distinction between surface radioactivity and that absorbed into the plant. In crops with an inedible peel (such as oranges or melons), the peel should be removed and analyzed separately to determine the distribution of radioactivity. Some crops are more challenging than others to homogenize. Crops such as lettuce or tomatoes contain significant amounts of water, which means the sample may not be homogenous. Other crops (such as potatoes or aubergines) are hard and may need to be roughly chopped first. Different types of homogenizer may be needed for different types of crop. Homogenizing with solid carbon dioxide may be useful for some types of crop.

If the TRR is >0.01 mg/kg, samples are further processed and analyzed, and the amounts of extractable and nonextractable residues are determined.



Summary

The key to successful crop metabolism studies is to start with an active ingredient that has been suitably radiolabeled; this may require the synthesis of more than one radiolabeled form of the active ingredient, with each ring system radiolabeled to ensure that you can track the whole transformation pathway.

It is also essential that the specific activity of the radiolabeled active ingredient is sufficient to measure concentrations down to at least 0.01 mg/kg.

The proposed GAP for the PPP should include details of the use and formulation, application rate and number and timing of applications to the target crop. Utilizing this proposed GAP for the PPP allows you to design and run a study that mimics the conditions used in the field as closely as possible.

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