Comments on ESE Alcohol Sampling Plan

General Comments

1. Given the large area of the fields being sampled, the variable nature of the wet distiller's grain (WDG) application method, and the inherent heterogeneity of the pesticide concentrations in the WDG, the EPA recommends that two fields from the "high quantity" group (or Group 1), be sampled in addition to one field from the "mid quantity" group (or Group 2). One of the Group 1 fields must be selected from the fields that received WDG most recently in 2021. This recommendation assumes that ESE Alcohol will fully execute the sampling approach outlined in Attachment A.

2. Typically, sampling plans include background information, a summary of the problem (problem formulation), a Conceptual Site Model (CSM), and the scientific decision to be made based on the results of the sampling. This information provides the context for the purpose of the sampling. Please revise the sampling plan to include this pertinent information.

3. The sampling plan should indicate that GPS coordinates will be provided for each individual sample or aliquot collected. GPS coordinates for the composite samples are not needed.

4. In several places, the sampling plan discusses when a parcel will no longer need to be sampled and removed from the Administrative Order on Consent (AOC). These provisions should be removed from the sampling plan, as the Administrative Order on Consent governs when a parcel may be released from AOC requirements.

Specific Comments

1. **Introduction.** (a) The sampling plan states that a tiered approach will be used to sample "worst case fields" first. It is unknown at this point whether those fields receiving the highest application rates of WDG will have the highest concentrations of seed treatment pesticides remaining in soil. Please revise the text in this section and the rest of the document by replacing "worst case fields" with "fields treated with the highest amount of WDG" or comparable language, as appropriate. (b) The EPA did agree to remove pesticides from the list; however, that agreement was contingent upon those specific pesticides not being detected during the proposed voluntary sampling event, which did not occur. Thus, ESE Alcohol must conduct laboratory analysis for all 24 pesticides listed in Table 1.

2. **Sample Plan Procedure and Media to be sampled.** (a) The title for section A should be revised from "Grain and/or Corn Fields- to "Corn Fields" because this sampling plan does not address sampling of grain. All references to sampling of grain should be removed from the sampling plan. (b) The phrase "…i.e. commercial application rates or higher may be established," should be deleted because it has no impact on whether additional sampling will be conducted and the meaning of this text is unclear.

3. **Field Soil Samples, Fields to be Sampled.** (a) As stated in General Comment 1, ESE Alcohol should sample two fields from the "high quantity" group (or Group 1), in addition to one field from the "mid quantity" group (or Group 2). If the sampling approach outlined in Attachment A is not followed and the parties agree to an alternate approach, then the number of fields requiring sampling will need to be revisited. (b) The method for selecting the fields in Group 1 and the field in Group 2 should be specified. (c) This section states that 21 fields comprising 2031 acres were treated, while the Introduction indicates the site is comprised of approximately 2600 acres of agricultural fields. Please revise the sampling plan to ensure the total acres are in agreement. (d) The last paragraph of this section mentions "FDA clearance levels." This text should be deleted from the sampling plan because no grain samples will be sampled by ESE Alcohol.

4. **Field Soil Samples, Sampling Procedures.** An adequate number of samples per field must be collected to ensure the sample is representative. In other words, in order to generate a reliable estimate of the mean soil concentration that represents the entire treated field, a sufficient number of core samples must be collected to account for the variability in concentrations across the field. The number of samples to estimate the mean concentration (statistically, the sample size) will then depend on the desired precision (i.e., standard deviation around the mean) and the variability of the pesticide concentration in the field (the field or population variability).

   Field dissipation studies typically use 15 or 16 non-composited soil cores based on a Coefficient of Variation (CV) of 110%, a precision of 60% (the estimate of the mean is no more precise than 60% of the CV) and a confidence level of 95% (Table 2 in EPA, 2008). Although this study proposes the same number of aliquots as the field dissipation guidance, the value from the field dissipation guidance is based on an estimated standard deviation of the mean at each sampling time during a dissipation study, not a standard deviation of the mean from a single sampling event. Further, the guidance recommends 15 to 16 <u>non-composited</u> samples, not one composite sample comprised of 15 aliquots. Finally, the size of the sample plots in a field dissipation study are much smaller in comparison to the fields being sampled at the site. For example, typical plot sizes for large-scale field dissipation studies range from 4 x 10 meters to 10 x 40 meters.

   The sampling plan must outline a valid statistical approach to determining sample size that is tailored for this site. Given the difficult task of estimating a representative mean for a large, treated field, the EPA recommends an Incremental Sampling Method (ISM) be used at the site. ISM is an ideal approach because it is a structured composite sampling and processing protocol that reduces data variability and provides a reasonably unbiased estimate of mean contaminant concentrations in the volume of soil targeted for sampling. The EPA has developed an ISM approach for the site that can be used as a frame of reference (Attachment A). However, please refer to guidance on ISM for a more detailed treatment of the method (ITRC, 2020).

5. **Field Soil Samples, Sampling Procedures**. According to OPP's "Fate, Transport and Transformation Test Guidelines, OPPTS 835.6100 Terrestrial Field Dissipation, October

2008," in order to fully characterize soil concentrations in each field, soil should be collected from a depth sufficient to encompass the vertical distribution of the pesticide. Moreover, the major transformation processes usually occur within the "biologically active" zone of the soil. For sampling purposes, this zone can be defined as the maximum depth of tillage, rooting depth of agronomic plants or the depth of an impermeable soil layer, whichever is deepest. For most studies, the biologically active zone encompasses a depth of one meter or about 3 feet.

Further, according to the University of Nebraska Lincoln, although the depth of corn roots can reach 5 or 6 feet, until late in the season, conservative irrigation management assumes a root depth of 3-feet, which is roughly one meter (Kranz et al., 2008). In addition, the effective rooting depth for corn is 3 to 4 feet and changes throughout the rooting season (Irmak and Rudnick, 2014). The effective rooting depth is the active crop zone where the majority of soil-water and nutrient uptake occurs. Thus, seed treatment pesticides that reach this depth will be taken up by the corn plant. More importantly, water extraction increases significantly from the second and third foot of the soil profile at early tasseling to silking stages (Irmak and Rudnick, 2014, https://www.pioneer.com/us/agronomy/corn-water-use.html), which coincides with the period when pollinator species would be most attracted to corn.

Therefore, the EPA recommends 36-inch soil cores be collected, and samples be collected from within 3 depth intervals (0-12 inches, 12-24 inches, and 24-36 inches).

6. **Staging and conditioning area.** (a) This section of the sampling plan should clearly indicate that a composite sample will be collected from each windrow and submitted for analysis. (b) After the windrows are removed and properly disposed of, sampling of the soil beneath the previous windrow locations must be conducted. (c) Sampling of the lagoon floors is required unless they are lined with manmade material (i.e., a synthetic liner). If a synthetic liner is in place, ESE Alcohol must provide technical details on its composition and placement.

7. **Treated Seed Receiving Area.** (a) This section states that "The level of concern at the facility shall be based on commercial/workplace levels for human/mammal exposure only." The values in Figure 5 are based on acute exposure resulting in lethality. Thus, these values are not protective of human health and cannot be used to "…remove non-crop land from the order." Rather, the EPA recommends using the Regional Screening Level calculator for an Outdoor Worker based on an excess individual lifetime cancer risk of 1×10^{-6} and a hazard quotient of 0.1 to account for the presence of multiple pesticides that may have similar modes of toxic action or toxic endpoints (e.g., same target organ or system) (see https://epa-prgs.ornl.gov/cgi-bin/chemicals/csl_search). (b) As discussed above under Comment 4, in order to generate a reliable estimate of the mean soil concentration, a sufficient number of core samples must be collected to account for the variability in concentrations across the treated seed area. The sampling plan does not provide a valid statistical based justification that this will be achieved with six aliquots. The EPA recommends either analyzing each soil sample for the 24 analytes or

increasing the number of aliquots. (c) It is unclear why ESE Alcohol has proposed to collect soil samples to a depth of 20 to 30 inches. Outdoor workers will not be exposed to soil at this depth. (d) A duplicate sample will be collected from this area. Because there is some uncertainty regarding the number of total samples to be collected, it could not be verified that the collection of this one duplicate sample meets the field duplicate frequency identified in the QAPP of one field duplicate per 20 investigative samples.

8. **Schedule.** The text indicates that sampling will be conducted within two weeks after the plan is accepted, while the table indicates sampling will be scheduled within 30 days of approval of the QAPP, DQOs, and HASP. The EPA requests that sampling begin no later than two weeks following approval of these documents.

References

Irmak, S. and D.R. Rudnick. 2014. Corn Soil-Water Extraction and Effective Rooting Depth in a Silt-Loam Soil. University of Nebraska Lincoln Extension – Institute of Agriculture and Natural Resources.

ITRC. 2020. Incremental Sampling Methodology Update. https://ism-2.itrcweb.org/

Kranz, W.L., S. Irmak, S.J. van Donk, C.D. Yonts, and D.L. Martin. 2008. Irrigation Management for Corn. University of Nebraska Lincoln Extension – Institute of Agriculture and Natural Resources.

United States Environmental Protection Agency. 2008. Fate Transport and Transformation Guidelines. OPPTS 835.6100. Terrestrial Field Dissipation. EPA-712-C-08-020. Washington, DC. October 2008.

Attachment A
Example Incremental Sampling Work Plan
ESE Alcohol Inc
Leoti, Kansas

Introduction

The ESE Alcohol Facility, located in Leoti, Kansas, is an ethanol plant that produced ethanol from seed corn. Because most of the seed corn in the U.S. is coated with pesticides, its use at this facility has resulted in pesticide contamination in the holding pond liquids, as well as in the solids (wet distillers grain) produced as a result of the ethanol production process.

The liquid and wet distiller's grain (WDG) have historically been managed by applying these waste streams to agricultural fields as a form of fertilizer. Records obtained from the facility indicate that over 19,000 acres across 116 tracts of land have been treated in this manner. In addition, facility records from 1998 to 2020 indicate that roughly 2 to 6 million gallons of process wastewater per year, and roughly 5 to 19 tons of WDG per year, have been applied to surrounding fields. Recent estimates show that in 2021, a total of 838 acres were treated with WDG at an average application rate of 5.26 tons per acre (tons/A).

Problem Formulation

Due to the potential risk from elevated levels of these pesticides in the environment, an Ecological Risk Assessment (ERA) was completed. Potential risk to birds, mammals, and honeybees in agricultural fields where WDG had been applied was evaluated. Of the three assessment endpoints evaluated, honeybees were found to be the most sensitive assessment endpoint. The mechanism of exposure is such that pesticides are taken up by the roots and translocated to the pollen and nectar, exposing foraging bees to pesticide residues, as can be seen in the Conceptual Site Model (Figure 1). It is important to keep in mind that any pollinator may be exposed in this manner; however, because honeybees are very sensitive to neonicotinoid pesticides, they are the primary receptor of concern and remedial actions selected to be protective of honeybees should also be protective of other less sensitive pollinators.

The ecological risk assessment evaluated potential risk to honeybees by converting the maximum concentration in WDG to a soil application rate in pounds of active ingredient per acre (lb a.i./A), using the arithmetic mean WDG application rate of 10.20 tons/A from 1998 through 2021. This estimated site-specific application rate was first compared to allowable maximum application rates for each pesticide to develop a list of Contaminants of Potential Concern (COPC). Clothianidin, thiamethoxam, ipconazole, tebuconazole, thiabendazole and chlorantraniliprole site-specific application rates exceeded the range of maximum label application rates identified by the Office of Pesticide Programs for these pesticides for crops typically grown in western Kansas.

Table 1. Estimated Site-Specific Application Rates of Pesticides Compared to Approved Application Rates.

Pesticide	Approved Application Rates (lb a.i./acre)	Estimated Site-Specific Application Rate (lb a.i./acre)
Clothianidin	0.1 to 0.2	12.91
Thiamethoxam	0.17 to 0.18	1.6
Tebuconazole	0.113 to 0.225	0.60
Thiabendazole	0.15	1.02
Ipconazole	0.0006 to 0.01	0.69
Chlorantranilipole	0.2	3.75

Using the EPA's Bee-REX model, the ecological risk assessment also calculated Risk Quotients (RQs) based on these application rates and found the highest potential risk was due to clothianidin. For adult bees, acute RQs range from 3.2 (queen) to 175 (worker foraging for nectar), and chronic RQs range from 32 (queen) to 1,801 (worker foraging for nectar).

Due to the results of the ERA, the RCRA 7003 Administrative Order for Imminent Hazard requires soil sampling of agricultural fields where WDG has been applied over the last 1 to 2 years to determine if pesticide concentrations remaining in soil pose a significant risk to pollinators. Therefore, recommended acceptable soil concentrations, or ecological Levels of Concern (LOCs) were derived using the maximum approved application rates for crops typically grown in western Kansas, which can be converted to a soil concentration using the equation 1 lb a.i./A = 0.5 mg/kg soil (USEPA, 2012; USEPA et al., 2014).

Table 2. Ecological Levels of Concern based on Allowable Approved Application Rates.

Pesticide	Approved Application Rates (lb a.i./acre)	Soil Concentration (mg/kg)
Clothianidin	0.1 to 0.2	50 to 100
Thiamethoxam	0.17 to 0.18	85 to 90
Tebuconazole	0.113 to 0.225	55 to 125
Thiabendazole	0.15	75
Ipconazole	0.0006 to 0.01	0.3 to 5
Chlorantranilipole	0.2	100

The purpose of the field sampling described in this work plan is to determine if the average concentration of pesticides within the investigation area (agricultural fields treated with WDG and process water) present unacceptable risk to pollinators based on the ecological LOCs outlined in Table 2. If soil concentrations exceed the LOC, this indicates that ecological risk to

pollinators is likely, and crops that are attractive to pollinators should not be planted until the soil concentration falls below the LOC.

Field Sampling Approach

Because of the fundamental heterogeneity of contaminant distribution in soil, the collection of a small number of traditional discrete or composite samples can underestimate or overestimate the actual mean concentration. Therefore, this sampling effort will incorporate an Incremental Sampling Method (ISM) to estimate the mean concentration. ISM provides representative samples of specific soil volumes defined as Decision Units (DUs) by collecting numerous increments of soil (typically 30 to 100 increments) that are combined, processed, and subsampled according to specific protocols. This allows a site to be reliably characterized with the collection of a relatively small number of analytical samples (ITRC, 2020).

For the purposes of this study, the Decision Unit (DU) is a single agricultural field. Honeybees can be exposed during foraging to large areas across any given field. Therefore, the lateral boundary of a DU is one agricultural field, with a standard size of 94 acres. Two fields that have received over 10 tons/acre of WDG will be evaluated (one of which as recently as 2021), as well as one field that has received 6 to 10 tons/acre.

Because neonicotinoid pesticides are taken up through the roots of the plant, three depth intervals will be analyzed in a 36-inch soil core (0-12 inches; 12 to 24 inches; 24 to 36 inches). This depth is based on the fact that the depth of corn roots can reach 5 or 6 feet, until late in the season, conservative irrigation management assumes a root depth of 3 feet (Kranz et al., 2008). In addition, the effective rooting depth for corn is 3 to 4 feet and changes throughout the rooting season (Irmak and Rudnick, 2014). Water extraction increases significantly from the second and third foot of the soil profile at early tasseling to silking stages (Irmak and Rudnick, 2014, https://www.pioneer.com/us/agronomy/corn-water-use.html), which coincides with the period when pollinator species would be most attracted to corn. Therefore, the vertical boundary of the DU is defined as 36 inches or 3 feet (Figure 2).

ISM can incorporate random, systematic, or systematic random sampling. Systematic random sampling was selected as it is in a sense a compromise approach, with elements of both simple random and systematic sampling. It is relatively easy to implement, but also avoids the appearance of over- or underrepresentation of subareas within a DU. Given the large size of the treated fields, the DU was completely divided into fifty 2-acre grids. Of those fifty, forty-six of the grids will be sampled as the corners will be excluded from sampling because the corners received lower applications of WDG. In general, 30 to 50 increments are sufficient for most DUs; however, given the large size of the fields, the potential heterogeneity of the WDG, and the variability associated with the WDG application method, 46 increments was selected (ITRC, 2020). DUs will be gridded out in the field by marking the corners of the 2-acre grids with flags or markers using established GPS locations.

If soil characteristics allow, a 2-centimeter (cm) soil core will be used to collect a random increment of each depth interval within each grid cell, resulting in 46 increments per DU per depth interval. If a larger coring device is needed due to soil conditions, a core diameter that allows for ease of sampling as well as a manageable sample volume will be selected.

An example of the systematic random sampling is provided in Figure 3. For the systematic random sampling design, the location of the first increment is determined randomly, and subsequent increments are collected in the same relative location within each grid, resulting in a serpentine collection pattern ending at the opposite corner or end of the DU from where sampling was started. A Global Positioning System (GPS) device will be used in the field to verify the sample location within each cell. Three field replicates will be collected at each depth interval following the same process. The purpose of the collection of replicates is to provide multiple estimates of the mean and a relative standard deviation. The collection of replicates is a crucial step in the ISM process as it is the only way of estimating the representativeness of the estimated mean.

In general, individual soil increments typically weigh 20 to 60 grams (g). Final ISM field samples typically weigh 500 to 2500 g. However, given the potentially large amount of soil collected at three depth intervals, inclusion of the entire core increment across a targeted depth interval in an ISM sample may be impractical. Therefore, to reduce the volume of soil collected, a representative *subsample* from each core increment will be collected using a "core wedge" approach (ITRC, 2020). For each DU, a total of nine ISM samples will be collected and transported to the laboratory for further processing (three replicates for each of three depth intervals).

Laboratory Processing and Handling

The ISM samples will be further processed in the laboratory. Samples will be air-dried for several days to remove moisture by spreading soil evenly on aluminum pans. Soil should be dry enough to crush agglomerates into a flowable matrix (usually a moisture content of 5 to 10%). To disaggregate the soil, the vegetation and debris will be removed and dry samples will be gently rubbed into a #10 sieve (2 mm) sieve to promote break-up of the soil agglomerates.

Analytical subsampling to provide representative aliquots will be accomplished using the two-dimensional Japanese slabcake method, which emulates the field incremental subsampling process in the controlled laboratory setting. The entire sample is spread evenly onto a 2-D surface at a depth that can be easily penetrated by a square scoop. Each sample will be spread across a 20 × 30-inch aluminum baker's tray. A tray can easily hold a 2-kilogram sample spread across it at a depth of no more than 1 to 2.5 cm. A scoopula will be used to push the sample around and spread it to an even depth and ideally as thin as practical. As the sample is spread, the fine particles tend to migrate downward toward the tray while the larger, less-dense particles rest on top. Therefore, a square-walled, blunt-end scoop with a minimum 16 mm width will be used to minimize the discrimination of taking more of the large particles on the top (See example in Figure 4).

Pesticides will be analyzed using EPA Method 8321b, which requires a 1 g portion of soil. If processing occurs in the laboratory, each scoop (increment) will ideally represent 1/30th of the desired target mass. Therefore, for a 1 g subsample, each increment should weigh about 30-40 milligrams (Figure 4). However, if the samples are processed in the field, each scoop will weigh 1 g and a 30 g sample will be sent to the laboratory for analysis.

Before starting the scooping process, a few trial scoops will be taken and weighed, to calibrate the amount needed for each scoop. This technique works best when used after disaggregation in conjunction with particle size selection via sieving to reduce the range of particle sizes. A process will be established to document that the increments are collected from systematic random locations over the entire exposed surface to ensure adequate representation of the sample.

Finally, most contaminant concentrations are reported on a dry-weight basis; thus, a subsample for percent moisture determination will be collected using the same techniques as for the contaminant. However, subsampling and percent moisture determination will be performed on the as-received ISM bulk sample prior to any processing in the lab.

Two laboratory replicates will be collected to assess the precision of the ISM subsampling processes. These replicates should then be carried through the rest of the analytical process. Note that there is a difference between replicates collected during sample processing and replicates collected during the field sampling effort. ISM replicates collected from a DU provide information on the variance in the estimate of the mean without specifically separating out the contribution of laboratory sample processing from other sources of variance.

Decision Endpoints

Three replicate ISM samples will be collected in the field from each DU and from each depth interval. These replicates will provide a measure of the variability of the entire sampling, preparation, and analytical process. The *mean concentration* of the replicates will then be calculated and compared to ecological LOCs.

Decision errors will be analyzed based on field and laboratory replicates. High Relative Standard Deviations (RSDs) (e.g., exceeding 30 to 35%) from field replicates, but with acceptable RSDs from laboratory replicates, strongly suggests a substantial degree of heterogeneity in the DU contaminant concentrations. For this study, high RSDs are problematic, as it means that estimates provided by the individual ISM replicates are quite variable and that the estimate of the average for the DU they provide may be unreliable. If the results are close enough to an action level that a Type 1 or 2 decision error is possible, resampling with an increased number of increments may need to be completed to reduce error.

References

Irmak, S. and D.R. Rudnick. 2014. Corn Soil-Water Extraction and Effective Rooting Depth in a Silt-Loam Soil. University of Nebraska Lincoln Extension – Institute of Agriculture and Natural Resources.

ITRC. 2020. Incremental Sampling Methodology Update. https://ism-2.itrcweb.org/

Kranz, W.L., S. Irmak, S.J. van Donk, C.D. Yonts, and D.L. Martin. 2008. Irrigation Management for Corn. University of Nebraska Lincoln Extension – Institute of Agriculture and Natural Resources.

United States Environmental Protection Agency (USEPA). 2012. Environmental Chemistry Methods Guidance. Office of Pesticides Programs. Environmental Fate and Effects Division.

USEPA Office of Pesticides Programs, Health Canada Pest Management Regulatory Agency, and California Department of Pesticide Regulation. 2014. Guidance for Assessing Pesticide Risks to Honeybees.

FIGURES

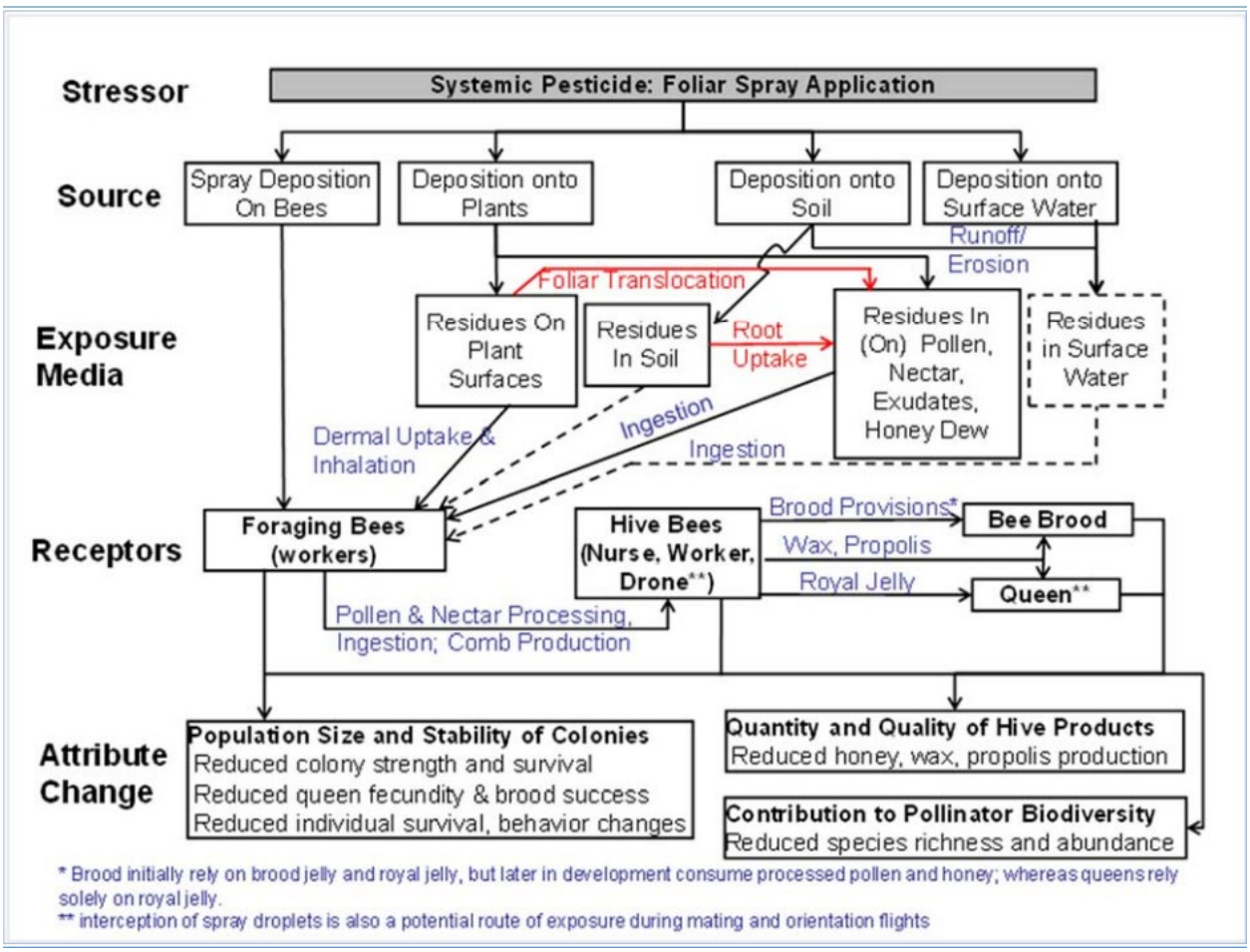

Figure 1. Conceptual Site Model for Honeybee Exposure to Pesticides.

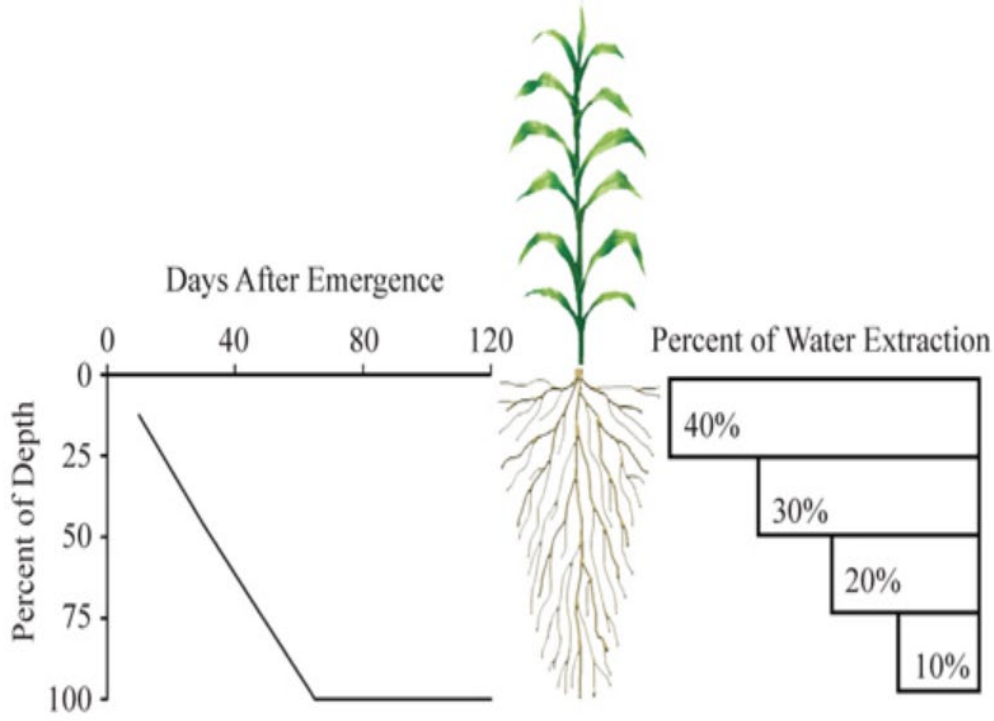

Figure 2. Depth of Corn Roots and Percentage of Water Extraction.

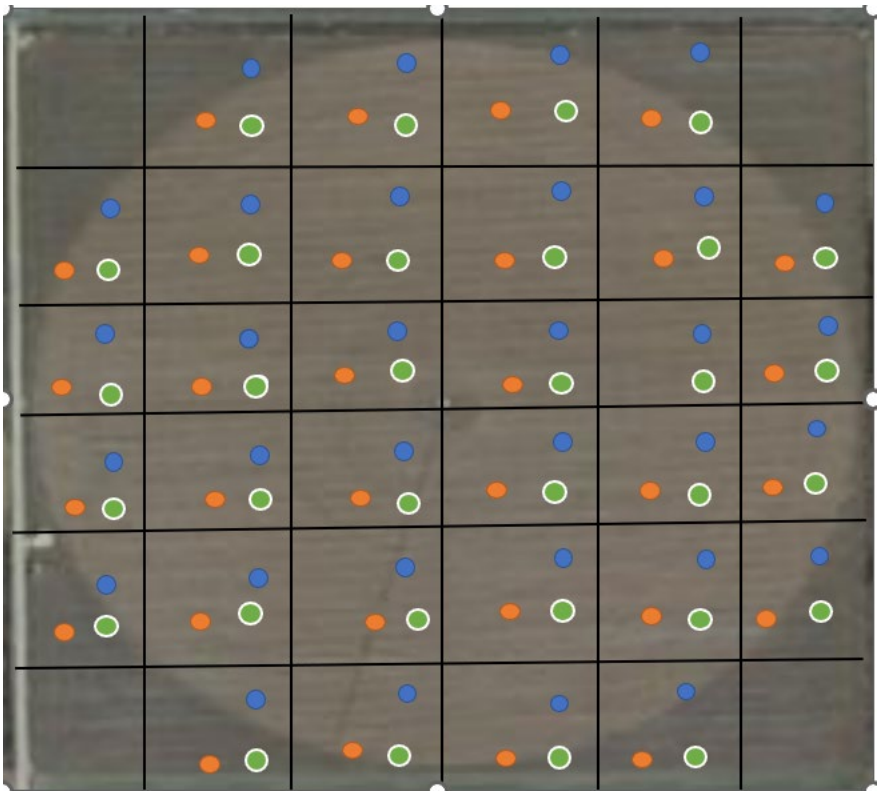

Figure 3. Example of Systematic Random Sampling of a single Decision Unit (one field).

Figure 4. Example of a 2D Japanese Slabcake Incremental Sample and Square Sampling Tool.