REPORT OF THE OIL MODIFICATION WORKING GROUP

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INTRODUCTION

Oilseed production represents a major sector of U.S. and world agricultural output. The most important oilseed crops are soybean, oil palm, rapeseed/canola, and sunflower. Fatty acids from such plants are a vital component of the human diet and can provide up to 25% of the caloric intake in developed countries. In addition, plant fatty acids have many industrial applications such as soaps, detergents, lubricants, biofuels, cosmetics, and paints. There are at least 200 different types of fatty acids that have been identified in plants, but the most abundant are linoleate, palmitate, laurate and oleate. The end use of the plant oil dictates which fatty acids are most desirable. Genetic engineering presents opportunities to modify oil content. This may be done to increase the proportion of “healthy” fatty acids in an oil, improve oil stability, expand the range of fatty acids that can be produced at low cost, and increase oil content to reduce cost. We considered two case studies in which oilseed crops were genetically engineered. In both cases, the inserted genes were under the control of seed-specific regulators.

The first case study was soybeans, genetically engineered for high oleic acid content. Commodity soybean varieties have over 50% linolenic which is an omega-6 fatty acid. Oleic acid is a monounsaturated fatty acid. Evidence is accumulating that incidence of coronary heart disease might be reduced by consuming a lower amount of omega-6 fatty acids than is typical in many western diets. The omega-6 content was reduced and oleic acid was increased by transformation with a delta-12 desaturase enzyme from soybean. The promoter was a sequence from beta conglycinin, a seed storage protein also from soybean. The inserted gene then inhibited synthesis of the target desaturase through a process known as sense suppression. As a result oleic acid content was greatly increased and omega-6 fatty acids were greatly decreased in the seeds. DuPont submitted a petition for determination of non-regulated status to APHIS, which was granted in 1997.

The second case study was canola, genetically engineered to accumulate laurate in seeds. Laurate is an important fatty acid for production of soaps, shampoos, and detergents. A thioesterase gene from California bay (Umbellularia californica) was inserted under the control of the napin storage protein promoter from rapeseed. This enzyme cleaves lauroyl-ACP to yield free laurate. The bay thioesterase is related to the native canola enzyme but has different specificity for fatty acid chain length, and, under the napin promoter, laurate accumulates in the seed. The accumulation of laurate induced several biochemical pathways to become active. It was shown that the β-oxidation pathway for breakdown of lipids, the glyoxylate pathway for fatty acid carbon re-utilization, and the fatty acid synthesis pathways all showed increased activity, giving rise to a futile cycle in seeds during the time period when the promoter was active. Interestingly, total oil yield was not reduced, indicating that increased fatty acid synthesis was able to compensate for any breakdown. Calgene submitted a petition for determination of non-regulated status to APHIS, which was granted in 1994.

In addition to these two case studies, the groups also considered possible future engineered oil modifications to plants for producing better food or animal feed or for industrial uses. In such discussions, we generally assumed that future modifica-

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tions would likely still involve seed-specific promoters, which may narrow the focus of concern to mostly seed factors.

**PHASE I: GENERAL DATA NEEDED**

"Given the regulatory criteria for field testing, what biochemical, physiological, or phenotypic changes may impact confinement of transgenic plants? How might these changes be detected prior to field testing?"

There was general agreement that certain phenotypic changes could have an impact on confinement of transgenic plants. However, such changes would need to be large in magnitude to really have the potential to affect confinement measures. Phenotypic changes that might impact confinement are typically factors that influence dispersal of plants such as:

- Pollen: amount, longevity, dispersal distance, factors influencing self-incompatibility
- Floral traits: morphology (e.g., anther extrusion), development, number of flowers, spatial separation of floral organs, attractiveness to pollinators, flowering time
- Seeds: number, longevity, dispersal distance, persistence
- Vegetative propagation: extent, dispersal

If the transgene is intended to induce such changes, current procedures would deal with the increased risk in the permit process. Small, unintended changes may be difficult to detect, no matter whether they are induced by a ‘first generation’ gene (such as Bt or RR) or from one of the ‘second generation’ genes (such as transcription factors, genes with a regulatory function), or by traditional breeding. On the other hand, large phenotypic changes would be likely identified in greenhouse or growth chamber trials already and would not progress to field testing given the typically applied screening procedure.

Because some changes will not be detected during growth chamber and greenhouse trials, field trials are an essential tool for detection of such important changes. Because initial field trials are quite limited in scale, the risk is considered to be minimal. Furthermore, most unintended effects are likely to disadvantage the plant with regard to fitness.

It was also agreed within the group that there is no such thing as absolute containment during a field trial. However, current procedures are adequately addressing this point. Regulation of field tests involves a risk-based approach for categorizing transgenic plants, such that suitable confinement conditions can be imposed.

Under the current procedure, the size of the field trials is not limited. It was noted that it is hypothetically possible that transgenic plants could be tested under large scale field trials that have not been previously tested under small scale trials. However, as a matter of practice, this is highly unlikely to happen, as there is no incentive to commit the necessary financial resources to perform such large scale field trials without prior screening in smaller trials. Once an event is put on a commercial track, large scale field trials usually become necessary. However, during these trials, a whole battery of regulatory questions has to be addressed, assuring that all the above-mentioned changes in phenotype will be captured and addressed.

"Do existing standards and methods for gene characterization and identification of secondary effects encompass monitoring for these changes?"

In general, yes. Small-scale trials are unlikely to negatively impact confinement issues. Larger-scale trials (e.g., 100 acres—the group knew of no basis defining a limit) would likely be established for commercial-track events. Evaluation of such events would adhere to current industrial guidelines consistent with evaluation for potential unintended effects (description of traits/phenotypes analyzed has been presented elsewhere). Industry and non-industry groups share processes/approaches toward identifying unintended effects. Different species may require different acreages, although this should be considered more the exception than the rule. Characterization of transgenic plants is typically initiated in the first generation, and intensified in subsequent generations. Events requiring planting on large acreages would already be substantially characterized upon such planting.
"What are the strengths of the industry approach (described in the morning plenary session) to characterize genes from genomic projects?"

The team decided to redefine the scope of this question to consider genes and phenotypes relating to regulatory, metabolic, and signaling pathways, rather than those from genomics projects. We agreed that there are many new types of genes that may be incorporated into field released plants over the next few years. Some of these plants may contain genes for which the function is not as well characterized as those in the first generation of de-regulated plants such as Bt or EPSPS genes.

Several strengths of the existing industry approach were noted. Early safety evaluations through reviews by an internal biosafety committee would potentially identify any genes or phenotypes that would require more stringent field testing requirements. Early consultation with USDA-APHIS also allows the researcher to gain valuable insight of potential risks or hazards that should be considered.

Extensive gene characterization prior to field testing is not always feasible (since some of the characterization requires growth under field conditions). However, some key information is usually available prior to a release. APHIS requires that gene function be known for genes tested under notification and has provided guidance on determining gene function. An example of information that should be considered prior to a release is the comparison of the protein to databases of known allergens and toxins. Information collected prior to field testing should be sufficient to conclude that the protein is not likely to be toxic to non-target organisms if the field test is to be authorized under the notification procedure.

Another important aspect of the industry approach is a commitment to stewardship. A stewardship program often includes committing dedicated resources to ensure that the proper procedures are in place and that protocols are strictly followed and documented. Utilization of the established and well-tested protocols will greatly minimize the potential for gene flow out of the experimental trial.

"Are there areas where the approach should be improved?"

Those in industry, who have gained considerable experience in producing and developing transgenic plants for commercial use, could offer to train others in the approach that has been successfully deployed in the past. Trainees might include those in academia or even emerging small companies. The training could encompass identification of the type of data that is typically needed at the various stages of development and the methodologies that have been used for gathering such data.

"Do any new environmental issues relevant to field releases and management arise when considering emerging genes and the phenotypes they affect?"

No, the phenotypes of these plants are not expected to be different enough from other GM plants to result in new types of environmental risks. We felt that existing regulations cover questions about gene escape and non-target effects that could occur during field testing. Existing procedures should be sufficient for field tests of plants with GM pathways. Due to the limited scope of this workshop, we did not consider environmental issues that should be evaluated prior to deregulation.

PHASE II: OIL MODIFICATION

"Does this gene/trait differ from currently commercialized genes/traits (e.g., Bt, herbicide tolerance, virus resistance, delayed fruit ripening) in ways that are relevant to regulatory criteria for field testing?"

No, as with previously commercialized genetically engineered plants, plants engineered for oil modification are not expected to be drastically altered relative to the fitness characteristics listed in our response to the first question. Therefore, we do not see a need to change the regulatory criteria for field testing. Most examples of plants genetically engineered for oil modification, including our two case studies, involve modifications to existing pathways to increase the amount of a certain fatty acid or to effect accumulation in certain tissues. Current and near future technol-
ogy will not likely increase total oil levels dramatically. In addition, we have no reason to believe that modified oils will be inherently more toxic to non-target organisms.

In anticipating new risks, it is important to consider promoters. For oil modification, we anticipate that new genes will always be under seed specific promoters, as in the case studies. This helps to focus the risk evaluation to seed factors. As new plants engineered for modified oil content progress into large scale field testing toward commercial development, changes in seed parameters that may have smaller effects on fitness should be examined. These factors could include: duration of seed production; seed dormancy; and germination and emergence under various conditions.

"Is there evidence to indicate engineering the pathway under consideration may produce effects (either directly or secondarily) that impact confinement of field trials?"

Unlike the other case studies presented at the workshop, the engineered plants considered in the modified oil case studies have been deregulated by APHIS. They have already been field tested extensively and have been found to be no different than their nontransgenic counterpart with respect to characteristics that may impact confinement. In addition, we have a long history of traditional breeding and release of oil-seed crops with modified oil quality or quantity. These practices have not led to the identification of any phenotypic changes of the type and magnitude that would affect confinement as in the case of field testing of regulated articles.

"Are there areas that would benefit from additional research? What data or experiments would address these areas?"

Several areas of research were identified that might produce information useful in helping to assess the risks of plants intended for commercialization. We noted that much of this information was not critical to field testing. Areas discussed were that following:

- Effects of distance and other parameters on pollination frequencies for various crops—such data would be helpful in validating current isolation distances required for various crops engineered with various categories of transgenes.

- Baseline data for ecological studies—this type of data is necessary to interpret ecological changes that may be detected during field testing. Such data would help to define normal ranges in agricultural settings and would be useful in determining whether such changes are beneficial, neutral, or deleterious.

- Transcript profiling—appears to be very useful as a tool for academic research, but we do not see an immediate application as a screen for safety.

**CONCLUSION**

As a general conclusion, we do not see a need for changing the regulatory criteria for field testing based on our case studies of crops genetically engineered for altered oil content. This does not mean that modification of lipid pathways or other metabolic pathways cannot have effects that can alter plants phenotypes in ways that may affect their ecology, but rather that we see no indication that such changes would be of the magnitude that they would significantly affect containment in field trials. As field testing is scaled up, as a result of favorable results and predictable behavior in initial trials, more extensive data should collected to detect smaller changes prior to commercialization.

**Primary Reference**


**Other Oil Modification References**


