Notices

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This section of the FEDERAL REGISTER contains documents other than rules or proposed rules that are applicable to the public. Notices of hearings and investigations, committee meetings, agency decisions and rulings, delegations of authority, filing of petitions and applications and agency statements of organization and functions are examples of documents appearing in this section.

DEPARTMENT OF AGRICULTURE

Animal and Plant Health Inspection Service

[Docket No. 94-052-2]

Availability of Determination of Nonregulated Status for Genetically Engineered Canola

AGENCY: Animal and Plant Health Inspection Service, USDA.

ACTION: Notice.

SUMMARY: We are advising the public of our determination that certain Laurate canola lines are no longer considered regulated articles under our regulations governing the introduction of certain genetically engineered organisms. Our determination is based on our evaluation of data submitted by Calgene, Inc., in its petition for a determination of nonregulated status, an analysis of other scientific data, and our review of comments received from the public in response to a June 1994 notice announcing our receipt of the Calgene petition. This notice also announces the availability of our written determination document and its associated environmental assessment and finding of no significant impact.

EFFECTIVE DATE: October 31, 1994.

ADDRESSES: The determination, an environmental assessment and finding of no significant impact, the petition, and all written comments received regarding the petition may be inspected at USDA, room 1141, South Building, 14th Street and Independence Avenue SW., Washington, DC, between 8 a.m. and 4:30 p.m., Monday through Friday, except holidays. Persons wishing to inspect those documents are asked to call in advance of visiting at (202) 690-2817.

FOR FURTHER INFORMATION CONTACT: Dr. Sivramiah Shantharam, Chief, Microorganisms Branch, Biotechnology Permits, BBEP, APHIS, USDA, room 850, Federal Building, 6505 Belcrest Road, Hyattsville, MD 20782, (301) 436-7612. To obtain a copy of the determination or the environmental assessment and finding of no significant impact, contact Ms. Kay Peterson at (301) 436-7601.

SUPPLEMENTARY INFORMATION:

Background

On March 31, 1994, the Animal and Plant Health Inspection Service (APHIS) received a petition from Calgene, Inc., of Davis, CA, seeking a determination that certain Laurate canola lines and their progeny do not present a plant pest risk and, therefore, are not regulated articles under APHIS' regulations in 7 CFR part 340.

On June 14, 1994, APHIS published a notice in the Federal Register (59 FR 30569, Docket No. 94-052-1) announcing the receipt of the Calgene petition and stating that the petition was available for public review. The notice also discussed the role of APHIS and the Food and Drug Administration in regulating the Laurate canola lines and food products derived from them. In the notice, APHIS solicited written comments from the public as to whether the Laurate canola lines posed a plant pest risk. The comments were to have been received by APHIS on or before August 15, 1994.

APHIS received a total of 17 comments on the Calgene petition. The comments were submitted by farmers, associations, universities, a farm cooperative, State officials, a seed company, and an environmental organization. Most of the comments were in favor of the petition; none were critical of this particular petition. APHIS has provided a discussion of the comments in the determination document, which is available upon request from the individual listed under FOR FURTHER INFORMATION CONTACT.

Analysis

Laurate canola has been described by Calgene as any *Brassica napus* cultivar or its progeny containing the 12:0 ACP thioesterase (TE) gene from California bay (*Umbellularia californica*) (referred to below as the bay TE gene) with its associated napin promoter and napin terminator regions. The bay TE gene encodes the 12:0 ACP thioesterase enzyme. Activity of the bay TE enzyme results in the accumulation of the 12-carbon saturated fatty acid, laurate, in the canola seed. Laurate canola may also contain a kanamycin resistance (nptII) gene, 35S promoter from cauliflower mosaic virus, *tml* 3' terminator, *ori* (origin of replication) pRi from *Agrobacterium rhizogenes*, a segment of the transposable element Tn5, right and left T-DNA border sequences from *Agrobacterium tumefaciens*, and a Lac Z' polylinker sequence. The bay TE gene is expressed only in the seed by a seed-specific napin promoter from *Brassica rapa*.

The Laurate canola lines have been considered "regulated articles" under APHIS' regulations in 7 CFR part 340 because their noncoding regulatory sequences were derived from the plant pathogens *A. tumefaciens* and cauliflower mosaic virus. However, 22 field tests of the Laurate canola lines have been conducted at approximately 21 sites in California, Georgia, and Michigan under permits issued by APHIS, and the field reports from those tests indicate that there were no deleterious effects on plants, nontarget organisms, or the environment as a result of the Laurate canola lines' release into the environment.

Determination

Based on its analysis of the data submitted by Calgene, a review of other scientific data, the comments received from the public, and a review of field tests of the original transformant lines pCGN3828-212/86-18 and pCGN3828-212/86-23 and other lines derived from those two transformants, APHIS has determined that the subject Laurate canola lines: (1) Exhibit no plant pathogenic properties; (2) are no more likely to become weeds than their nonengineered parental varieties; (3) are unlikely to increase the weediness potential of any other cultivated plant or native wild species with which the organisms can interbreed; (4) will not cause damage to processed agricultural commodities; and (5) are unlikely to harm other organisms, such as bees or earthworms, that are beneficial to agriculture. APHIS has also concluded that there is a reasonable certainty that new progeny varieties bred from the Laurate canola lines pCGN3828-212/86-18 and pCGN3828-212/86-23 will not exhibit new plant pest properties, i.e., properties substantially different from any observed in the field-tested

Laurate canola lines, or those observed in standard canola in traditional breeding programs.

The effect of this determination is that the two original transformant Laurate canola lines designated pCGN3828–212/86–18 and pCGN3828–212/86–23, and all other lines bred from those two transformants by sexual or asexual reproduction involving Mendelian inheritance, are no longer considered regulated articles under APHIS' regulations in 7 CFR part 340. Therefore, the permit and notification requirements pertaining to regulated articles under those regulations no longer apply to the field testing, importation, or interstate movement of the subject Laurate canola lines or their progeny. However, the importation of the Laurate canola lines and any Laurate canola nursery stock or seeds capable of propagation is still subject to the restrictions found in APHIS' foreign quarantine notices in 7 CFR part 319.

National Environmental Policy Act

An environmental assessment (EA) has been prepared to examine the potential environmental impacts associated with this determination. The EA was prepared in accordance with: (1) The National Environmental Policy Act (NEPA) of 1969 (42 U.S.C. 4321 *et seq.*), (2) Regulations of the Council on Environmental Quality for Implementing the Procedural Provisions of NEPA (40 CFR parts 1500–1508), (3) USDA Regulations Implementing NEPA (7 CFR part 1b), and (4) APHIS Guidelines Implementing NEPA (44 FR 50381–50384, August 28, 1979, and 44 FR 51272–51274, August 31, 1979). Based on that EA, APHIS has reached a finding of no significant impact (FONSI) with regard to its determination that the Laurate canola lines designated as pCGN3828–212/86–18 and pCGN3828–212/86–23, and other lines bred from those two transformants by sexual or asexual reproduction involving Mendelian inheritance, are no longer regulated articles under its regulations in 7 CFR part 340. Copies of the EA and the FONSI are available upon request from the individual listed under **FOR FURTHER INFORMATION CONTACT.**

Done in Washington, DC, this 31st day of October, 1994.

Terry L. Medley,

Acting Administrator, Animal and Plant Health Inspection Service.

[FR Doc. 94–27405 Filed 11–3–94; 8:45 am]

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Response to Calgene Petition 94-090-01p for Determination of Nonregulated Status for Laurate Canola Lines

Environmental Assessment and Finding of No Significant Impact

October 1994

Finding of No Significant Impact

The Animal and Plant Health Inspection Service (APHIS), United States Department of Agriculture, has prepared an environmental assessment prior to issuing a determination in response to a petition (APHIS Number 94-090-01p) received from Calgene, Inc. regarding the status of Laurate canola under APHIS regulations at 7 CFR Part 340. The plants have been engineered with a gene that results in accumulation of laurate, a saturated fatty acid, in canola seed. Based upon the analysis documented in its environmental assessment, APHIS has reached a finding of no significant impact on the environment from its determination that certain lines of Laurate canola shall no longer be regulated articles.

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Acting Director
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Animal and Plant Health Inspection Service
U.S. Department of Agriculture
Date: OCT 31 1994

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APPENDICES

I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS), U.S. Department of Agriculture (USDA), has prepared an Environmental Assessment (EA) in response to a petition (APHIS Number 94-090-01p) from Calgene, Inc. regarding Laurate canola. Calgene seeks a determination that certain Laurate canola lines do not present a plant pest risk and should therefore no longer be a regulated article under regulations at 7 CFR Part 340. Laurate canola has been genetically engineered to express a gene that results in accumulation of laurate, a saturated fatty acid, in canola seed.

Calgene submitted its petition after the completion of field tests of Laurate canola at 21 sites in 3 States, conducted since 1991 under 5 APHIS permits. Field trial reports from these tests demonstrate no deleterious effects on plants, nontarget organisms, or the environment. All field trials were performed under conditions of physical and reproductive confinement.

An Environmental Assessment (EA) was prepared prior to granting each of the permits for a field trial using Laurate canola. The EAs for the previous introductions of Laurate canola addressed plant pest risk issues relative to the conduct of field trials under physical and reproductive confinement. This EA specifically addresses the potential for impacts to the human environment through use in agriculture of Laurate canola.

APHIS has considered the information provided by Calgene in its petition as well as other scientific data and comments received from the public relating to the potential plant pest risk of Laurate canola. A thorough evaluation of the potential for significant impact to the human environment through the unconfined, agricultural use of Laurate canola has brought APHIS to a Finding of No Significant Impact (FONSI). This conclusion is based upon (1) the purpose of the genetic modification; (2) the fact that this modification will not increase the weediness of canola or any sexually compatible plants; and (3) the fact that this modification will not negatively effect any nontarget organisms, including beneficials. In conjunction with the FONSI, APHIS has made the determination that certain Laurate canola lines and their progeny have no potential to pose a plant pest risk, and are, therefore, no longer a regulated articles. Our documentation of that determination is attached as Appendix A.

II. INTRODUCTION

This EA examines potential environmental impacts from the unrestricted introduction of Laurate canola. Laurate canola has been extensively field tested under permit by Calgene since 1991. The genetic material introduced into this line has been discussed in detail in EAs prepared for field tests under APHIS permits 91-346-01r, 92-156-01r, 92-163-01r, 92-244-01r, and 92-363-01r. Calgene has presented field data

reports for all these release permits. These reports give information on rates of seed germination, seedling vigor, stand establishment, days to flowering, days to maturity, yield, oil content, and laurate content. The only significant consistent difference between Laurate canola and the parent variety is the increase in laurate content from less than 0.1% to greater than 10%. In some trials there is also a reduced yield in Laurate canola that is significant. Such yield losses are often encountered during variety development.

All field trials were performed under conditions of physical and reproductive confinement. Further discussions of the biology of canola as well as of the genetic components of Laurate canola are found in APHIS Determination of Nonregulated Status. Because this information is included as Appendix A, it will not be described in detail in the body of this document.

Prior to issuing a permit for a field release, APHIS analyzes the potential impacts associated with the proposed introduction, and prepares an environmental assessment which documents the analysis in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969 (42 USC 4321 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274). APHIS also evaluates the potential for significant impact to the human environment from its determination of nonregulated status.

A genetically engineered organism is considered a regulated article if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in the regulation and is also a plant pest, or if there is reason to believe that it is a plant pest. The transgenic canola plants described in the Calgene petition have been considered regulated articles because noncoding DNA regulatory sequences are derived from the plant pathogens *Agrobacterium tumefaciens*, *A. rhizogenes*, and cauliflower mosaic virus, and because *A. tumefaciens* was used as a vector agent.

III. PURPOSE AND NEED

The purpose of this EA is to ascertain whether the approval of a petition submitted to USDA/APHIS for the determination of nonregulated status of Laurate canola, which will allow the unconfined introduction of the article, will have a significant impact on the environment. A petition was submitted to APHIS pursuant to regulations codified in 7 CFR Part 340 entitled "Introduction of Organisms and Products Altered or Produced Through Genetic Engineering Which Are Plant Pests or Which There is Reason to Believe Are Plant Pests." The regulations govern the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is not subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status," provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be granted, thereby allowing for unregulated introduction of the article in question. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of that article or its progeny. Normal agronomic practices with it, e.g., cultivation, propagation, movement, and cross-breeding could then be conducted without APHIS approval.

The Food and Drug Administration (FDA) has authority to ensure the safety and wholesomeness of all food(s), other than meat and poultry. FDA's policy statement concerning the regulation of foods derived from new plant varieties, including genetically engineered plants, was published in the Federal Register on May 29, 1992 (57 FR 22984-23005). Regulatory oversight for the safety of any food or feed products derived from laurate canola lines is under the jurisdiction of the FDA.

IV. ALTERNATIVES

In the course of preparing the environmental assessment for Calgene's petition, APHIS considered the following three alternatives: (1) deny the petition, so that Laurate canola would continue to be regulated under 7 CFR Part 340; (2) approve the petition, with geographical limitations; and (3) approve the petition, so that permits would no longer be required from APHIS under 7 CFR Part 340 for Laurate canola when grown in the United States and its territories. Based on the biology of canola, the nature of the genetic change, data and information presented by Calgene, scientific literature, and information and comment provided by the public, APHIS could find no basis for denying the petition (Alternative 1), or for imposing geographical limitations on the use of Laurate canola (Alternative 2).

V. AFFECTED ENVIRONMENT AND POTENTIAL ENVIRONMENTAL IMPACTS

Potential impacts to be addressed in this EA are those that pertain to the use of Laurate canola in the absence of confinement.

Potential impacts based on increased weediness of Laurate canola relative to traditionally bred canolas

Almost all definitions of weediness stress as core attributes the undesirable nature of weeds from the point of view of humans; from this core, individual definitions differ in approach and emphasis (Baker, 1965; de Wet and Harlan, 1975; Muenscher, 1980). In further analysis of weediness, Baker (1965) listed 12 common weed attributes, almost all pertaining to sexual and asexual reproduction, which can be used as an imperfect guide to the likelihood that a plant will behave as a weed. Keeler (1989) and Tiedje et al. (1989) have adapted and analyzed Baker's list to develop admittedly imperfect guides to the weediness potential of transgenic plants; both authors emphasize the importance of looking at the parent plant and the nature of the specific genetic changes.

Despite its ability to volunteer, escape from cultivated fields, and form temporary occasional populations, the parent plant in this petition, *Brassica napus*, is not a weed under conditions found in the United States. *B. napus* is not listed as a weed in Weed Science Society of America (1989). The comprehensive world list of Holm et al. (1991) does not list it as a serious or principal weed anywhere in the world; they do, however, give two listings as a common weed: one in Finland and one in Kenya. *B. napus* is mentioned as an "occasional weed" by Munz (1968), and "sometimes escaped" by Bailey (1949). Calgene has submitted substantial evidence to indicate the lack of weedy nature of transformed and nontransformed canolas under U.S. agricultural conditions. They have reviewed the weed literature, conducted experiments on dormancy, germination, and persistence in both greenhouse and field conditions.

The relevant introduced trait, laurate content, is extremely unlikely to increase weediness of this canola. Such an alteration, because it does not confer any pest resistance or alter reproductive biology or change any physiology related to survival, does not confer a competitive advantage favoring the canola plants over unmodified varieties. To increase weediness of the canola plant there would have to be selection pressure on Laurate canola (Tiedje et al., 1989; Office of Technology Assessment, 1988). Calgene data from field trials show no obvious increase in volunteers from seed, increase in seed dormancy, or other variation indicative of increased weediness.

While isolated environments have fragile ecologies that have frequently been damaged through human intervention, Calgene data from a number of test sites show no potential differences in impacts on such environments from traditional canola varieties that are not subject to regulation under 7 CFR Part 340.

Potential impacts from outcrossing of Laurate canola to wild relatives

Whereas intra-specific crosses between *B. napus* cultivars occur readily, inter-specific crosses between *B. napus* and related species occur with varying degrees of success and are influenced greatly by the direction of the cross. Even where there is a possibility of hybridization between *B. napus* and a related species growing in the vicinity of a release, poor vigour and high sterility in the hybrids will generally mean that hybrids and their progeny will not survive in either an agicultural or natural habitat (Scheffler and Dale, 1994).

The potential of a gene movement, at very low level, from *B. napus* to other *Brassica* spp. such as *B. juncea* or *B. rapa*, will be subject to the availability of the target organism and the reduced fertility of the hybrids. *B. napus* can cross with *B. rapa* (under co-cultivation 1.3% hybrid seed was formed) and produce hybrids of much reduced fertility; *B. napus* can also cross at low frequency with *B. juncea* (under field co-cultivation 4.7% hybrid seed formed) and these hybrids can produce a small amount of seed and fertile progeny (Bing 1991). As with Laurate canola, the gene that codes for high amounts of laurate should not confer a competitive advantage in any other species.

Transgenic canola with kanamycin tolerance and glufosinate tolerance have been field tested to test the increased invasiveness under field conditions in the United Kingdom (Cherfas, 1991, Crawley, 1992; Crawley et al. 1993). The major conclusions of these studies are that transgenic canola is not any more aggressive than the nontransgenic canola, transgenic rapeseeds do not invade undisturbed habitats, and they do not persist in the environment into which they were introduced any more than their parents did.

Potential impact on nontarget organisms, including beneficial organisms such as bees and earthworms

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from the cultivation of Laurate canola. Neither the laurate nor the gene that produces increased laurate is known to have any toxic properties.

There is no reason to believe that deleterious effects or significant impacts on nontarget organisms, including beneficial organisms, would result from kanamycin resistance, which is used as a marker of Laurate Canola. Safety concerns for human and animal consumption of products with kanamycin resistance are not addressed under 7 CFR Part 340. They are specifically addressed by the Food and Drug Administration in 21 CFR Parts 173 and 573.

Consideration of potential environmental impacts associated with the cultivation of Laurate canola outside the United States

APHIS has also considered potential environmental impacts outside the United States and its territories associated with the potential approval of this Laurate canola in the United States.

Several factors contribute to the conclusion that there should be no impacts abroad from cultivation of this canola line or its progeny.

Any international traffic in the canolas subject to this determination would be fully subject to national and regional phytosanitary standards promulgated under the International Plant Protection Convention (IPPC).

The IPPC has set a standard for the reciprocal acceptance of phytosanitary certification among the nations that have signed or acceded to the Convention (98 countries as of December, 1992). The treaty, now administered by a Secretariat housed with the Food and Agriculture Organization in Rome, came into force on April 3, 1952, and establishes standards to facilitate the safe movement of plant materials across international boundaries. Plant biotechnology products are fully subject to national legislation and regulations, or regional standards and guidelines promulgated under the IPPC. The vast majority of IPPC signatories have promulgated, and are now adminstering, such legislation or guidelines. The IPPC has also led to the creation of Regional Plant Protection Organizations (RPPOs) to facilitate regional harmonization of phytosanitary standards.

Issues that may relate to commercialization of particular agricultural commodities produced through biotechnology are being addressed in international forums. APHIS has played a role in working toward harmonization of biosafety and biotechnology guidelines and regulations included within the RPPO for our region, the North American Plant Protection Organization (NAPPO), which includes Mexico, Canada, and the United States. NAPPO's Biotechnology Panel advises NAPPO on biotechnology issues as they relate to plant protection.

APHIS participates regularly in biotechnology policy discussions at forums sponsored by the European Union and the Organization for Economic Cooperation and Development. In addition, APHIS periodically holds bilateral or quadrilateral discussions on biotechnology regulatory issues with other countries, most often Canada and Mexico. APHIS also acts as a consultant for the development of biotechnology guidelines and regulations, and has interacted with governments around the world in this manner, including those in regions where canola originated or is cultivated in significant quantities (e.g., China, Japan, Korea, Association of South East Asian Nations member States, India, Pakistan, African States, and more). We have participated in numerous conferences intended to enhance international cooperation on safety in biotechnology, and sponsored several workshops on safeguards for planned introductions of transgenic crops (crucifers, maize, wheat, potatoes, rice, tomatoes) most of which have included consideration of international biosafety issues.

In the course of these wide-ranging studies and interactions, APHIS has not identified any impacts on the environment that might be relevant to Laurate canola or follow from the unconfined cultivation of this canola line in the United States and its territories, or abroad. In addition to the assurance provided by the analysis leading APHIS to a finding of no significant impact for the introduction of this canola variety, it should be noted that all the considerable, existing national and international regulatory authorities and phytosanitary regimes that currently apply to introductions of new canola cultivars internationally apply equally to those covered by this determination.

VI. CONCLUSIONS

In accordance with the requirements of NEPA, APHIS has considered the potential for significant impact on the environment of a proposed action, i.e, reaching the determination that Laurate canola has no potential to pose a plant pest risk and should no longer be considered a regulated article under the regulations at 7 CFR Part 340. After careful analysis of the available information, APHIS concludes that its proposed action will not have a significant impact on the environment, and that the proper alternative is to approve the petition. This conclusion is based on factors discussed herein or in the determination included as appendix A, as well as the following conclusions:

1. A gene that results in accumulation of laurate has been inserted into a canola chromosome in Laurate canola. In nature, chromosomal genetic material from plants can only be transferred to another sexually compatible flowering plant by cross-pollination.

2. Neither the gene that results in accumulation of laurate, nor the laurate itself, nor its associated regulatory sequences, confers on Laurate canola or its progeny any plant pest characteristic.

3. In nature, the gene that results in accumulation of laurate will not provide Laurate canola or its progeny with any measurable selective advantage over nontransformed canola plants in their ability to disseminate or to become established in the environment. There is no reason to believe that Laurate canola exhibits any increased weediness relative to that of traditional varieties.

4. The use of Laurate canola or its progeny in agriculture will not lead to an increase in weediness in any plant with which it can successfully interbreed.

5. The use of Laurate canola or its progeny in agriculture will not have a significant impact on any beneficial organisms in the environment, or on any threatened or endangered species.

VII. LITERATURE CITED

Baker, H. G. 1965. Characteristics and Modes of Origin of Weeds. In: The Genetics of Colonizing Species. pp. 147-172. Baker, H. G., Stebbins, G. L. (eds.). Academic Press, New York and London.

Bailey, L. H. 1949. Manual of Cultivated Plants. Macmillan Publishing Company, New York. 1116 pp.

Bing, D. J. 1991. Potential of Gene Transfer Among Oilseed Brassica and their Weedy Relatives. Master of Science Thesis, University of Saskatchewan. 155 pp.

Cherfas, J. 1991. Transgenic crops get a test in the wild. Science 251:878.

Crawley, M. J. 1992. The comparative ecology of transgenic and conventional crops. Proceedings of 2nd International Symposium on the Biosafety Results of Field Tèsts of Genetically Modified Plants and Microorganisms. Goslar, Germany.

Crawley, M. J., Hails, R. S., Rees, M., Kohn, D., and Buxton, J. 1993. Ecology of transgenic oilseed rape in natural habitats. Nature 363: 620-623.

de Wet, J. M. J., Harlan, J. R. 1975. Weeds and Domesticates: Evolution in the Man-Made Habitat. Economic Botany. 29:99-107.

Holm, L., Pancho, J. V., Herbarger, J. P., Plucknett, D. L. 1979. A Geographical Atlas of World Weeds. John Wiley and Sons, New York. 391 pp.

Keeler, K. 1989. Can genetically engineered crops become weeds? Bio/Technology 7:1134-1139.

Muenscher, W. C. 1980. Weeds. Second Edition. Cornell University Press, Ithaca and London. 586 pp.

Munz, P. A. 1968. A California Flora. University of California Press, Berkeley and Los Angeles. 1681 pp.

Office of Technology Assessment, United States Congress. 1988. New Developments in Biotechnology-- 3. Field-Testing Engineered Organisms: Genetic and Ecological Issues. U.S. Government Printing Office, Washington, DC. 150 pp.

Reed, C. F. 1970. Selected Weeds of the United States. Agriculture Handbook No. 366. Agricultural Research Service, United States Department of Agriculture, Washington, D.C. 463 pp.

Scheffler, J. A. and Dale P. J. 1994. Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. Transgenic Research 3:263-278.

Tiedje, J. M., Colwell, R. K., Grossman, Y. L., Hodson, R. E., Lenski, R. E., Mack, R. N., Regal, P. J. 1989. The Planned Introduction of Genetically Engineered Organisms: Ecological Considerations and Recommendations. Ecology 70:298-315.

Weed Science Society of America. 1989. Composite List of Weeds. WSSA. Champaign, Illinois.

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RESPONSE TO CALGENE PETITION 94-090-01p FOR DETERMINATION OF NONREGULATED STATUS FOR LAURATE CANOLA LINES

October 1994

Prepared by
United States Department of Agriculture
Animal and Plant Health Inspection Service
Biotechnology, Biologics, and Environmental Protection

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I. SUMMARY

The Animal and Plant Health Inspection Service (APHIS) has determined, based on a review of scientific data and information that two original transformed Laurate canola lines designated pCGN3828-212/86-18 and pCGN3828-212/86-23 and all other lines bred or otherwise derived from these two transformants by sexual or asexual reproduction involving Mendelian inheritance do not present a plant pest risk, and are therefore no longer regulated articles under 7 CFR Part 340. As a result of this determination, approval under those regulations will no longer be required from APHIS for planting, importation, or interstate movement of the above mentioned Laurate canola lines or their progeny. (Importation of Laurate canola lines mentioned above, and nursery stock or seeds capable of propagation are still subject to restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319. Variety registration and/or seed certification for individual Laurate canola lines may involve future actions by the U.S. Plant Variety Protection Office and State Seed Certification officials.

APHIS determination has been made in response to a petition from Calgene, Inc., Davis, California, received on March 31, 1994. The petition seeks a determination from APHIS that Laurate canola lines and their progeny do not present a plant pest risk and are therefore no longer regulated articles. On June 14, 1994, APHIS announced receipt of the Calgene petition in the Federal Register (58 FR Vol. 59, No. 113, 30569) and stated that the petition was available for public review. APHIS also indicated its role, as well as those of the Food and Drug Administration (FDA), in regulation of Laurate canola lines, and food products derived from it. APHIS invited written comments on whether Laurate canola lines pose a plant pest risk, to be submitted on or before August 15, 1994.

Laurate canola lines have been described by Calgene as any *B. napus* cultivar or progeny containing the bay TE gene with its associated napin promoter, napin terminator regions, and it may also contain a kanamycin resistance (nptII) gene, 35S promoter from the cauliflower mosaic virus, *tml* terminator, *ori* (origin of replication) pRi from *Agrobacterium rhizogenes*, a segment of the transposable element Tn5, right and left T-DNA border sequences from *A. tumefaciens*, and a lac Z' polylinker sequence. The bay TE gene isolated from California bay (*Umbellularia californica*) encodes the 12:0 thioesterase enzyme that results in the accumulation of the 12 carbon saturated fatty acid, Laurate, in the canola seeds. The bay TE gene is expressed only in the seed by a seed specific promoter, napin, from *B. rapa*. Each of the significant introduced sequences will be discussed in detail in Section IV of this determination.

APHIS regulations 7 CFR Part 340 promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation,

interstate movement, or release into the environment) of certain genetically engineered organisms and products. An organism is not subject to the regulatory requirements of Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data, and determine that a particular regulated article does not present a plant pest risk, and therefore should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition would be granted that would allow for introduction of the regulated article (organisms) in question without permits or notifications under 7 CFR Part 340. In this instance, they are Laurate canola lines.

The Laurate canola lines have been considered "regulated articles" because they contain components from organisms that are known plant pathogens, i.e., the bacteria *Agrobacterium tumefaciens* and *A. rhizogenes*, and cauliflower mosaic virus. Field testing of the Laurate canola lines has been conducted with APHIS approval since 1991. Calgene submitted its petition after the completion of field tests of Laurate canola lines under 5 APHIS permits on 21 sites spread over 3 states. All field trials were performed under conditions of physical and reproductive confinement.

APHIS has determined that the Laurate canola lines identified in the petition do not present a plant pest risk, and therefore, will no longer be considered a regulated article under APHIS regulations at 7 CFR Part 340. The Agency's decision is based on an analysis of data provided to APHIS by Calgene as well as other scientific data, and comments received from the public relating to the potential plant pest risk of Laurate canola lines. Calgene provided both general and specific information and data from field testing of Laurate canola lines. From our review, we have determined that Laurate canola lines: (1) exhibit no plant pathogenic properties; (2) are no more likely to become a weed than their non-engineered parental varieties; (3) are unlikely to increase the weediness potential of any other cultivated plant or native wild species with which they can breed; (4) will not cause damage to processed agricultural commodities; and (5) are unlikely to harm other organisms, such as bees and earthworms that are beneficial to agriculture. APHIS has also concluded that there is a reasonable certainty that new progeny varieties bred from Laurate canola lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the field tested Laurate canola lines, or those observed for canola in traditional breeding programs.

The potential environmental impacts associated with this determination have been examined in accordance with regulations and guidelines implementing the National Environmental Policy Act of 1969 (42 USC 4331 *et seq.*; 40 CFR 1500-1508; 7 CFR Part 1b; 44 FR 50381-50384; and 44 FR 51272-51274). An Environmental Assessment (EA) was prepared and

a Finding of No Significant Impact (FONSI) was reached by APHIS for the determination that Laurate canola lines are no longer regulated articles under its regulations at 7 CFR Part 340. The EA and FONSI are available from APHIS upon written request.

This document consists of two parts: (1) background information which provides the regulatory framework under which APHIS has regulated the field testing, interstate movement, and importation of Laurate canola lines, as well as a summary of public comments provided to APHIS on its proposed action; and (2) analysis of the key factors relevant to APHIS' decision that Laurate canola lines do not present a plant pest risk.

II. BACKGROUND

USDA Regulatory Authority. APHIS regulations, which were promulgated pursuant to authority granted by the Federal Plant Pest Act (FPPA), (7 U.S.C. 150aa-150jj) as amended, and the Plant Quarantine Act (PQA), (7 U.S.C. 151-164a, 166-167) as amended, regulate the introduction (importation, interstate movement, or release into the environment) of certain genetically engineered organisms and products. A genetically engineered organism is deemed a regulated article either if the donor organism, recipient organism, vector or vector agent used in engineering the organism belongs to one of the taxa listed in § 340.2 of the regulations and is also a plant pest; if it is unclassified; or if APHIS has reason to believe that the genetically engineered organism presents a plant pest risk.

Prior to the introduction of a regulated article, a person is required under § 340.1 of the regulations to either (1) notify APHIS in accordance with § 340.3 or (2) obtain a permit in accordance with § 340.4. Introduction under notification (§ 340.3) requires that the introduction meets specified eligibility criteria and performance standards. The eligibility criteria impose limitations on the types of genetic modifications that qualify for notification, and the performance standards impose limitations on how the introduction may be conducted. Under § 340.4, a permit is granted for a field trial when APHIS has determined that the conduct of the field trial, under the conditions specified by the applicant and/or stipulated by APHIS, does not pose a plant pest risk.

The FPPA gives USDA authority to regulate plant pests and other articles to prevent direct or indirect injury, disease, or damage to plants, plant products, and crops. The PQA provides an additional level of protection by enabling USDA to regulate the importation and movement of nursery stock and other plants which may harbor injurious pests or diseases, and requires that they be grown under certain conditions after importation. For certain genetically engineered organisms, field testing may be required to verify that they exhibit the expected biological properties, and to demonstrate that although

derived using components from plant pests, they do not possess plant pest characteristics.

An organism is no longer subject to the regulatory requirements of 7 CFR Part 340 when it is demonstrated not to present a plant pest risk. Section 340.6 of the regulations, entitled "Petition Process for Determination of Nonregulated Status", provides that a person may petition the Agency to evaluate submitted data and determine that a particular regulated article does not present a plant pest risk and should no longer be regulated. If the agency determines that the regulated article does not present a risk of introduction or dissemination of a plant pest, the petition may be granted. A petition may be granted in whole or in part.

Laurate canola lines have been considered "regulated articles" for field testing under Part 340.0 of the regulations because the noncoding regulatory sequences were derived from the plant pathogens *A. tumefaciens*, *A. rhizogenes*, and cauliflower mosaic virus.

APHIS believes it prudent to provide assurance prior to commercialization that organisms, such as the Laurate canola lines developed in part from plant pest sequences, do not pose any potential plant pest risk. Such assurance may aid the entry of new plant varieties into commerce or into breeding and development programs. The decision by APHIS that Laurate canola lines are no longer a regulated article is based in part on evidence provided by Calgene concerning the biological properties of the Laurate canola lines, and their similarity to other varieties of canola grown using standard agricultural practices for commercial sale or private use. Laurate canola lines have field tested under 5 permits (91-346-01, 92-156-01, 92-163-01r, 92-244-01r, and 92-363-01r). The 22 completed field tests took place at approximately 21 sites in the following 3 states: California, Georgia, and Michigan. Field trial reports from these tests show no deleterious effects on plants, nontarget organisms, or the environment as a result of these releases.

The fact that APHIS regulates genetically engineered organisms having plant pest components does not carry with it the presumption that the presence of part of a plant pest makes a whole plant pest or that plants or genes are pathogenic. The regulations are based on the premise that when plants are developed using biological vectors from pathogenic sources, transforming material from pathogenic sources, or pathogens are used as vector agents, that they should be evaluated to assure that there is not a plant pest risk (McCammon and Medley, 1990). For each release permit application APHIS performs a review that allows a verification of the biology and procedures used; assesses the degree of uncertainty and familiarity; evaluate mitigating factors and agricultural practices of the crop in question and allows the identification of any predictable hazards. The overall aims of APHIS regulations in the Code of Federal Regulations at 7 CFR Part 340 are to allow for the safe testing of genetically engineered organisms under an appropriate level of oversight, and to enable any

issues of potential or hypothetical risks to be addressed early enough in the development of the new organisms to allow for the safe use and application of biotechnology in agriculture.

A certification that an organism does not present a plant pest risk means that there is reasonable certainty that the organism cannot directly or indirectly cause disease, injury, or damage either when grown in the field, or when stored, sold, or processed. This approach is considerably broader than a narrow definition of plant pest risk arising from microbial or animal pathogens, including insect pests. Other traits, such as increased weediness, and harmful effects on beneficial organisms, such as earthworms and bees, are clearly subsumed within what is meant by direct or indirect plant pest risk. In APHIS' regulations at 7 CFR Part 340, a "plant pest" is defined as: "Any living stage (including active and dormant forms) of insects, mites, nematodes, slugs, snails, protozoa, or other invertebrate animals, bacteria, fungi, other parasitic plants or reproductive parts thereof; viruses; or any organisms similar to or allied with any of the foregoing; or any infectious agents or substances, which can directly or indirectly injure or cause disease or damage in or to any plants or parts thereof, or any processed, manufactured, or other products of plants."

Lack of plant pest risk may be arrived at when there is evidence that the plant under consideration: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than its non-engineered parental varieties; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organism can interbreed; (4) does not cause damage to processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture. In addition, because the Calgene petition seeks a determination regarding Laurate canola lines, it should be established that there is a reasonable certainty that any new Laurate canola varieties bred from these Laurate canola lines will exhibit plant pest properties not different from any observed for canola in traditional breeding programs or as seen in the development of Laurate canola lines.

Oversight by Other Federal Agencies. The EPA regulates the use of pesticide chemicals, including herbicides, in the environment. Under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) (7 U.S.C. 136 et seq.), EPA has the authority to regulate the testing, sale, distribution, use, storage, and disposal of pesticides. Before a pesticide may be sold, distributed, or used in the United States, it must be registered under FIFRA Section 3. For a pesticide that is already registered, the use of the pesticide on a new crop plant (i.e., use on a crop for which the pesticide is not already registered) requires EPA approval of an amendment to the registration. In determining whether to approve the new use of the pesticide, EPA considers the possibility of adverse effects to human health and the environment from the new use. Under the Federal Food, Drug and

Cosmetic Act (FFDCA) (21 U.S.C. 201 et seq.), EPA also has responsibility for establishing tolerances for pesticide residues on food or feed. No pesticidal substances are at issue for this determination.

The FFDCA provides FDA with authority to ensure the safety and wholesomeness of all food(s), other than meat and poultry. FDA's policy statement concerning the regulation of foods derived from new plant varieties, including genetically engineered plants, was published in the Federal Register on May 29, 1992 (57 FR 22984-23005). Regulatory oversight for the safety of any food or feed products derived from Laurate canola lines is under the jurisdiction of the FDA.

III. SUMMARY AND RESPONSE TO COMMENTS

Summary and Analysis of Comments

APHIS received 17 comments from the following: farmers (5); farm cooperatives (2); associations (3); seed company (1); universities (3); States (2); and an environmental group (1). Most comments were in favor of the petition. None were critical of this specific petition.

Comments Favorable

According to the applicant and commenters, Laurate canola offers much needed agricultural diversification for the American farmer in upper Midwest and Southeast. Laurate canola may provide a domestic source of an important raw material worth approximately $400 million for soaps and detergents. Laurate canola may work well in crop rotation programs without incurring additional expenses in terms of agricultural equipment and facilities both on the farm, and related agriculture industry. Cultivation of Laurate canola may expand the use of existing elevators and crushing plants. Laurate canola may serve as an alternative for imported coconut and palm oil and may indirectly benefit many other businesses. There may be an opportunity to provide profit opportunities in economically deprived regions.

Comments Unfavorable

None. But two comments from active researchers in the area of population ecology, risks of gene escape and its consequences, and weed risk potentials of genetically engineered canola in general and Laurate canola in particular have asked USDA/APHIS to closely monitor the large scale or commercial scale plantings for the escape of Laurate canola, and any increase in the dormancy of feral Laurate canola seeds. Also, they have urged that hybridization and backcrossing of Laurate canola with wild populations of *B. rapa* be closely monitored through some public funding mechanism as this will be an ideal opportunity study such phenomena.

There is not a single comment that identified any plant pest risk issue associated with the commercialization or large scale plantings of Laurate canola lines.

Another comment, from an environment group, which cautioned APHIS about granting the non-regulatory status routinely for other genetically engineered canolas if they happen to be engineered to express novel genes such as disease resistance genes. The comment that backcrossing of F1 hybrids with *B. rapa* might introgress genes that confer weedier properties to the progenies. This situation is likely to happen if the conditions are favorable to such cross pollinations like the presence of a large populations of *B. rapa*, and an adequate number of insect pollinators, and the introduced gene is

controlled by a regulatory element that functions under all circumstances in all parts of the plant body. This comment does not pertain to the specific canola lines that are the subject of this petition, but to future petitions of other lines of canola. Any future petitions of canola that involve other traits will be evaluated on the nature of the trait and other characteristics of the specific transformed canola lines.

Response to Comments

All comments received expressed a support in favor of determining that these Laurate canola lines be no longer regulated. APHIS agrees with all the comments that there are no plant pest risks, or weediness issue associated with Laurate canola, and that commercialization or large scale plantings of Laurate canola lines will have no significant impact on the environment.

To the suggestion that USDA provide public funding to monitor the escape of Laurate canola and increase in the seed dormancy of feral canola populations either during or following large scale plantings of Laurate canola, APHIS would like to point out that USDA/Cooperative State Research Service through its Biotechnology Risk Assessment Grants would be the venue to seek funds as it is an open and peer reviewed process.

The comment that backcrossing of F1 hybrids with *B. rapa* might introgress genes that confer weedier properties on to the progenies for transformations of canola lines that may be seen in future petitions. Results from the studies, which involved other traits that Laurate canola, of Adler et al. (1993) indicate that varied germination responses to environmental cues may be the result of different selective pressures on each type. The direction of the hybrid cross, which affected germination and dormancy, may influence the ability of crop X wild hybrids to persist in natural environments. This situation may happen if conditions are favorable to such cross pollinations, as when large populations of *B. rapa* are present, adequate number of insect pollinators are present, and the introduced gene is controlled by a regulatory element that functions under all circumstances in all parts of the plant body. The potential transfer of the Bay TE gene from Laurate canola lines should not cause much concern as such, because acquisition of the Bay TE gene will not present a plant pest risk or the risk of increased weediness.

IV. ANALYSIS OF THE PROPERTIES OF LAURATE CANOLA

A brief description of the biology of canola and canola cultivation practices is expected to be helpful in specific environmental and biosafety issues applicable to Laurate canola lines. In addition, to reach its determination that Laurate canola lines do not present a plant pest risk, APHIS has analyzed not only public comments and basic information on the biology of canola but also data presented by Calgene and scientific data on other topics relevant to a discussion of plant pest risk. Based on the data, APHIS has arrived at a series of conclusions regarding the properties of Laurate canola lines.

Biology and Cultivation of Canola

Brassica napus L., is a mustard crop grown primarily for its seed which yields about forty percent oil and a high-protein animal feed. Varieties of *B. napus* are known by the common names of rapeseed, rape, oilseed rape, and canola.

Cultivar 212/86, developed by ProDana of Denmark, a spring canola variety was used for transformation. Recent interest in the crop has centered around cultivars that have low erucic acid, and thus contain desirable edible oils. Rapeseed oils have been used for lamp oils, soap making, plastics manufacturing, and high-temperature and tenacious high-erucic acid lubricating oils (Röbbelen et al. 1989; Weiss 1983). Other species of *Brassica* are also grown for rapeseed oil, but they are not the subject of this determination.

World production of rapeseed oil in 1987-1988 was 7.5 million metric tons, ranking it number three behind canola (15.4) and palm (11.7), and before sunflower (7.0), cottonseed (3.4), and peanut (2.8) (Jewell 1989). China, India, Europe, and Canada are the top world producers (Niewiadomski 1990). Current production in the United States is limited.

Taxonomy of Rapeseed. *Brassica* is a genus within the plant family Brassicaceae (Cruciferae), which is commonly known as the mustard family. This family, of about 375 genera and 3200 species, includes species recognized as crops, condiments, ornamentals, and many weeds. *Brassica* contains about 100 species, including cabbage, cauliflower, broccoli, brussels sprouts, turnip, various mustards and weeds (Willis 1973).

B. napus belongs to a group of six genetically related species (Röbbelen et al. 1989):

> *B. nigra* (Linnaeus) Koch, black mustard, a diploid species n=8, originally spread by trade over much of the Old World, and now spread as a weed throughout much of the New World, including virtually all of the United States.

B. oleracea Linnaeus, cabbage, broccoli, brussels sprouts, cauliflower, kale, a diploid species n=9, originally confined to the Mediterranean, but now widely grown in temperate gardens.

B. rapa Linnaeus (=*B. campestris* Linnaeus), field mustard, turnip, turnip rape, bird rape, a diploid species n=10, originally spread throughout much of Europe, Asia, northern India, and northern Africa, and now either grown as a vegetable or oil crop, or spread as an occasional weed in much of the United States.

B. carinata A. Braun, Abyssinian mustard, Ethiopian mustard, an allotetraploid species n=17, derived from *B. nigra* and *B. oleracea*, presumed to come from an ancient cross or crosses in northeast Africa, and occasionally grown in the United States as a novelty.

B. juncea (Linnaeus) Czerniakowska et Cosson, Indian mustard, brown mustard, mustard greens, an allotetraploid species n=18, derived from Old World crosses of *B. nigra* and *B. rapa*, and now grown for the leaves, or spread as an occasional weed in crops or waste places.

B. napus Linnaeus, the subject of this petition, an allotetraploid species n=19, derived from ancient crosses between *B. oleracea* and *B. campestris*, and now grown widely for its oil, and an occasional weed or volunteer in cultivated fields.

Sexual Reproduction and Inter-specific Crosses in Rapeseed.

B. napus produces an inflorescence of yellow, nectar-bearing flowers. The plants are capable of both self-fertilization and intra-specific cross-fertilization. Honeybees are the primary pollinators. Partial sexual compatibility exists with some related *Brassica* spp. and other closely related species outside the genus.

Rapeseed has unexceptional entomophilous flowers capable of both self- and cross-pollination. In cultivated fields, cross-pollination has been reported at about 35%, but varies depending on the availability of insect pollinators, cultivar, and weather. Downey and Bing (1990) reported outcrossing rates of 2.1, 1.1, and 0.6 percent for isolation plots located 46, 137, and 366 meters from a pollen source. Seed certification requires a reproductive isolation distance of 660 feet for the production of Foundation Seed for B. napus, and even greater distance (1320 feet) for self-incompatible species such as B. rapa. At these distances there is a tolerance of 0.05 percent offtypes, presumably derived from pollen contamination by sources beyond the specified distance (7 CFR Part 201.76).

Honey bees are the primary pollinators of rapeseed. Although a honeybee colony may collect nectar and pollen from many species, and

potential foraging flights can be quite distant (to 10 km), several factors limit the potential for spread (Seeley, 1985) to those distances noted in the above paragraph. First, each individual honeybee forager almost always collects nectar and pollen from a single plant species during a single visit. Second, given abundant flowers, such as in a cultivated field, individual honeybee foragers tend to collect nectar and pollen from flowers in the same or immediately adjacent plants. Third, honeybees are very sensitive to barometric pressure, and decrease foraging distances in response to impending adverse weather. Fourth, honeybees are subject to the pressures of energy economics, and do not forage at great distances from the nest when abundant nectar and pollen sources are close by, as in many agricultural settings.

Whereas intra-specific crosses between *B. napus* cultivars occur readily, inter-specific crosses between *B. napus* and related species occur with varying degrees of success and are influenced greatly by the direction of the cross. The three allotetraploid species mentioned above undoubtedly arose from ancient natural crosses of diploid species, and therefore demonstrate the potential for gene movement among all these species. Bing (1991) reported the following crosses and attempted crosses of plants that may be outside cultivation or escapes from cultivation. Data reported are, in order, (1) cross performed (pistillate plant listed first, pollen plant listed second), (2) the number of hybrid seed per 100 pollinated buds, and (3) the results of co-cultivation.

> *Sinapis arvensis* x *B. napus*, no hybrid seeds, and no hybrids from field co-cultivation.
>
> *B. nigra* x *B. napus*, 0.1 hybrid seeds, and no hybrids from field co-cultivation.
>
> *B. rapa* x *B. napus*, 933.8 hybrid seeds, and 1.3% hybrids from field co-cultivation.
>
> *B. juncea* x *B. napus*, 401.9 hybrid seeds, 4.7% hybrids from field co-cultivation.

The potential of a gene movement, at very low level, from *B. napus* to other *Brassica* spp. such as *B. juncea* or *B. rapa*, will be subject to the availability of the target organism and the reduced fertility of the hybrids. *B. napus* can cross with *B. rapa* (under co-cultivation 1.3% hybrid seed was formed) and produce hybrids of much reduced fertility; (2) *B. napus* can also cross at low frequency with *B. juncea* (under field co-cultivation 4.7% hybrid seed formed) and these hybrids can produce a small amount of seed and fertile progeny (Bing 1991).

There is no published evidence for the existence of any mechanism, other than sexual crossing of compatible *Brassica* species, by which the introduced genetic sequences can be transferred to other

organisms. Another mechanism by which *B. napus* can transfer genetic material to sexually non-compatible plants is through "bridging." Bridging is defined as "a mating made between two incompatible or reproductively isolated species by first transferring the genetic material to an intermediate species that is sexually compatible with the two sexually incompatible species" (King and Stansfield, 1985). Such a possibility of the "bridging" phenomenon may occur with *B. juncea* acting as the intermediate species. The occurrence of hybrids between *B. napus* and *B. juncea* is rare, and moreover, the hybrids do not persist long enough in the environment due to poor fertility, poor germination, and high seedling mortality, to serve as a bridge species. Furthermore, crosses between *B. juncea* and *B. nigra* are not fully compatible, and it follows that crosses between *B. napus* hybrids, and *B. nigra* would be even less compatible. Another genetic barrier for gene transfer is that it has to take place by chromosomal crossing over to the bb genome in the *B. napus* and *B. juncea* hybrid to be stably introduced into *B. nigra* (Scheffler and Dale, 1994).

Comparative analyses of numerous gene sequences from microorganisms and plants have never, to our knowledge, yielded any published evidence of strong inter-kingdom gene homologies that would be indicative of recent or frequent gene exchanges between plants and microorganisms with the exception of T-DNA of the Ti-plasmid of *Agrobacterium*. A certain amount of information can be found in the scientific literature (e.g., Carlson and Chelm, 1986; Wakabayashi et al., 1986; Doolittle et al., 1990) that provides a suggestion that transfer of genes from plants to microorganisms may have occurred over evolutionary time, i.e., in the eons since the various times of divergence between the kingdoms. Bryngelsson et al. (1988) has suggested that plant DNA can be taken up by a parasitic fungus, but no evidence has ever been forthcoming that such DNA uptake has resulted in the frequent transfer of a functional DNA sequence. Even if a rare plant-to-microbe gene transfer were to take place, there is no reason to believe that such a transfer of any of the sequences would pose any plant pest risk. We conclude that concerns regarding DNA transfer from Laurate canola lines to microorganisms are, at best, highly speculative, and improbable, if not altogether impossible.

The risk of crosses between wild *B. rapa* x *B. napus* Laurate canola hybrids is lower than feral *B. napus* Laurate canola. Wild *B. rapa* x *B. napus* canola hybrids not only have much lower dormancy than the persistent wild *B. rapa* control, their dormancy level is lower than that of nontransgenic hybrid control. This finding coupled with the reduced fertility of the inter-specific hybrids makes it very unlikely that populations of hybrids will persist. There is a small chance that the hybrids could backcross to wild *B. rapa* and thereby transfer the Laurate transgene to wild populations (Crawley et al. 1993).

Neither the introduced genes, and their products, nor the added regulatory sequences controlling their expression presents a plant pest risk in Laurate canola.

The standard recombinant DNA technology to introduce the genes into plant cells (transformation) uses a recombinant plasmid (vector) molecule which is complex chimera of DNA sequences drawn from various organisms. Some of these organisms from which these DNA sequences are derived are known plant pests, and as such the transgenic crop plants or organisms become regulated articles under 7 CFR 340.

The introduction of the vector DNA does not present a plant pest risk in Laurate canola lines identified in the present petition. The vector system used to transfer the Bay TE gene into the canola nuclear genome, pCGN3828, is a derivative of a high copy *Escherichia coli* plasmid pUC19 (Viera and Messing, 1987), and does not contain any disease causing sequences from the native tumor-inducing (Ti) plasmid system used by the plant pathogenic bacterium *Agrobacterium tumefaciens* for plant infection and gene transfer (Zambryski, 1988). Additionally, there are DNA sequences derived from *A. rhizogenes*, and cauliflower mosaic virus, both of which are well known plant pathogens that are on the list of regulated articles in 7 CFR Part 340. *A. tumefaciens* is the causal agent of a plant disease called crown gall, and *A. rhizogenes* causes hairy root disease. In Laurate canola lines, none of the introduced coding regions, or the regulatory sequences or the marker genes used for the selection of the transformants confer any plant pest risk. The vector system was used as a part of transformation method known as agro-infection that involves incubating the hypocotyl explants from 7 day old seedlings of canola with A. tumefaciens EHA101 (Hood et al. 1986) containing a binary vector to accomplish the stable gene transfer. Calgene has presented evidence in Table 12 of its petition that the Bay TE gene in Laurate canola lines is transmitted through meiosis in a Mendelian fashion.

Calgene analyzed the physical structure of the integrated genetic material in Laurate canola lines (See Figures 7 in the petition, and Appendix 3, Section V)). This analysis revealed that none of the vector pCGN3828 DNA was present in the plant's genome. Southern blot analyses of the T1 and T2 generations of the Laurate canola lines clearly demonstrate that there are three copies of the Bay TE gene, and that they are transmitted to offspring in a stable Mendelian manner. The segregation analysis was done by scoring for the Geniticin marker, an analog of kanamycin (p.50-51 of the petition). These copies of the Bay TE gene contain the napin promoter and terminator sequences, and right and left hand border sequences derived from *B. rapa* storage protein gene, 35S promoter derived from cauliflower mosaic virus, APH(3')II originally isolated from the bacterial transposon Tn5, and another terminator tml 3' from *A. tumefaciens*. The bay TE gene from California bay (*U. californica*) codes for an acid in the fatty acid biosynthetic pathway found in

developing seeds. As the gene is being regulated by the napin promoter, the bay TE gene is expressed only in the seeds resulting in the accumulation of medium chain fatty acids, Laurate and myristate. Bay TE gene results in cleavage of lauroyl-ACP to release free Laurate. Laurate is normally present at no more than 0.1% in canola. The bay TE enzyme is functionally similar to canola native thioesterase enzyme, and bears 25-30% amino acid similarity between the two. It is important to note that ACP thioesterase enzyme is native to and functions in canola. There is no reason to believe that this gene or its protein product could impart any disease or cause damage to either Laurate canola lines or any other plant with which they are sexually compatible.

The gene conferring kanamycin resistance is described in a subsequent section. Neither it or any other genetic components of the construct confer a plant pest risk.

Despite the presence of certain pathogen-derived sequences in the Laurate canola lines genome, no crown gall, hairy root or CaMV disease symptoms were observed by Calgene under the field conditions. Furthermore, Calgene provides evidence that expression of the introduced gene does not result in disease symptoms or the synthesis of products toxic to other organisms. Calgene monitored the Laurate canola line field trials to verify that the severity of any disease or insect infestation of the transgenic plants and found that they did not differ from that of the parental line. No difference in disease and insect susceptibility was observed at all the sites where Laurate canola lines were tested in the United States.

Laurate Canola Lines are neither weeds nor have any significant potential to become a weed, and do not transmit weedy characteristics to sexually compatible plants.

Weediness can be broadly defined as any capacity for invasion of natural habitats (Peter Kareiva, public comment of July 25, 1994). Many species of *Brassica* and related mustards are weeds or have weedy tendencies. *B. napus* is mentioned as an occasional weed, escape, or volunteer in cultivated fields (Munz 1968, Bailey 1949, Muenscher 1980). *B. juncea*, *B. nigra*, *B. rapa*, and *S. arvensis* (=*B. kaber*) to some degree are agricultural weeds, sometimes serious, in much of the United States (Gleason 1952; Slife et al. 1960; Reed 1970; Muenscher 1980).

B. napus is the only *Brassica* species naturalized in the United States, and is not considered to be a weed in the United States (Holm et al. 1991; Ecological Society of America, 1989). Generally most crop plants are bred and carefully selected to express agriculturally useful traits, and therefore, they are not usually competitive in unmanaged or untended natural environments. In other words, they are not ecological fit to survive. Canola and other rapeseeds are very well adapted for cultivation (fertilization, herbicide, and pesticide

application), but not so for growth outside agricultural environments. Without favorable conditions, and intensive cultivation, domesticated types of *B. napus* cannot compete successfully with naturalized forms of *B. napus* in the United States. Naturalized types of *B. napus* are sporadically distributed in Canadian environments, whereas in the United Kingdom, they are widespread in the wild, although they have not been classified as weeds (Mitchell-Olds, 1992; Holm et al., 1991). Efforts are under way to confirm whether these widespread canolas are self sustaining populations or are a result of repeated introductions (van der Meijden and de Vries, 1992). In any event, non-transgenic canolas are not weeds, and the only question that arises is whether Laurate canola lines are weeds or have the potential to become weeds. From the experimental data submitted by Calgene to directly address the question, it becomes very clear that agronomic and morphological characteristics observed on Laurate canola lines do not lead to suggest that Laurate canola lines are either weeds or have the potential to become weeds (Table 13a, and 13b). None of the Laurate canola lines showed increased seedling vigor, or overwintering ability.

Transgenic canola that are not Laurate canola, have been field tested to test the increased invasiveness under field conditions in the United Kingdom (Cherfas, 1991, Crawley, 1992; Crawley et al. 1993). The major conclusions of these studies are that transgenic canola is not any more aggressive than the nontransgenic canola, transgenic rapeseeds do not invade undisturbed habitats, and they do not persist in the environment into which they were introduced any more than their parents did. More importantly, the reproductive rate of transgenic rapeseed was less than one in the presence of inter-specific competition in the uncultivated plots during the first year of the study, whereas in the cultivated plots the inter-specific competition was less than one in the second year of the study.

Peter Kareiva and Ingrid Parker of the University of Washington, Seattle, in a study carried out for Calgene showed that out of the 3600 seeds planted in cultivated soils, only 17 seeds germinated under harsh summer conditions at two week census. In the uncultivated plots, the germination was less than 9 out of 3600 seeds. Chi square analysis revealed no significance for this attribute. At the end of 12 weeks, no survivors were found. But, many other common weeds became well established in both cultivated and uncultivated plots suggesting that increased Laurate content of the seeds did not contribute to the seed vigor and germination rate.

Weediness may be affected by seed dormancy and seed persistence. Growth chamber studies conducted on Calgene Laurate canola do not show significant dormancy changes in the transformants (Linder, 1994). Persistence of seeds is a direct manifestation of the increased shattering and dormancy of the seeds. This is best exemplified by the seeds of *B. rapa* which are much more dormant. To address the question of seed dormancy PROSAMO conducted studies in the United Kingdom

(Crawley et al., 1993). Such studies have been conducted in the United States as well. Results tabulated in Tables 16a and 16b of the petition showed that seeds buried were found to be intact and dormant after 24 months. These results suggested that transgenic canola seeds were no more dormant than nontransgenic canolas; seed decay was rapid; survival rate was not even close to 60% observed for the closest weedy relative, *S. arvensis*. Results from the United States study (Table 17) clearly show that Laurate canola seeds show no more persistence than standard canolas, show no persistence characteristics of weeds, and do not become more dormant after being exposed to temperature extremes. In summary, the genetic change in Laurate content did not change the persistence characteristics of the Laurate canola lines.

Cultivated canola plants are outcrossing species of plants aided by honey bees. The petition references outcrossing studies with oil modified *B. rapa*. These studies have been extrapolated with the belief that Laurate canola lines would behave similarly. These studies show that cross pollinations decline rapidly as the separating distances increase from the transgenic plots. At a distance of 4.5 meters from the transgenic plants, the percentage of cross pollination drops to 0.5%, and is likely to be unmeasurable beyond that distance. Visiting honey bees showed no preference to either transgenic or non-transgenic canolas. This extrapolation of the high stearate canola studies are justifiable because the similarity in the oil profile of the two genetically modified canolas and the mode of expression of the introduced genes. Should movement of genetic material take place to any receptive plants, and the increased Laurate content be transferred, no competitive advantage would be conferred as discussed earlier.

Laurate canola lines will not cause damage to agricultural commodities.

Canola, by definition is specifically bred to have extremely low levels of toxicants, although *B. napus* rapeseed and its close relatives are known to carry several toxicants (Bell, 1984; Busch et al. 1994; Cheeke, 1989). Canola varieties have very low levels (30 micromole/g of alkenyl glucosinolates in the defatted meal).

Calgene has been in direct and continuous consultation with the Food and Drug Administration to assure that both oil and meal from Laurate canola are safe for human and animal consumption. In addition to the bay TE gene, the Laurate canola lines also express the APH(3')II protein that degrades the antibiotic kanamycin. This is also known as the antibiotic resistance marker gene used exclusively for selection of transformed cells under the control of a 35S cauliflower mosaic virus promoter which is a constitutive promoter. The enzyme is degraded by the digestive enzymes of the gut, is not functional in the absence of the energy molecule adenosine triphosphate (ATP), and low pH of the gut. In addition, kanamycin is rarely used in chemotherapy in humans or animals. Therefore, kanamycin resistance gene in Laurate canola will not compromise chemotherapy by kanamycin in humans and

animals. APH(3')II has not been shown to have significant homology with known toxins and allergens, and should not cause allergenic reactions from food products derived form Laurate canola. Recently, Food and Drug Administration issued a Final Rule permitting the use of APH(3')II as safe in feed and drinking water (21 CFR Parts 173 and 573). This final rule provides for the safe use of APH(3')II as a processing aid in the development of new varieties of oilseed rape, and cotton, in response to a petition filed by Calgene. FDA has examined all the relevant safety issues for the use of APH(3')II in foods, and reached the conclusions that supports the use of APH(3')II in foods and feeds. APH(3')II should not present any environmental risk, as the enzyme will be rapidly degraded in the soil, and similar antibiotic markers are present in soil microorganisms in any case.

Coconut oil, and palm oil are the most important sources of laurate already in food use. They are considered by the Food and Drug Administration to be safe food substances. Laurate presence is variable from plant species to species (Table 14a and 14b). Most of the insects feed on plants that are rich in Laurate without any ill effects. It follows that Laurate canola will have no ill effects on humans, animals, and insects that have not been hitherto undetected. Moreover, the Bay TE gene is expressed only in the seeds, and therefore, when other vegetative parts of the Laurate canola plants are ingested, there should not be any other toxic effects. Bay TE enzyme is not known to be a toxicant, and is broken down in the digestive system.

Erucic acid is a monounsaturated fatty acid (22:1) normally produced in very high concentrations (20-60%) in rapeseed. Canola, by definition has less than 2% of erucic acid which is considered safe. Field production of crops that produce high levels of erucic acid for industrial purposes is not restricted or otherwise regulated in the United States. Erucic acid and glucosinolates are the only two toxicants known in rapeseed. Laurate canola lines have been developed from low erucic acid and low glucosinolate canola varieties, thus meeting the regulatory specifications for their levels. As such Laurate canola lines should not present any concerns as far as toxicological properties of Laurate canola lines.

Information provided by Calgene regarding the components and processing characteristics of Laurate canola lines revealed no differences in any component that could have a direct or indirect plant pest effect on any processed commodity.

Laurate canola lines will not be harmful to beneficial organisms, including bees.

There is no reason to believe that deleterious effects on beneficial organisms could result from the cultivation of Laurate canola. Laurate is a normal part of the diets of animals, humans and insects.

Some birds even produce their own Laurate (Kolattukudy et al. 1993). All fatty acids are easily digested by the action of lipases in the guts of insects, and digestive system of higher animals, and humans. Cabbage seedpod weevil (*Ceutorhynchis assimilis*) and other *Lygus* species are common pests of canola. These insects are not on the list of threatened and endangered species. Laurate canola does not contain elevated level of toxic oils, and therefore, insects that may feed on Laurate canola will not be unduly affected in their ability to reproduce or function normally after feeding. Knowledge of this enzymes's mode of action, and the lack of known toxicity for this protein suggest no potential for deleterious effects on beneficial organisms such as bees and earthworms. APHIS has not identified any other potential mechanisms for deleterious effects on beneficial organisms.

V. CONCLUSIONS

APHIS has determined that Laurate canola lines will no longer be considered regulated articles under APHIS regulations at 7 CFR Part 340. Permits under those regulations will no longer be required from APHIS for field testing, importation, or interstate movement of Laurate canola lines or their progeny. Importation of Laurate canola lines, and nursery stock or seeds capable of propagation, is still, however, subject to the restrictions found in the Foreign Quarantine Notice regulations at 7 CFR Part 319. This determination has been made based on an analysis which revealed that the Laurate canola lines: (1) exhibits no plant pathogenic properties; (2) is no more likely to become a weed than its non-engineered parental variety; (3) is unlikely to increase the weediness potential for any other cultivated plant or native wild species with which the organisms can interbreed; (4) will not cause damage to processed agricultural commodities; and (5) is unlikely to harm other organisms, such as bees, that are beneficial to agriculture. APHIS has also concluded that there is a reasonable certainty that new progeny varieties bred from the Laurate canola lines will not exhibit new plant pest properties, i.e., properties substantially different from any observed for the field tested Laurate canola lines, or those observed for standard canola in traditional breeding programs.

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VI. REFERENCES

Adler, L. S., Winkler, K., Wyndham, F. S. , Linder, C. R., and Schmitt, J. 1993. Potential for persistence of genes escaped from canola: germination cues in crop, wild, and crop-wild hybrid *Brassica rapa*.

Bell, J. M. 1984. Nutrients and toxicants in rapeseed meal: A review. Journal Animal Science 58:996-1010.

Bryngelsson, T., Gustafsson, B., Green, B., Lind, M. 1988. Uptake of host DNA by the parasitic fungus *Plasmodiophora brassicae*. Physiological and Molecular Plant Pathology 33:163-171.

Busch, L., Gunter, V., Mentele, T, Tachikawa, M., and Tanaka, K. 1994. Socializing Nature: Technoscience and the transformation of rapeseed into Canola. Crop Science 34:607-614.

Cheeke, P. R. 1989. Toxicants of Plant Origin (vol. II) CRC Press, Boca Raton, Florida.

Cherfas, J. 1991. Transgenic crops get a test in the wild. Science 251:878.

Crawley, M. J. 1992. The comparative ecology of transgenic and conventional crops. Proceedings of 2nd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms. Goslar, Germany.

Crawley, M. J., Hails, R. S., Rees, M., Kohn, D., and Buxton, J. 1993. Ecology of transgenic oilseed rape in natural habitats. Nature 363: 620-623.

Carlson, T. A. and Chelm, B. K. 1986. Apparent eukaryotic origin of glutamine synthetase II from the bacterium *Bradyrhizobium japoncium* Nature 322:568-570.

Downey, R. K., and Bing, D. J. 1990. Biosafety of Transgenic Oilseed Crucifers. In: Workshop on Safeguards for Planned Introductions of Transgenic Oilseed Crucifers, Ithaca, NY, October 9, 1990. USDA-APHIS, Hyattsville, Maryland.

Gleason, H. A. 1952. The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada. Vol 3. Hafner Press, New York. 595 pp.

Linder, C. L. 1994. Determinants of Population Performance and Persistence in Crop-Wild Hybrids of Wild *Brassica rapa* and cultivated *Brassica rapa* and *Brassica napus*. Ph. D. Thesis, Brown University, Rhode Island.

Mitchell-Olds, T. 1990. Genetically engineered crucifers in the field. pp24-25. In: McCammon, S. L., and Dwyer, S. G. (eds.), Workshop on Safeguards for planned introduction of transgenic oilseed crucifers. October 9, 1990, Cornell University, Ithaca, New York.

Muenscher, W. C. 1980. Weeds. Second Edition. Cornell University Press, Ithaca and London. 586 pp.

Munroe, S. H. 1988. Antisense RNA inhibits splicing of pre-mRNA in vitro. EMBO Journal 7:2523-2532.

Reed, C. F. 1970. Selected Weeds of the United States. Agriculture Handbook No. 366. Agricultural Research Service, United States Department of Agriculture, Washington, D.C. 463 pp.

Röbbelen, G., Downey, R. K., and Ashri, A. 1989. Oil Crops of the World. McGraw-Hill, New York et alibi. 554 pp.

Scheffler, J. A. and Dale P. J. 1994. Opportunities for gene transfer from transgenic oilseed rape (*Brassica napus*) to related species. Transgenic Research 3:263-278.

Schmitt, J., and C. R. Linder. 1994. Will escaped transgenes lead to ecological release? Molecular Ecology, 3:71-74.

Seeley, T. D. 1985. Honeybee Ecology. Princeton University Press, Princeton, New Jersey. 201 pp.

Slife, F. W. 1960. Weeds of the North Central States. Circular 718. University of Illinois Agricultural Experiment Station, Urbana, Illinois. 262 pp.

van der Meijden, R., and de Vries, F. T. 1992. Real chances for spontaneous gene flow from cultivated plants to the wild flora of The Netherlands. Proceedings of the 2nd International Symposium on the Biosafety Results of Field Tests of Genetically Modified Plants and Microorganisms, Goslar, Germany.

Viera, J., Messing, J. 1987. Production of single-stranded plasmid DNA. Methods in Enzymology 153:3-11.

Wakabayashi, S., Matsubara, H., Webster, D. A. 1986. Primary sequence of a dimeric bacterial hemoglobin from vitreoscilla. Nature 322:481-483.

Weiss, E. A. 1983. Oilseed Crops. Longman, London and New York. 660 pp.

Willis, J. C. 1973. A Dictionary of the Flowering Plants and Ferns. Eighth Edition. Cambridge University Press, Cambridge et alibi. 1245 pp.

Yadav, N. S., Postle, K., Saiki, R. K., Thomashow, M., Chilton, M. D. 1980. T-DNA of a crown gall tumor is covalently joined to host plant DNA. Nature 287:458-461.

Yang, F. M., Montoya, A. L., Merlo, D. J., Drummond, M. H., Chilton, M. D., Nester, E. W., Gordon, M. P. 1980. Foreign DNA sequences in crown gall teratomas and their fate during the loss of the tumorous traits. Molecular and General Genetics 117:704-714.

Zambryski, P. 1988. Basic processes underlying *Agrobacterium* mediated DNA transfer to plant cells. Annual Review of Genetics 22:1-

Zambryski, P., Depicker, A., Kruger, K., Goodman, H. 1982. Tumor induction by Agrobacterium tumefaciens: Analysis of the boundaries of T-DNA. Journal of Molecular and Applied Genetics 1:361-370.

Zamecnik, P. C., Stephenson, M. L. 1978. Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucelotide. Proceedings of the National Academy of Sciences (USA) 75:280-284.