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CONSENSUS DOCUMENT ON GENERAL INFORMATION CONCERNING THE BIOSAFETY OF CROP PLANTS MADE VIRUS RESISTANT THROUGH COAT PROTEIN GENE-MEDIATED PROTECTION

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

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Series on Harmonization of
Regulatory Oversight in Biotechnology

No. 5

Consensus Document on General Information concerning the Biosafety of Crop Plants Made Virus Resistant through Coat Protein Gene-Mediated Protection

Environment Directorate

ORGANISATION FOR ECONOMIC CO-OPERATION AND DEVELOPMENT

Paris 1996

There is a list of OECD Environmental Health and Safety publications beginning on page 43.

Foreword

The OECD's Expert Group on Harmonization of Regulatory Oversight in Biotechnology decided at its first session to focus its work on the development of consensus documents which would be mutually acceptable among OECD Member countries. These consensus documents were to contain information for use during the regulatory assessment of the particular product. In the area of plant biosafety, consensus documents would be initiated on the biology of certain crop plants and on selected traits.

The first trait chosen to be reviewed was virus resistance. The Expert Group charged a task group to develop a document, and possible a workshop, that would identify information relevant to consideration of virus resistant crop plants developed through the use of viral coat protein genes. The Expert Group, through recommendations from the task group, also identified at least two "core" issues that should be addressed: (1) creation of new viruses through mechanisms such as recombination and transencapsidation (also referred to as "transcapsidation"); and (2) the potential for synergistic effects when a transgenic plant is infected by other indigenous viruses.

The first draft of this document was forwarded to National Co-ordinators for Environmental Biosafety of Transgenic Plants in September 1995 for technical comments. A Working Group subsequently met in Washington, DC, in October 1995 to review these comments and provide others.

A first revision was forwarded to OECD National Co-ordinators for a second round of technical comments in January 1996. Further comments were provided at a Working Group meeting which took place in Paris in March 1996, in conjunction with the meeting of the Expert Group.

The second revision was then forwarded to the Joint Meeting of the Chemicals Group and Management Committee for review. The Joint Meeting recommended that this document be derestricted. It is published on the responsibility of the Secretary-General of the OECD.

Table of Contents

Preamble

OECD Member countries are moving rapidly towards the commercialisation and marketing of agricultural and industrial products of modern biotechnology. They have therefore identified the need for harmonization of regulatory approaches to assess these products, in order to avoid unnecessary trade barriers.

In 1993, the "Commercialisation of Agricultural Products Derived through Modern Biotechnology" was instituted as a joint project of the OECD's Environment Policy Committee and Committee on Agriculture. The project's objective is to assist countries in their regulatory oversight of agricultural products derived through modern biotechnology - specificallly in their efforts to ensure safety, to make oversight policies more transparent and efficient, and to facilitate trade. Its focus is on the review of national policies, with respect to regulatory oversight, that will affect the movement of these products into the marketplace.

The first step in this project was a survey which focused on national policies with regard to regulatory oversight of these products. Data requirements for products produced through modern biotechnology, and mechanisms for data assessment, were also surveyed. The results are published in OECD Environment Monograph No. 99, *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results* (1995).

Subsequently, an OECD Workshop was held on 3-4 June 1994 in Washington, DC, with the aim of improving awareness and understanding of the various systems of regulatory oversight developed for agricultural products of biotechnology; identifying similarities and differences in the various approaches; and identifying the most appropriate role for OECD in further work towards harmonization of these approaches. Approximately 80 experts in the areas of environmental biosafety, food safety and varietal seed certification, representing 16 Member countries, eight non-Member countries, the European Commission and several international organisations, participated in the Workshop. The *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* was published as Environment Monograph No. 107 (1995).

As a next step towards harmonization, the Expert Group on Harmonization of Regulatory Oversight in Biotechnology instituted the development of **consensus documents** which are *mutually acceptable* among Member countries. The goal is to identify common elements in the safety assessment of a new plant variety developed through modern biotechnology, in order to encourage information sharing and prevent duplication of effort among countries. These common elements fall into two general categories: the biology of the host species, or crop; and the gene product.

The first gene product chosen to be reviewed was virus resistance. The Expert Group charged a task group to develop a document that would identify information relevant to consideration of virus resistant crop plants specifically developed through the use of viral coat protein genes. The Expert Group identified two "core" issues that should be explored: (1) creation of new viruses through mechanisms such as recombination and transencapsidation (also referred to as transcapsidation); and (2) the potential for synergistic effects when a transgenic plant is infected by other indigenous viruses.

In order to reflect the most current scientific and technical developments, the consensus documents will be “living documents”, developed in a “modular” fashion. This will allow for flexibility through their updating and the addition of new components relevant to the subject addressed in the initial document. Therefore, users of the consensus documents are invited to inform the OECD Secretariat of such relevant new scientific information and proposals for additional components.

This document was prepared by a lead country, the United States. It is based upon material developed by Member countries, either during specific risk assessments or from national conferences and scientific meetings. It is intended as technical assistance for use by regulatory authorities and others who have responsibility for the assessments of transgenic plants for commercialisation, or those who are involved in the design and development of transgenic plants to be commercialised in the future**.**

Summary Note

This document, developed under the auspices of the OECD's Expert Group on Harmonization of Regulatory Oversight in Biotechnology, is intended to provide information that may be of assistance to regulatory officials, developers of virus resistant plants,and other interested parties. Any decision on the use of virus resistant plants at the small- or large-scale stages of product development, or their commercial use, will require a case-by-case review by each Member country, as the specific environment in which such plants will be grown is a component of each of the issues addressed in this document.

The focus of this report is limited to issues that can be discussed in a general fashion without reference to the specific environment in which the transgenic plant is to be introduced. Therefore, any issues relating to the cultivation of the virus resistant plants or to the potential for, or potential effects of, gene transfer from a virus resistant plant to another crop plant or to a wild relative are outside the scope of this document. This document is not intended as an encyclopedic review of all the scientific experimentation on the use of genes to make plant species virus resistant.

The Expert Group identified three topics to be considered in this document as they relate to the use of one specific gene, the viral coat protein, whose expression in plants often results in a resistant phenotype. Two of these topics, transcapsidation and recombination, are known molecular mechanisms through which new virus types may arise; the third topic is the potential for specific synergistic effects that modify symptom development. These are biologically complex phenomena that may sometimes involve at least four different organisms: two viruses, an organism (most often an arthropod) that transmits the virus from plant to plant, and one or more host plant species. These phenomena are not understood in complete detail, and there is considerable research ongoing to address less well-understood aspects.

In some instances, the discussions in this document focus on particular taxa of plant viruses, either those for which the most information is available or those for which the risk issues can be most clearly identified. In addition, the document provides guidance on the biological and molecular information needed to characterise the virus from which the coat protein gene was isolated and the gene inserted into the transgenic plant. Also provided is a list of references that may be helpful in locating such information.

Further research on the basic biology of plant viruses may speed the development of genes for use in virus resistant plants that minimise the potential agronomic or environmental concerns associated with their use, and potentially reduce the likelihood that viral strains will arise that overcome the resistance trait.

Section I - General Introduction

The following document, developed by a Task Group under the auspices of the Expert Group on Harmonization of Regulatory Oversight in Biotechnology, is intended to be one in a series of documents of use in providing information to regulatory officials, developers of new products produced through biotechnology, and other interested parties.

This document is not intended as a definitive or encyclopaedic review of all the scientific experimentation pertaining to the use of viral coat protein genes to make plant species virus resistant, nor is it intended to dictate to regulatory authorities in any country how they should review requests for field testing, deregulation, or commercialisation of such plants. (For other information on virus resistant transgenic plants, see Hull, 1990, 1994; de Zoeten, 1991; Mansky and Hill, 1993; Falk and Bruening, 1994; Palukaitis, 1991; Tepfer, 1993, 1995; Wilson, 1993.) Rather, the document attempts to describe the current state of experience in Member countries with a particular set of issues pertaining to crop plants made virus resistant through coat protein gene-mediated protection. It draws upon a wide range of information sources, including not only the scientific literature but also risk assessments from Member countries and reports from national conferences and scientific meetings. In an effort to capture the current "state of the art", it also contains preliminary information that may not yet have received full and critical evaluation by the scientific community. Where such information appears, it is indicated as "preliminary".

The issues discussed in this document are a subset of issues that regulatory officials may consider in relation to crop plants made virus resistant through coat protein gene-mediated protection. The focus is limited to issues that can be discussed without reference to the specific environment in which the organism is to be introduced. Therefore, any issues relating to the cultivation of virus resistant plants or to the potential for, or potential effects of, gene transfer from a virus resistant plant to another crop plant or to a wild relative are outside the scope of this document, although these issues are valid considerations that may enter into regulatory deliberations by regulatory authorities in any country. Neither does this document address potential agronomic impacts in any Member country or any other issues that may relate to the potential international marketing of such crop plants.

This document focuses instead on the potential for effects of such genetically modified plants on some natural virus populations or on the severity of viral infections. Specifically, the Expert Group identified three topics to be considered in this document as they relate to the use of one specific viral gene, the gene encoding the viral coat protein, to confer virus resistance. Two of these topics, transcapsidation and recombination, are known molecular mechanisms through which new virus types may arise; the third topic is the potential for specific synergistic effects of particular introduced viral genes on infections with other viruses. These are biologically complex phenomena which may sometimes involve at least four different organisms: two viruses, a viral vector, and a host plant species. These phenomena are not understood in complete detail for all viruses, and there is considerable research ongoing in Member countries to address some of their less well-understood aspects. In some instances, the discussions in this document focus on particular taxa of plant viruses either for which the most information is known, or for which the risk issues can be most clearly identified.

This document represents a consensus of Member countries' positions on factors relevant to addressing the biosafety concerns raised in considering the three identified molecular mechanisms affecting viral populations and viral diseases of plants, as they relate to virus resistant crop plants mediated by CP genes. Current scientific information on these subjects may be sufficient to enable the conduct of case-specific, scientifically sound risk assessments and biosafety evaluations of currently developed varieties. This may enable competent authorities or regulatory officials in countries, after such reviews, to

give authorisation for release or commercialisation of particular varieties. The document does not attempt to provide detailed, definitive, or general conclusions on the outcomes of such considerations, nor does it attempt to advise countries on how any such deliberations should be concluded. Member countries have agreed that such deliberations are conducted on a case-by-case basis. Results of particular evaluations of certain issues, as they relate to individual virus resistant crops, are presented for illustrative purposes.

Section II - Technical Introduction to Plant Viruses

Viral diseases cause significant economic losses to agriculture. Viral infections cause damage to fruits, leaves, seeds, flowers, stems, and roots of many important crop species. Under natural conditions, certain plant viruses are nearly always present on particular crop(s) or weed host(s). The types of symptoms produced in a specific plant vary depending on the virus, the specific strain of the virus, whether the plant is infected with another virus or other viruses, the cultivar of the host plant, and the environment. The severity of infection by a particular virus often varies from location to location and from one growing season to the next, reflecting the importance of the environment on symptom development and vector transmission rates for the virus. Some virus outbreaks have been sufficiently severe that entire plantings of target crops (e.g., sugar beet, citrus, and rice) have been destroyed in specific areas. Most crop species are routinely infected with several different viruses. The American Phytopathology Society's Compendium of Plant Disease series lists the important viruses affecting the major crops of the world. *Viruses of Tropical Plants* by Brunt et al. (1990) is a useful resource for viral disease of tropical plants.

Plant viruses may be spread in various ways, depending on virus type. Means of spread include: vector-mediated transmission, seed or pollen transmission, and mechanical transmission (whether by transfer of plant sap or by vegetative propagation of infected host tissue). Virus vectors may be nematodes, mites, fungi, or insects. In some environments and for certain viral diseases, substantial inputs of pesticides are needed to control particular vector organisms (typically insects) for serious viral diseases, even though the insects do not themselves cause significant damage to the target crop or could possibly be controlled by biological means. Control of the vector organism does not always result in complete or effective control of the viral disease. In addition, in certain environments particular crop species cannot be grown profitably because of the presence of persistent populations of infected plants and potential vectors. The situation with soil-borne (nematode or fungus transmitted) viruses is even more dire. If these infested vectors become established at a site, eradication or even satisfactory control is usually impossible or environmentally untenable. Unless resistant cultivars are available, cultivation of the susceptible crop at that site may have to be abandoned. Examples include infections of Indian peanut clump furovirus in groundnut in parts of India, and rhizomania disease in sugar beets [caused by beet necrotic yellow vein virus (BNYVV)] in the United Kingdom.

Plant viruses are relatively simple pathogens, in essence protein coats (capsids) wrapped around genomes of either DNA or RNA. Some capsids may also contain carbohydrates and/or lipids. The viral genome encodes at least its own nucleic acid replicating enzyme, (a) protein(s) required for movement of the virus throughout the plant, the viral coat protein(s), and often other necessary proteins. After entering a host plant cell, a virus particle (virion) uncoats, replicates copies of its genome, uses its CP gene to manufacture the protein subunits for the virus protein coat, and then assembles new virions. The new virions or infectious agents may spread to adjacent cells or be transported by vector organisms to other host plants.

Plant viruses are usually named according to the plant species in which they were first detected and the type of symptoms observed in infected plants. The genome of each plant virus is of a characteristic composition, DNA or RNA, either single-stranded or double-stranded depending on the virus. Some plant viruses contain more than one nucleic acid molecule within each virus particle. For other viruses, the genomes consist of more than one nucleic acid molecule, each packaged in a separate virion. Some viral infections are also associated with the production of satellite RNAs or satellite viruses. Satellite RNAs depend on a specific virus (called helper virus) for the replication enzymes needed to replicate their own RNA, are usually smaller in size than their helper viral genome, have no significant sequence homology to the helper virus genome, and affect disease symptoms (at least in some hosts) (Matthews, 1991). In

satellite viruses, the satellite codes for its own coat protein, while satellite RNAs are packaged in the coat protein of the helper virus. Plant viruses are taxonomically grouped according to their nucleic acid composition and other physical properties of the virions. Nucleic acid sequencing of hundreds of animal and plant viral genomes has revealed the evolutionary relationships among many viruses. An important reference for viral taxonomy is Murphy et al. (1995), a publication of the International Committee on Taxonomy of Viruses (ICTV).

Plant viruses have traditionally been controlled in agriculture using a variety of strategies with varying effectiveness: exclusion of contaminated material at national or state borders with accompanying virus identification (often by indexing); rogueing of infected crops; plant eradication measures, when appropriate; certification of virus-free stock or seeds (e.g., to control plum pox potyvirus in fruit trees and for many viruses in potatoes); use of agronomic practices designed to minimise virus spread or persistence (e.g., not planting a particular crop for a specified period of time in a given locality); conventional breeding for virus-resistant cultivars; and conventional cross-protection (i.e., pre-inoculation of plants with a mild strain of the virus to protect against severe infection by another strain of the same virus) (used to varying degrees to control tomato mosaic tobamovirus in tomatoes in Europe and Japan and citrus tristeza closterovirus in Brazil). Conventional cross-protection, while commercially important for some crops in certain localities, is only effective for certain viruses. It involves intentional infection of crop plants with a suitable closely related mild virus strain, provided that such a virus strain is available. Two of the main issues associated with the development of useful conventionally-bred, virus resistant crop lines have been 1) identification of breeding stock containing an appropriate resistant trait/gene(s); and 2) potential trade-offs between introgression of the virus resistant trait and other agronomically important traits in the crop itself.

Until 1994, when Whitman et al. cloned and sequenced the tobacco N gene conferring resistance to TMV, no plant-derived traditional viral resistance gene had been cloned or sequenced. The exact function of the N gene is still not understood, although recent evidence suggests its involvement in a common signal transduction mechanism for general pathogen resistance (Staskawicz et al., 1995). Nonetheless, introduction of traditional resistance genes into agronomically desirable cultivars has been used for decades to protect plants from viral infections even though their mode of action has not been understood. The lack of detailed understanding of the mechanism of traditional resistance genes, or traditional cross protection measures, has not prevented their use.

Another type of strategy for protecting a plant against viral disease involves introduction and expression of a gene encoding the viral CP in the genome of the plant itself. This type of strategy is referred to as "coat protein gene-mediated protection", and its effectiveness was first demonstrated on tobacco mosaic tobamovirus infection of tobacco in 1986 by Powell Abel et al. It provides heritable protection of the recipient plant species against the target virus, and frequently against related strains as well. This strategy has been demonstrated in laboratory or field experiments to be effective against at least 50 different viruses to date. Since that time, viral genes other than CP genes [dedicated movement proteins, replicase (polymerase), viral genes modified to contain ribozymes, satellite and defective interfering RNAs] have also been shown to confer a virus resistant phenotype on recipient plants. The growing number of genes used to encode virus resistance is more illustrative of the diversity of the viruses against which resistance is targeted than the plant species they infect. However, this document focuses exclusively on the biosafety of those genetically modified plants made virus resistant through the introduction of a viral CP gene, and on biosafety with respect to interactions of the modified recipient plant with other plant viruses in the environment.

Section III - Basic Information for Virus Characterisation

The information relevant for a biosafety review of an organism includes that which establishes the identity of the organism in question and that which describes the environments in which the organism is to be used. Any genetically modified plant protected against viral infection through CP gene-mediated protection will potentially interact with the range of organisms with which the parental plant species can interact within the same environment. Characterisation of the virus which provided the transgene would include information on virus biology, taxonomy, genetics, and known viral interactions in the environment. This necessary information would include:

a) the taxonomic name of the virus, including family, genus, and strain designation, including any synonyms;
b) the type of nucleic acid contained in the virus;
c) whether the infection is systemic or localised;
d) whether the virus is restricted to specific tissues (e.g., phloem-limited);
e) whether the virus is associated with any satellite or helper viruses;
f) the natural host range of the virus;
g) how the virus is transmitted;
h) if the virus transmitted by a vector, the identity of the vector including mode of transmission (e.g., persistent or non-persistent) and the identity of the viral gene(s) (if known) involved in vector transmission; and
i) whether any synergistic or transcapsidation interactions with other viruses under field situations have been reported in the literature.

In order to evaluate any potential biosafety concerns posed by the use of viral genes, viral sequences engineered into the plant should be well-characterised sequences that are derived from well-characterised viruses, and the specific biological properties of the actual strain utilised should be known. Characterisation of the strain from which the transgene is derived may enable determination of whether that strain is identical or nearly identical to the strain found in other countries. For example, beet necrotic yellow vein furovirus strain A that is widely prevalent in Japan, the United Kingdom, the Netherlands, and other parts of Europe is virtually identical to the strain found in the United States (Kruse et al., 1994). A considerable amount of data on viral strains is readily available in scientific publications and from publicly accessible data bases.

For appropriate designations of most plant viruses, the official taxonomic body for virology is the International Committee on Taxonomy of Viruses (ICTV), which has published the accepted taxonomic names for most plant viruses (Murphy et al., 1995). Relevant types of information to describe the virus in question are the complete name of the virus (including any synonyms), the family and genus names, the strain designation, the name of the disease incited, and the locality where the strain was first isolated. The molecular characteristics of a plant virus most important for describing the properties of viral infections are the type of nucleic acids contained, RNA or DNA, and whether those nucleic acids are single- or double-stranded (Murphy et al., 1995). It is important to describe whether the virus replicates in all cells (e.g., tobamoviruses) or is limited to certain cells (e.g., phloem cells for luteoviruses).

Although an up-to-date, definitive, and concise list of the host ranges of all plant viruses is not available, several publications and Internet sites have a significant amount of useful information:

a) The Commonwealth Mycological Institute/Association of Applied Biologists' "Description of Plant Viruses" is a series of pamphlets describing the biology of several hundred plant viruses.
b) The USDA's *Plant Pests of Importance to North American Agriculture, Index of Plant Virus Disease* (Agriculture Handbook No. 307, 1966) provides a list of plants and the viruses that infect them.
c) The American Phytopathology Society's series on plant diseases of crops has up-to-date listings of viral diseases for the major crops. The Society also has a list of names of U.S. plant diseases and their causal agents.
d) The Australian Virus Identification Data Exchange (VIDE) is currently being promoted by the ICTV to establish a worldwide database dealing with plant viruses. The World Wide Web site for the database is: http://life.anu.edu.au/viruses/lctv/index.hmtl.
e) The British Society for Plant Pathology's *Names of British Plant Diseases and their Causes*, published in 1984, lists the English and European names of the diseases and the scientific names of the causal organisms, arranged by host plant.
f) The *European Handbook of Plant Diseases* by Smith et al. (1988) provides descriptions of the viral, bacterial, and fungal pathogens of European plants.
g) A World Wide Web site maintained by the Garry Laboratory at Tulane University (USA) has as its goal to provide a list of all virology sites on the Word Wide Web. This site can be accessed at http://www.tulane.edu/~dmsander/garryfavweb.html. A mirror site has been established to facilitate access in Europe at the University of Leicester (United Kingdom) (http://www-micro.msb.le.ac.uk/335/garryfavweb.html).

Many publications describe the host range of a particular virus. However, most lists do not describe the host range of specific viral strains. Host range is an important consideration for the three issues with which this document is concerned. Because of the number of different strains of a particular virus, information on the natural host range of the specific viral strain used as a donor organism may be more easily provided by the person who has engineered that plant than by a literature search. Information on the natural host range of a viral strain in managed and unmanaged ecosystems is probably more relevant than information on its "artificial" host range. The natural host range of a virus lists the plants growing in managed and unmanaged ecosystems that are commonly infected with the virus. The artificial host range includes plants that become infected when intentionally inoculated by man under controlled conditions but are not necessarily infected under natural conditions. The artificial host range of a virus includes more plant species than the natural host range (Matthews, 1991).

A definitive worldwide list of the geographical distribution of plant viruses is also unavailable. However, limited information on the geographical distribution of many plant viruses can be found in the references listed above. The United States Department of Agriculture (USDA) has a state-by-state list of occurrences of widely prevalent viruses on its World Wide Web Site (http://www.usda.gov/bbep/bp).

Viruses are transmitted by many vectors, including whiteflies, mites, nematodes, aphids, planthoppers, leafhoppers, beetles, thrips, and fungi. They can also be transmitted mechanically and through seed or pollen. For those viruses that are vector-transmitted, a single virus is transmitted under field conditions by a single vector group. Thus, as an example, three viruses from three different genera of the family Potyviridae, potato Y potyvirus (PVY) (genus *Potyvirus*), ryegrass mosaic virus (genus *Rymovirus*), and barley yellow mosaic virus (genus *Bymovirus*) are transmitted by unrelated types of

vectors. In this example several aphid species transmit the first virus, the mite *Aceria tulipae* transmits the second, and the fungus *Polymyxa graminis* transmits the third virus. Each group of vectors transmits particular viruses worldwide (Murphy et al., 1995).

Although these three viruses are all in the same family, they are transmitted by only one specific type of vector. The high specificity of this virus-vector relationship is a result of interaction between specific vector-encoded receptors and the specific virus-encoded protein(s) that is unique to each virus (Murphy et al., 1995; Murant et al., 1988b). Identifying the major vectors of field importance (both scientific and common names) is part of the characterisation of both the virus itself and the recipient environments. In addition, if any viral genes have been identified as being implicated as required for vector transmission, the nature of the genes and, briefly, how they are believed to be involved in vector transmission should be described.

Section IV - Expression of Coat Protein Gene in Transgenic Plants Results in a Virus Resistant Phenotype

Powell Abel et al. (1986) showed that transgenic plants expressing the CP of tobacco mosaic tobamovirus (TMV) imparted resistance to TMV. Since that time, over 30 plants, both monocots and dicots, have been engineered to express more than 50 viral CP genes from ten taxa. Many of these have been field tested. One of the catalysts for this research has been knowledge of the phenomenon of cross protection, in which a plant infected with a mild strain of a virus is often protected from infection by a severe strain of the same virus. Although the exact mechanism by which cross protection works is not clear, evidence suggests that CP is involved with some viruses (Matthews, 1991).

Cross protection has been used in agriculture for many decades worldwide. Currently in Japan, more than half a million tomato plants (for both fresh market and processing uses) are cross protected against cucumber mosaic cucumovirus (CMV) (containing a satellite RNA of Japanese origin) (Sayama et al., 1993; Sayama, unpublished data). Tomatoes cross protected with tomato mosaic tobamovirus have been or are being consumed in Europe and Japan (classically-bred resistant cultivars are also widely used), citrus trees have been protected against citrus tristeza closterovirus in Brazil (Fulton, 1986), papaya trees have been protected with papaya ringspot potyvirus (as reviewed by Yeh et al., 1988), and zucchini plants have been protected with zucchini yellow mosaic potyvirus. Before indexing was widely used for virus elimination in potato, many potato seed pieces were infected with mild strains of many common viruses, including potato leaf roll luteovirus, potato X potexvirus, and potato Y potyvirus (Hooker, 1981), and therefore were cross protected using traditional techniques. These methods are still used for many vegetatively propagated plants, like strawberries, as well as florist and nursery crops.

Coat protein gene-mediated protection is best understood with TMV and tobacco. A brief summary of the current state of knowledge of this system is summarised below. For protection of tobacco to be effective, TMV CP must accumulate. Development of protection does not seem to involve the induction of the plant's natural disease resistance system. Resistance appears mainly to be based on blocking the uncoating of the CP in the incoming TMV. There is, however, evidence that a later step in infection is also affected (Reimann-Phillip and Beachy, 1993). It has been observed that protection is better when the CP is derived from a viral strain that naturally infects the recipient plant than when the CP is derived from a closely related strain that infects another host plant. Tomato plants expressing tomato mosaic tobamovirus (ToMV) CP gene, the tobamovirus most closely related to TMV, are better protected from ToMV infection in the field than tomato plants expressing tobacco mosaic tobamovirus CP (Sanders et al., 1992). Resistance derived from the CP gene of other viruses may have modes of action different from TMV.

Based on the success of CP gene-mediated protection during field testing, most plant virologists believe that CP gene-mediated resistance may be successfully applied for many but not all (Ploeg et al., 1993) single-stranded, positive sense RNA viruses, a group which includes over 75 per cent of all plant viruses (Beachy, 1993). More field tests of virus resistant plants have occurred in the U.S. than in any other OECD Member country. In the U.S., most but not all of the CP genes have been derived from viruses that commonly infect the recipient crop. A majority of the viral sequences that have been introduced into transgenic plants and field tested thus far have not been modified from the original sequence found in the parental virus, except for modifications related to cloning of the gene. A few CP genes have been modified so that the ability of the virus to be transmitted by its vector is significantly reduced; others have been isolated from strains that were non-transmissible by the vector under natural conditions. In some cases, expression of CP gene from a viral strain that does not naturally infect the plant

can provide resistance to taxonomically related virus that may or may not naturally infect the plant (Stark and Beachy, 1989; Namba et al., 1992).

Another approach involves using a CP gene that has been modified by removing some of the nucleotide sequences from the gene, resulting in a truncated CP (Lindbo and Dougherty, 1992 a,b). Depending on how much of the gene is deleted, the CP derived from the truncated transgene may or may not be able to function in virion assembly (Lindbo et al., 1993). Dougherty's laboratory (Smith et al., 1994; Lindbo and Dougherty, 1992 a,b) has shown that a modified CP transgene that encodes a non-translatable mRNA may also provide protection. This resistance may result from direct interaction of transgene RNA and viral RNA, commonly referred to as RNA-mediated resistance, although host factors may also play a role in resistance (Smith et al., 1995).

Antisense expression (the production of complementary, non-coding transcript of a gene) of coat protein gene has generally not been as effective as sense expression in protecting plants against viral infections, although there are some notable exceptions (Hammond and Kamo, 1995; Kawchuk et al., 1991; Lindbo and Dougherty, 1992 a,b). This low success rate as compared to sense expression may not be unexpected, since antisense strategies act at the level of gene expression in the nucleus whereas most plant viruses multiply in the cytoplasm (Beachy, 1993). Whether antisense-, truncated sense-, or untranslatable sense-mediated resistance is as effective in providing immunity or resistance as sense CP-gene-mediated protection under field conditions needs further investigation. If the CP-derived transgene produces a CP which cannot encapsidate viral nucleic acid or does not produce a CP, this minimises the issues addressed in Section V(transcapsidation and synergy).

CP-gene mediated resistance will probably not be totally effective against virus strains that have satellite RNAs associated with them. These small RNAs can often modify the symptoms expressed by infected plants. Depending on the genotype of the host plant, the sequence of the small RNA, the helper virus, and environmental conditions, the symptoms may be attenuated or more severe (Matthews, 1991). Although satellites and defective-interfering RNAs have been detected in some viruses, their role in disease development under natural conditions is unclear. For the majority of viruses for which satellite RNAs have been detected, satellites are rarely found in virus-infected plants in the field, nor have they ever been shown to have caused a severe epidemic. There are two major exceptions. One is that of the satellites of CMV, which have caused serious disease epidemics in China, Italy, Japan and Spain in the past decade (Tien and Wu, 1991; Kaper et al., 1990). The other is groundnut rosette virus, of which all the isolates that cause rosette symptoms contain satellite RNAs (Murant et al., 1988a). Coat protein gene-mediated protection alone does not protect plants against infection if the virus contains satellite RNAs, so that additional measures are likely to be necessary for engineering effective protection against such satellite-containing viruses (Yie and Tien, 1993).

In the Sixth Report of the International Committee on Taxonomy of Viruses (ICTV), the genus *Umbravirus* was recognised with carrot mottle as the type species (Murphy et al., 1995). Umbraviruses have worldwide distribution, but have been found only in plants co-infected with luteovirus. Umbraviruses can be distinguished from luteoviruses based on the fact that Umbraviruses are manually transmissible, whereas luteoviruses are only aphid-transmitted. However, in the field Umbraviruses are genomically masked by luteoviral coat protein and thus aphid-transmitted. On the basis of biological properties, four Umbraspecies have been recognised by the ICTV and four additional candidate species have been proposed. No reports have been published regarding transgenic plants engineered to be resistant to Umbraviruses, and the luteovirus resistant plant likely to commercialised within the next few years (see Section V) contains a non-capsid gene as the source of the resistant phenotype. For further information, see the papers cited in the second paragraph of Section I.

Section V - Issues Related to Potential Effects of CP Gene-mediated Virus Resistance on Virus Infections

Although more than 50 virus resistant plants using CP gene-mediated resistance have been field tested worldwide to date, it is likely that only a limited number of these will be commercially available in the next few years. Some of the virus resistant plants that may be eligible to be considered for commercialisation in the next few years in OECD Member countries might be:

beet necrotic yellow vein furovirus resistant sugar beets;
tomato mosaic tobamovirus resistant tomatoes;
potato leaf roll luteovirus resistant potatoes; *
potato X potexvirus resistant potatoes;
cucumber mosaic cucumovirus resistant tomatoes, peppers and cucurbits;
zucchini yellow mosaic potyvirus, watermelon mosaic potyvirus 2, and papaya ringspot potyvirus resistant cucurbits;
potato Y potyvirus resistant potatoes;
potato Y potyvirus resistant tobacco;
cucumber mosaic cucumovirus resistant tobacco; and
papaya ringspot potyvirus resistant papayas.

**In North America, Europe and Japan the PLRV resistant lines likely to be commercialised use a non-CP gene as source of the resistance phenotype. PLRV CP-mediated resistance has also been field tested in many countries.*

Some plants that may be commercialised could contain combinations of the above resistance genes. An attempt is made in this document to highlight information or data which may be particularly relevant to the above listed viruses.

Three distinct interactions, transcapsidation, synergy, and recombination, have been observed to occur when two plant viruses (or two different strains of the same plant virus) simultaneously infect a cell. A brief description of each of these interactions is provided, followed by an analysis of how each may play a role when transgenic plants are made virus resistant through the use of CP gene-mediated resistance.

A. Transcapsidation

When a single plant cell is simultaneously infected by two different strains of a virus (or two viruses), it may be possible for the genome of one virus to become encapsidated by coat protein of the second virus. If the virus is encapsidated by coat proteins of both viral strains, the phenomenon is called phenotypic mixing (mixed encapsidation). If the virus is encapsidated by only one of the coat proteins, this is termed genomic masking or transcapsidation. (For simplicity, it will be assumed that the terms transcapsidation and genomic masking include the phenotypic mixing phenomenon, since the issues for all are identical). Transcapsidation has been reported to be important in only a few instances in field situations in insect transmission of viruses (Falk et al., 1995), even though field grown plants and trees are known to be infected with multiple viruses (Abdalla et al., 1985; Falk and Bruening, 1994).

Transcapsidation has been best studied with infections with different strains of the barley yellow dwarf luteovirus, where the phenomenon can be important in field situations in that coat protein determines which specific aphid vector transmits the virus (Matthews, 1991). This phenomenon has also been detected with potyviruses (Bourdin and Lecoq, 1991; Lecoq et al., 1993) and tombusviruses (Dalmay et al., 1992). (Similar preliminary results have also been reported with nepoviruses (Hiriart, 1995). The result of transcapsidation, a "masked" virion, has a mismatched coat that may or may not be sufficiently functional to allow transmission of the viral genome it contains to another host plant. The "mismatched" or heterologous viral coat is not maintained in subsequent rounds of viral infection, because subsequent production of coat protein subunits is directed by the viral coat protein gene carried in the genome. Therefore, transcapsidation events are transient and any potential impacts can only persist with the first round of infection of the masked virus if it infects a susceptible host plant.

For some viral taxa, a protein other than CP is the primary determinant of whether a specific organism can successfully transmit a virus. These taxa include potyviruses, caulimoviruses, and waikaviruses (Murphy et al. 1995). This vector transmission protein is called a "helper component" in potyviruses and an "aphid helper transmission factor" in caulimoviruses (Murphy et al., 1995). Unless the appropriate vector transmission protein is present and functional, transcapsidated virions assembled with CP from a vector transmissible strain will not be efficiently transmitted by the "heterologous" insect vector (Berger et al., 1989; Atreya et al., 1990). In contrast, viral CPs apparently are the primary determinants for insect-transmissibility for geminiviruses and cucumoviruses (Matthews, 1991). In the fungus-transmitted furoviruses and the aphid-transmitted luteoviruses, the vector transmission protein is synthesised by read-through of the CP termination codon (Zaccomer et al., 1995; Schmitt et al., 1992; Wang et al., 1995). Rice tungro waikavirus is required for aphid-transmission of rice tungro bacilliform badnavirus, and thus probably encodes an aphid-transmission protein (Dasgupta et al., 1991). For some taxa, little is yet known about the nature of the protein(s) involved in vector transmission.

Two issues are important to be addressed in considering the likelihood and significance in any potential instance of transcapsidation in transgenic plants. As stated above, if a resistant plant was engineered with a gene that does not produce a CP, or produces one that cannot function in the assembly of virions, these issues need not be addressed:

1) Is there a sufficient amount of coat protein being produced by the transgenic plant to produce a masked virus? Is the CP found in the same or different tissue(s) where the virus is detected in a non-transgenic plant?

2) If a masked virus were produced, would it have any new biological properties (vector transmission and host range) and would any effects resulting from transcapsidation be measurable or significant?

As mixed infections by plant viruses of all taxonomic types are common in nature (Zink and Duffus, 1972; Davis and Mizuki, 1987; Duffus, 1963), it is likely that there are many as yet unrecognised examples of heterologous transcapsidation interaction that naturally occur between plant viruses. However, research thus far indicates that heterologous transcapsidation interactions occur only in specific interactions in most mixed infections. There is evidence for both traditional and transgenic virus resistant plants that transcapsidation may occur (Rochow, 1972; Matthews, 1991; Farnelli et al., 1992; Osbourn et al., 1990; Dalmay et al. 1992; Holt and Beachy, 1992; Lecoq et al., 1993; Maiss et al., 1994; Candelier and Hull, 1993).

With the impending commercialisation of transgenic virus resistant plants, an important consideration is whether the use of viral CP-expressing transgenic plants might increase the possibility for heterologous transcapsidation interactions to occur and, if the possibility is increased, whether it poses a significant risk. One way in which scientists have sought to assess potential transcapsidation frequency in transgenic virus resistant plants has been to compare the amount of the engineered coat protein in the transgenic plants with the amount of coat protein in a similar, but susceptible, non-transgenic plant (Issue 1 above). One hypothesis has been that comparable or smaller amounts of coat protein would lead to the prediction that the transcapsidation frequency will be comparable or reduced from the frequency that occurs in naturally occurring mixed infections.

A second consideration would be whether the transgene CP is synthesised in the same tissues that the virus naturally infects in non-transgenic plants. If CP synthesis takes place in the same tissues, then no new interactions with other viruses that may be limited to other plant tissues can occur. The amount of transgene CP that can be detected in a transgenic plant may increase if the plant is infected by a related virus to which it is susceptible (Farnelli et al., 1992). The increase in detectable CP transgene may be a result of the CP being stabilised in masked virus particles rather than to an increase in transgene mRNA. It may be prudent to ascertain the amount of detectable CP transgene and mRNA in a transgenic plant when inoculated with common viruses with which the transgenic plant would routinely become infected in field situations.

One example of these considerations having entered into the regulatory assessment process in an OECD Member country is the Asgrow Seed Company's ZW20 squash, which is engineered to be resistant to zucchini yellow mosaic potyvirus (ZYMV) and watermelon mosaic potyvirus 2 (WMV2) by the expression of their respective CP genes. The review of ZW20 was conducted by the United States. At the time of preparation of this consensus document, it is the only virus resistant plant that has completed the reviews necessary to allow agricultural use of the plant in an OECD Member country. In ZW20 plants, the review concluded that the CPs are expressed in the same plant tissues in which the corresponding viruses are normally detected, and that the amount of CP produced in ZW20 plants is less than, or equal to, the amount in naturally infected plants. The amount of transgene CP detected increased in ZW20 plants after infection with papaya ringspot potyvirus (PRSV), although transgene RNA concentration did not increase. The amount of transgene CP detected in PRSV-infected ZW20 was still less than that found in PRSV-infected squash plants. In a review of Asgrow's next squash line (CZW-3), which is resistant to cucumber mosaic cucumovirus, ZYMV, and WMV 2, no increase in transgene CPs was detected when the transgenic plants were challenged with PRSV.

It has been demonstrated that heterologous transcapsidation can occur in transgenic plants that express viral CP (Osbourn et al., 1990; Dalmay et al., 1992; Holt and Beachy, 1992). Lecoq et al., 1993 showed that when plants expressing a CP transgene derived from an aphid-transmissible strain were challenged with a non-aphid transmissible strain (defective in CP not aphid transmission factor), a heterologous aphid transmissible strain was detected. Another important question is whether transcapsidation can occur with more distantly related viruses. Candelier-Harvey and Hull (1993) have shown that when plants expressing the CP of alfalfa mosaic alfalmovirus (AlMV) are infected with cucumber mosaic cucumovirus (both members of the family Bromoviridae), the CMV genome is encapsidated in particles that contain AlMV CP. Since AlMV has no known insect vector, it was not possible to evaluate changes in vector specificity. It seems that if heterologous transcapsidation occurs in these plants as a result of virus infection in the field, there are at least two biologically significant outcomes to be considered. These are: 1) that heterologous transcapsidation events in the transgenic plants could alter or facilitate vector transmissibility of the new progeny virions (those generated as a result of heterologous transcapsidation); and 2) that heterologous transcapsidation events in transgenic plants could facilitate systemic movement of the resulting progeny virions within the transgenic plants when they

belong to a plant species in which the "normal" virus (that not resulting from heterologous transcapsidation interactions) does not readily move systematically. If the first scenario were to occur, and a virus were to gain vector transmissibility via heterologous transcapsidation with the transgenic plant, would the potential for new disease development be great either within the transgenic crop or in other plants? It is impossible to predict the answer for all situations, because cropping situations, geographic location, type of vector and its abundance, local crops and other factors will vary greatly from one country to another (Falk et al., 1995). Each of these scenarios will be discussed.

Scenario 1A. **Altered vector transmission and disease development in the transgenic crop**. If vector transmission of a plant virus were altered or facilitated as a result of heterologous transcapsidation interactions resulting from infection of a CP-expressing transgenic plant, it is not known whether this would cause significantly greater virus spread and disease development with the transgenic crop. In this scenario, any virus spread to a new transgenic plant as a result of heterologous transcapsidation would contain CPs derived from the CP-expressing transgenic plant. If these masked viruses were subsequently vector-transmitted to another CP-expressing plant within the same field (secondary spread), they might or might not be able to infect such a plant. In one experiment, Osbourn et al. (1990) challenged transgenic tobacco plants expressing the functional coat protein derived from U1 strain with a strain (DT1) of TMV that exists only as unencapsidated RNA. (The CP of this strain is defective.) Virions were produced that contained DT1 RNA encapsidated by U1 CP. When the masked virions were inoculated onto transgenic tobacco plants expressing U1 CP, the plants were resistant to infection. Control non-transgenic plants showed the expected symptoms. This supports the notion that secondary spread of masked virions is unlikely to occur within the transgenic crop, as the plants would be resistant to the masked virions.

Although transcapsidation may be detected under laboratory conditions, field tests under natural conditions will indicate whether the secondary spread of heterologous transcapsidated virions is likely. As part of an ongoing multi-year experiment to determine the potential biological impacts of transcapsidation, Dr. Gonsalves and co-workers have been attempting to determine whether there are biological impacts of transcapsidation in a field situation (Gonsalves et al., 1994; Fuchs and Gonsalves, 1995). Melon, squash, and cucurbit plants were developed that express the CP from a highly aphid-transmissible strain of CMV, strain WL. (The CP is known to be the only determinant in aphid-transmission in cucumoviruses.) Depending on the plant line used, the CP transgene may be expressed at relatively high or low concentrations. In the 1993 and 1994 growing season, these plants were grown in the field and challenge inoculated with a strain of CMV (strain C) that was not aphic-transmissible. The researchers looked in their inoculated transgenic plants and healthy, non-inoculated control plants for transcapsidated aphid-transmitted CMV. This transcapsidated CMV would have contained RNA from strain C, encapsidated with CP from WL strain derived from the plant transgene. Thus far, the spread of CMV C from inoculated transgenic to healthy non-transformed plants has not been detected. [Similar indications are also apparently emerging from the 1995 field trial (Fuchs, unpublished data)]. These experiments have been performed in a locality where the crops are routinely grown, the aphid vectors are abundant, and CMV is a serious problem in these crops. Further studies with other virus-crop systems will be useful in trying to confirm these findings (for scenario 1a) for other virus-plant systems and environmental conditions.

Scenario 1B. **Altered vector transmission and disease development with another plant**. It is also possible that if heterologous transcapsidation were to occur in CP-expressing transgenic plants, the resulting masked virion might be transmitted by the "new" vector to another plant or crop that is not transgenic. In this scenario, the transgenic crop would serve as a new virus reservoir (for the heterologous transcapsidated virus), allowing virus spread to a new plant. However, spread of heterologous

transcapsidated virions to the second plant would only be primary spread, i.e., spread from the transgenic plants where transcapsidation took place to a different plant species. Once the chimeric transcapsidated virions infect a new, non-transgenic host, they will again resort to the phenotype determined by the viral nucleic acid, as the only capsid protein source in these plants would now be their own genome. For these viruses to spread secondarily through the non-transgenic plant population, they would now have to be spread by their original vector, which may or may not be present (Falk et al., 1995). With respect to the potential effects of any primary spread, for many plant viruses, especially those of annual crops, the most common and economically important form of virus spread is secondary spread (Simons, 1959; Alderz, 1978). Primary spread generally involves few, or a limited number of, plants and in most cases does not result in economically important losses. Secondary spread, in contrast, can be rapid and involves spread from the initial, primary infected plants to the large numbers of remaining healthy plants (Matthews, 1991). Thus, if in the above example the transgenic plants were to serve as sources for primary spread of chimeric transcapsidated virion to another crop, disease and virus incidence from the primary spread would likely be limited in scope. Secondary spread in the non-transgenic plant could only occur if the natural vector(s) of the wildtype virus were already present. However, if the natural vector were already present, then it is possible that the natural vector could provide for primary as well as secondary spread, and both would spread wildtype virus (Falk et al., 1995). Of course this scenario, which deals with epidemiology of virus spread, depends greatly on the virus, vector, and local-site specific conditions, which might require case-by-case review.

Scenario 2. Disease development resulting from new systemic spread within transgenic plants. The movement of a virus from the initial site of infection throughout a plant, called systemic infection, requires expression of one or more viral genes (a dedicated movement protein, coat protein, and/or viral proteins) and a permissible host plant (Hull, 1989; Maule, 1991; Dawson et al., 1988; Marchoux et al., 1993; Dolja et al., 1995; Cronin et al., 1995; Valkonen and Somersalo, 1995). If a virus is unable to move from the initial site of infection, these infections are called subliminal. In a limited number of cases, viruses that cause subliminal infections in a host species may no longer be restricted when the host is infected by a second virus. In a large number of these studies (Atebekov and Talinsky, 1990) it has not been determined whether the coat protein is solely responsible for this helper dependent movement, but for viral taxa where a dedicated movement protein has not been described, consideration that the coat protein is the primary determinant of movement should be noted. If the coat protein expressed in the transgenic plant can facilitate the movement of viruses that cause subliminal infections, this would be a significant concern only if that CP was from a virus that rarely or never infects the recipient host plant. If CP is derived from a virus that is widely prevalent in the recipient plant, there would be no new novel interactions with subliminally-infecting viruses. This situation is true for the transgenic plants that are likely to be commercialised during the next few years (see Section V). This assumes the transgene is expressed in the same cells as virus. There are several situations in which this type of interaction may need further review.

a) Although the virus that provided the transgene may be widely prevalent in many countries, different strains may be present in different countries. Whether the biological properties of the transgene CP are identical to those of the CP from the viral strains present in another country would require a review.

b) If the virus that provided the CP transgene was not present in a country, then there could be new interactions between the transgene CP and viruses that cause subliminal infections. However, it is unlikely, but not inconceivable, that regulatory agencies in a country would be asked to approve a virus resistant plant where the virus was not an economically important pathogen.

c) If the virus that provided the transgene CP was present in the country, but was usually found in a different plant species from that of the recipient transgenic plant, there could be new interactions between the transgene CP and subliminally-infecting viruses.

In all these cases, if the viruses that cause subliminal infection of the recipient host are known, then easily performed tests can be conducted to determine whether CP facilitates their systemic movement. Whether the movement of the virus in the transgenic plant results in significant disease loss will depend on the virus, plant, and environmental conditions in each locality. Whether the virus can move from the transgenic plant will depend on its mode of transmission, especially whether viral vectors are present and feed on the transgenic crop.

Although not all of the useful experiments regarding the potential effects of heterologous transcapsidation have been completed, reports published in two OECD Member countries have reached certain conclusions about the potential risk concerns posed by heterologous transcapsidation. The conclusions reached in these two countries may not necessarily apply to all Member countries. In its report to the United Kingdom's Ministry of Agriculture, Fisheries, and Food entitled "Risks to the Agricultural Environment Associated with Current Strategies to Develop Virus Tolerant Plants Using Genetic Modification," Henry et al. (1995) state: "The general view is that transcapsidation is not a problem, because it is limited to a single transfer, i.e. once a transcapsidated genome is introduced into a new host, it reverts to using its own CP." In the report of a workshop on virus resistance prepared by the American Institute of Biological Sciences (AIBS) for the U.S. Department of Agriculture, a similar finding is reached (AIBS, 1995): "Transcapsidation of viral RNAs with coat protein produced by transgenic plants should not have long-term effects, since the genome of the infecting virus is not modified."

In conclusion, the potential impacts of transcapsidated viruses from viral CP-gene protected plants is generally expected to be no more serious than the impacts that occur in multiple viral infections of susceptible crops. However, there are a few cases with certain viral taxa where questions remain. Many of these potential impacts can be addressed via currently funded research or during variety development.

B. Synergy

Occasionally, when two viruses simultaneously naturally infect a plant, the symptoms can be more severe than when either of the viruses infects the plant singly. This phenomenon is called synergy (Matthews, 1991). Synergistic infections can often result in severely diseased, unmarketable crops. Synergy was first described and is best studied with PVX and PVY. The majority of synergistic viral combinations include, as one the viruses, a potyvirus (see **Table 1**, listing some synergistic interactions, which was prepared by Dr. V. Vance, University of South Carolina, U.S.A.). [The discussion here is limited to viral interactions that affect symptom development. Other specific interactions, e.g. the ability of TMV to move systemically in barley in the presence of brome mosaic bromovirus (Hamilton and Nichols, 1977), which probably result from movement protein complementation, will not be discussed.]

Will coat protein-mediated resistance produce unintended synergistic symptom expression when the resistant plant is infected by other plant viruses? Since potyvirus CP genes are not involved in synergism, it is unlikely that infection of a transgenic potyvirus resistant plant with any other virus would result in a synergistic interaction. It should also be noted that the specific potyviral gene involved in synergy is likely to be identified within the next few years. The identity of that gene is under investigation, and the search has been narrowed to three potential candidate genes on the 5'-end of the genome, the N-protease, helper component/protease, and the 50 kilodalton protein of unknown function (Vance et al.,

1995). [Preliminary indications are that the single gene responsible for the synergism symptom is the helper component-protease gene in PVY and potato X potexvirus and that the *same* gene is responsible for another synergistic symptom between PVY and tobacco mosaic tobamovirus (Vance, unpublished data).]

Because synergy, unlike recombination and transcapsidation, is not related to the potential for creation of new viruses, its effects can in a sense be considered to be agronomic rather than environmental. Evaluating the potential for interactions will be an important part of assessing the agronomic performance of a transgenic crop plant, and potential interactions would likely be assessed during the standard evaluations used in cultivar development.

C. Recombination

Recombination is defined as an exchange of nucleotide sequences between two nucleic acid molecules. Recombination between viral genomes results in heritable, permanent change. The persistence of a recombined viral genome will depend upon its fitness with respect to its ability to replicate within the original host cell, its ability to replicate in the presence of parental viruses, its ability to spread systemically within the host, or its successful transmission to other host plants.

Factors that influence recombination rates and detection of a viable recombinant include: sequence and structural similarity between the nucleic acid molecules, subcellular location and concentration of the nucleic acids, and the number of recombinational events required to form a viable recombinant viral genome (Lai, 1992). The frequency of recombination between two naturally occurring viruses or two viral strains in field-grown plants in the absence of selection pressure has not been determined (Henry et al., 1995), and is difficult or impossible to measure meaningfully. Recombination is hypothesised as an important mechanism for virus change over evolutionary time frames, during which they may have been quite frequent (Simon and Bujarski, 1994). Recently, the nucleotide sequences of numerous viral strains from many of the known genera have been published. Sequencing data have shown that certain genes in quite different taxa probably arose from recombinational events. In other cases, a single strain of a virus has been found to contain sequences apparently derived from a virus for a different taxa, while all other closely related strains do not have these sequences. [Listing all these events is outside the scope of this document. However, several references can provide readers additional information (Koonin and Dolja, 1993; Murphy et al., 1995; Sano et al., 1992; Edwards et al., 1992; LeGall et al., 1995; Pappu et al., 1994; Goulden et al., 1991; Mayo and Jolly, 1991; Revers et al., 1995; Gibbs and Cooper, 1995]. Currently, it is not possible to determine whether these recombinational events occurred, since for example the development of modern agricultural cropping practices or in much longer time frames. However, there is evidence of virus genome stability in shorter time frames, i.e., since the establishment of plant virology as a science. First, the biological properties of TMV have remained remarkably stable over the past century (Ford and Tolin, 1983; Dawson, 1992); and second, the Dutch and Wisconsin (U.S.A.) substrains of alfalfa mosaic alfalmovirus strain 425 have acquired, in approximately 20 years of laboratory use in each country, several nucleotide changes leading to five amino acid changes with apparently no significant changes in biological properties (Jaspars, 1985).

The potential use of virus resistant transgenic plants in agriculture highlights the following questions regarding recombination when transgenic plants are used:

a) Will the overall rate of viral recombination in nature be increased when these transgenic plants are used because there will be increased opportunity for recombination?
b) What factors may affect the rate of recombination, and will that rate be proportional to the concentrations to parent molecules?
c) Are any recombinants thus formed likely to be successful in competition with parental viruses?

Most transgenic plants are likely to be engineered in the near term with CP genes from viruses that regularly infect the host plant, because damage by those viruses poses the most constant potential for loss in the crop species. Sequences from those viruses, when available for recombination, would be unlikely to pose the potential for generating *novel* recombinants in comparison with natural mixed infections in the recipient plant, given certain conditions described below. (Genes from viruses that do not regularly infect the host plant might sometimes be introduced for experimental or other purposes, and the arguments herein would not necessarily apply in those instances.) In most virus resistant plants that have been experimentally engineered to date, transgenes that yield effective resistance to a target virus are usually expressed at very low levels compared with the levels seen in virus-infected plants. For example, in Asgrow's ZW20 squash, infected non-transgenic squash plants had a 100-fold higher concentration of viral RNA than the corresponding CP-transformed ZW20 plants. It is unlikely, though not impossible, that any compelling reason will emerge for scientists or breeders to develop new plant varieties in which high levels of transgene products are expressed, inasmuch as low level expression appears effective in conferring virus resistance. With regard to this issue, the AIBS report notes: "The implications of these low expression levels for recombination are not clear. Even assuming that the higher concentration of transgene RNA the greater the chance for recombination, we do not know what a meaningful range is; what are low and high concentrations of transgene transcript relative to unacceptable recombination rates? Currently, this information (concentration of transgene RNA) is of no use to regulatory agencies because there is no way to factor concentrations of RNA or protein into risk determination in a meaningful manner" (AIBS, 1995). Even given these quantitative uncertainties, however, the type of background information about virus identity, environment, and disease pressure characterisation indicated in Section III above is helpful.

The use of CP gene-mediated resistance might open the possibility of novel interactions between tissue-specific viruses and other viruses. In cases where the plant is systemically infected (i.e., virus can be found in all cell types), the cellular location of the transgene is probably not a major issue. In contrast, if a coat protein transgene from a phloem-limited virus is used for resistance, this might increase the probability of new interactions between the transgene transcript or its gene product and other viruses that replicate only in non-phloem tissues. These new interactions may result in modified symptoms, insect transmission of the infecting virus, or modified movement of the infecting virus within the transgenic plant. However, unless a recombinational event occurred between the transgene and the infecting virus and the resulting recombinant virus was competitive, the effect would be limited and restricted to the transgenic crop. If viral infections that result in subliminal infections are known in this crop, the interactions of the transgene with these viruses in terms of important parameters (movement, symptoms, and insect transmission) can be evaluated experimentally.

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Table 1

Reported Viral Synergisms

Potyviral Synergistic Interactions		**References**
Potato Y potyvirus (PVY)	Potato X potexvirus (PVX)	Rochow, W. F., Ross, A. F. 1955. Plant Disease (Reporter) 52:344-358.
Tobacco vein mottling potyvirus	PVX	Vance, V., B. Berger, P. H., Carrington, J. C., Hunt, A. G., Shi, X. M. 1995. Virology 206:583-590.
Tobacco etch potyvirus (TEV)	PVX	see above
Pepper mottle potyvirus	PVX	see above
Blackeye cowpea mosaic potyvirus	Cucumber mosaic cucumovirus (CMV)	Pio-Ribeiro, G., Wyatt, S. D., Kuhn, C.W. 1978. Phytopathology 68: 1260-1265.
Cowpea aphid borne potyvirus	CMV	Fisher, H. U., Lockhart, B. E. 1976. Phytopathology Z. 85:132-138.
Bean yellow mosaic potyvirus	CMV	Harrison, A. N., Gudauskas, R. T. 1968. Plant Disease (Reporter) 52:509-511.
Zucchini yellow mosaic potyvirus	CMV	Poolpol, P., Inouye, T. 1968. Annal Phytopathology Society of Japan 52:22-30.
Soybean mosaic potyvirus	Bean pod mottle comovirus	Calvert, L. A., Ghabrial, S. A. 1983 .Phytopathology 73:992-997. Les, Y-S., Ross, J. P. 1968. Phytopathology 62:839-845. Quiniones, S. S., Dunleavy, J. M. 1971. Phytopathology 763-766. Ross, J. P. 1968. Plant Disease (Reporter) 52:344-348.
SMV	Cowpea mosaic comovirus	Anjos, J. R., Jarlfors, U., Ghabrial, S. A. 1992. Phytopathology 82:17-23.

Maize dwarf mosaic potyvirus	Maize chlorotic mottle virus? (MCMV)	Goldberg, K-B., Brakke, M. K. 1987. Phytopathology 77:162-177. Niblett, C. I., Claflin, L. E. 977. Plant Disease (Reporter) 62:15-19. Uyemoto, J. K., Claflin, L. E., Wilson, D. L., Raney, R. J. 1981. Plant Disease 65:39-41.
Wheat streak mosaic potyvirus	MCMV	see above
PVY	TMV	Clark, R. L., Hill, J. H., Ellis, M. D. 1980. Phytopathology 70:131-134.
Turnip mosaic potyvirus	Cauliflower mosaic caulimovirus	Kahn, M. A., Demski, J. W. 1982. Plant Disease 66:253-256.
MDMV	Barley yellow dwarf luteovirus	Belli, G., Cinquanta, S., Soneini, C. 1980. Rivista Pathol. Veg. 16:83-86.
TEV	Dodder latent mosaic virus	Bennett, C. W. 1949. Phytopathology 39:637-646.
Non-potyviral Synergistic Interactions		
TMV	PVX	Vanterpool, T. C. 1926. Phytopathology 16:311-331.
TMV	CMV	Garces-Orejuela, C., Pound, G. S. 1957. Phytopathology 47:232-239.
TMV	Tobacco ringspot nepovirus	see above
TMV	Tomato aspermy cucumovirus	Holmes, F. O. 1956. Virology 611-617.
Cowpea chlorotic mottle bromovirus	Southern bean mosaic sobemovirus	Kuhn, C. W., Dawson, W. O. 1973. Phytopathology 63:1380-1385.
Alfalfa mosaic alfamovirus	potato acuba potexvirus	Kassanis, B. 1963. Advances in Virus Research 66:253-256.

There have been attempts to use transgenic plants to estimate experimentally the potential frequency of recombination between the transgene mRNA and the genome of a challenging virus, and/or to determine the rate of recombination between two viruses (or two viral strains). In transgenic plants expressing sequences derived from either a DNA virus (Schoelz and Wintermantel, 1993) or RNA virus (Greene and Allison, 1994), it has been demonstrated in some experiments that recombination between a viral transgene and a defective challenge virus can restore a functional, infective virus under high selection pressure. These results demonstrate that recombinational events can eventually occur in plants expressing viral sequences when inoculated with defective viruses. Because of the great interest in this area, it is expected that in the next several years additional information on the factors that influence recombination will be better understood. The results of all experiments dealing with recombination must be interpreted carefully before conclusions can be drawn, since no single experimental design is ideal to address each potential environmental condition, and each virus taxon, and certain assumptions and conditions are part of each experimental design. Some points to consider in interpreting these experiments are:

a) Are the transgenic plants susceptible or resistant to viral infection? Some scientists have developed experimental systems to study recombination, in which the transgenic plants expressing a viral sequence are susceptible to infection by the virus which provides the transgene. In a susceptible transgenic plant the amount of viral RNA from the infecting virus would be greater than in a resistant plant; thus, higher concentrations of RNA might increase the likelihood of recombination in the experimental system. Most, if not all, transgenic plants containing CP genes that are commercialised are likely to be resistant to infection by the virus (or strain) that provided the transgene sequence.

b) What is the selection pressure in the experiment? The AIBS report (1995) provided the following definition: "[H]igh selection pressure is defined as conditions that favour the recombinant virus, for example, a situation where the virus is not viable unless a recombination event occurs. Low selection pressure would be a situation where the novel phenotype does not confer a competitive advantage to the recombinant under the conditions of the experiment." A clear understanding of selection pressure in the experiment between a viral transgene and an infecting virus is important, since the recombination rate must be compared to natural recombination rates between the two viruses (or strains) to provide a meaningful comparison. The natural recombination rates between two viruses (or strains) may be high or low.

c) Were the experiments performed in the natural hosts for the viruses? If a recombinant virus is formed, is it competitive with wild-type virus? Recombination rates may be affected by the host organism (Lai, 1992). The host plant also affects the mutation rates of the infecting virus (Dawson, 1992). Often, virologists have used *Nicotiana* species as experimental hosts because they are easy to transform and grow, although they are not the natural hosts of the viruses being studied. As one example, the natural host range of cauliflower mosaic virus is limited to the Brassica family (Matthews, 1991), but experiments on this virus have been performed in Solanaceous plants (Takahashi et al., 1989; Baughman et al., 1989; Schoelz and Shepherd, 1988). However, recombinant viruses can frequently be observed to have increased virulence (i.e., more severe symptoms) on model host plants that are not the natural host of either viral parent (infecting virus or virus that the transgene was derived from). If a recombinant virus is generated, determining whether it is competitive with wild type virus in the natural host of the infecting virus and the virus that provided the transgene sequence is most relevant.

d) Do experiments performed in field situations provide additional benefits as compared to laboratory or greenhouse experiments? Whether there is any logistical or conceptual advantage for a field experiment versus a test under contained conditions depends on the experiment. However, in a field test plants are grown under natural stresses that would be found in a commercialised crop, including inoculation of the plants by vectors containing widely prevalent viral strains of that locality, and the presence of other diseases and pests, including other viruses, etc.

If a recombinant virus is formed in a cell (either in a transgenic plant or during a mixed infection), will that recombinant participate in the replication process in that cell, move systemically in the plant, or cause a new disease? The vast majority of progeny viruses do not apparently function in the replication process. For many viruses, the RNA is encapsidated by CP, viral RNA synthesis in the cell ceases or declines to undetectable levels, and, depending on the virus and whether it is transmitted to another plant or via progeny, is degraded when the plant cell dies (Matthews, 1991). The likelihood of a recombinant becoming established depends on many factors, including its competitiveness with infecting virus and other viruses that naturally infect the plant, and all the additional factors that may affect selection pressure (e.g., temperature, vectors, host plants). Thus, to predict the probability of development of new virus disease resulting from recombination of two viruses, or between a virus and a viral derived transgene, requires a considerable level of understanding of the population biology of viruses in cells and virus movement within plants, and a better understanding of the mechanisms of how viruses cause disease.

Much of the discussion of formation of recombinant virus or the detection of new viral strains may leave the impression that a strain of virus is homogenous with respect to plant-induced symptoms or nucleotide sequence. All the single-stranded RNA genomes that have been examined have been found to exist not as a unique nucleotide sequence, but as a collection of related sequence variants around a consensus sequence. This sequence microheterogeneity is always present in natural populations (Holland et al., 1982; Domingo et al., 1985; Morch et al., 1988). This microheterogeneity in viral sequence has led to the concept of "quasi-species" for some viruses (Eigen, 1993). It is thought to be a result of the lack of proof-reading function in the viral replicases and of the large quantity of viral RNA produced per cell.

Most variants have one or two nucleotide changes, although some viruses (e.g. soilborne wheat mosaic furovirus) are known to have large deletions in some genes (Matthews, 1991). Variants can also be detected by changes in symptomatology. A PVX strain that produces chlorotic local lesions on tobacco plants frequently gave rise to ring spot local lesion production (Matthews, 1949). A tobacco necrosis necrovirus strain that produced white lesions on cowpea frequently gave rise to strains giving red lesions (Fulton, 1952). Thus, the microheterogeneity of viral RNA may result in sequence variation with no visible differences to major symptom alterations. Of course, even more variability in both sequence and plant-induced symptoms exists in a single virus because many viruses have well-characterised, stable strains that are sufficiently different to have been given a unique identifier (Matthews, 1991).

Although additional research is currently being funded on viral recombination, reports in two OECD Member countries have reached certain conclusions about the potential risk concerns posed by the appearance of new viruses. The conclusions reached in these two countries may not necessarily apply to all Member countries. In a report to Agricultural and Agri-Food Canada, Rochon et al. (1995) conclude: "It is likely that current means of detecting and controlling new diseases in this country would be adequate to control any new virus resulting from recombination between a transgene and another virus." The AIBS report to USDA (1995) concludes by stating: "With or without the use of transgenic plants, new plant virus diseases will develop that will require attention."

Undoubtedly, many new crop varieties will need to be developed to resist emerging viruses or new strains of existing viruses. The appropriate application of scientific analysis to ensure the biosafety of new varieties will allow effective control of these diseases while protecting long-term agricultural productivity and the environment.

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No. 31, *Workshop on Emergency Preparedness and Response and on Research in Accident Prevention, Preparedness and Response* (1990)[F]

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No. 39, *International Survey on Biotechnology Use and Regulations* (1990)[F]

OCDE/GD(91)102 *Users Guide to Hazardous Substance Data Banks Available in OECD Member Countries* (1991)[F] ***[out of print]***

OCDE/GD(91)103 *Users Guide to Information Systems Useful to Emergency Planners and Responders Available in OECD Member Countries* (1991)[F] ***[out of print]***

[The two Users Guides above were translated into Spanish by UNEP IE.]

No. 43, *International Directory of Emergency Response Centres* (1992)[F]
[under revision by the OECD and UNEP IE]

[The International Directory is a co-operative project of OECD and UNEP IE. The emergency response centres in the Directory are located in OECD and non-OECD countries.]

No. 44, *Workshop on Prevention of Accidents Involving Hazardous Substances: The Role of the Human Factor in Plant Operations* (1992)

No. 45, *The OECD Principles of Good Laboratory Practice* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 1]*** (1992)[F]

No. 46, *Guides for Compliance Monitoring Procedures for Good Laboratory Practice* (1992)[F]

[***superseded*** by Environment Monograph No. 110, *Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice* (1995)]

No. 47, *Guidance for the Conduct of Laboratory Inspections and Study Audits* (1992)[F]

[***superseded*** by Environment Monograph No. 111, *Revised Guidance for the Conduct of Laboratory Inspections and Study Audits* (1995)]

No. 48, *Quality Assurance and GLP* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 4]*** (1992)[F]

No. 49, *Compliance of Laboratory Suppliers with GLP Principles* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 5]*** (1992)[F]

No. 50, *The Application of the GLP Principles to Field Studies* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 6]*** (1992)[F]

No. 51, *Guiding Principles for Chemical Accident Prevention, Preparedness and Response: Guidance for Public Authorities, Industry, Labour and Others for the Establishment of Programmes and Policies related to Prevention of, Preparedness for, and Response to Accidents Involving Hazardous Substances Areas* (1992)[F]

[The Guiding Principles are also available from the OECD in Russian and may be translated into other languages. In 1996, two Guidance Documents to be used in conjunction with the Guiding Principles were published (see below). For more information, please contact the Environmental Health and Safety Division.]

No. 52, *Report of the OECD Workshop on Monitoring of Organisms Introduced into the Environment* (1992)

No. 58, *Report of the OECD Workshop on Quantitative Structure Activity Relationships (QSARS) in Aquatic Effects Assessment* (1992)

No. 59, *Report of the OECD Workshop on the Extrapolation of Laboratory Aquatic Toxicity Data to the Real Environment* (1992)

No. 60, *Report of the OECD Workshop on Effects Assessment of Chemicals in Sediment* (1992)

No. 65, *Risk Reduction Monograph No. 1: Lead. Background and National Experience with Reducing Risk* ***[Series on Risk Reduction No. 1]*** (1993)

No. 66, *Report of the OECD Workshop on Strategies for Transporting Dangerous Goods by Road: Safety and Environmental Protection* (1993)

[The OECD's Chemical Accidents Programme and Road Transport Research Programme co-operated in organising this workshop.]

No. 67, *Application of Structure-Activity Relationships to the Estimation of Properties Important in Exposure Assessment* (1993)

No. 68, *Structure-Activity Relationships for Biodegradation* (1993)

No. 69, *Report of the OECD Workshop on the Application of Simple Models for Exposure Assessment* (1993)

No. 70, *Occupational and Consumer Exposure Assessments* (1993)

No. 73, *The Application of the GLP Principles to Short-term Studies* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 7]*** (1993)[F]

No. 74, *The Role and Responsibilities of the Study Director in GLP Studies* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 8]*** (1993)[F]

No. 76, *Guidance Document for the Development of OECD Guidelines for Testing of Chemicals* (1993; reformatted 1995) ***[Series on Testing and Assessment No. 1]***[F]

No. 77, *Data Requirements for Pesticide Registration in OECD Member Countries: Survey Results* ***[Series on Pesticides No. 1]*** (1993)

No. 81, *Health Aspects of Chemical Accidents: Guidance on Chemical Accident Awareness, Preparedness and Response for Health Professionals and Emergency Responders Areas* (1994)[F]

[Four international organisations collaborated in the preparation of this publication: the International Programme on Chemical Safety (IPCS), OECD, UNEP IE, and the World Health Organization - European Centre for Environment and Health (WHO-ECEH).]

No. 88, *US EPA/EC Joint Project on the Evaluation of (Quantitative) Structure Activity Relationships* (1994)

No. 90, *Ottawa '92: The OECD Workshop on Methods for Monitoring Organisms in the Environment* (1994)

No. 91, *Compendium of Methods for Monitoring Organisms in the Environment* (1994)

[Monographs No. 90 and 91 are companion documents.]

No. 92, *Guidance Document for Aquatic Effects Assessment [**Series on Testing and Assessment No. 3]*** (1995)

No. 93, *Report of the OECD Workshop on Chemical Safety in Port Areas* (1994)

[This Workshop was co-sponsored by OECD, the International Maritime Organization (IMO) and UNEP. Also see Monograph No. 188.]

No. 94, *Report of the OECD Special Session on Chemical Accident Prevention, Preparedness and Response at Transport Interfaces Areas* (1995)

No. 95, *Report of the OECD Workshop on Small and Medium-sized Enterprises in Relation to Chemical Accident Prevention, Preparedness and Response Areas* (1995)

No. 98, *Detailed Review Paper on Biodegradability Testing **[Series on Testing and Assessment No. 2]*** (1995)

No. 99, *Commercialisation of Agricultural Products Derived through Modern Biotechnology: Survey Results **[Series on Harmonization of Regulatory Oversight in Biotechnology No. 1]*** (1995)

No. 100, *Analysis of Information Elements Used in the Assessment of Certain Products of Modern Biotechnology **[Series on Harmonization of Regulatory Oversight in Biotechnology No. 2]*** (1995)

No. 101, *Risk Reduction Monograph No. 2: Methylene Chloride. Background and National Experience with Reducing Risk **[Series on Risk Reduction No. 2]*** (1994)

No. 102, *Risk Reduction Monograph No. 3: Selected Brominated Flame Retardants. Background and National Experience with Reducing Risk* ***[Series on Risk Reduction No. 3]*** (1994)

No. 103, *Risk Reduction Monograph No. 4: Mercury. Background and National Experience with Reducing Risk* ***[Series on Risk Reduction No. 4]*** (1994)

No. 104, *Risk Reduction Monograph No. 5: Cadmium. Background and National Experience with Reducing Risk* ***[Series on Risk Reduction No. 5]*** (1994)

No. 105, *Report of the OECD Workshop on Environmental Hazard/Risk Assessment* ***[Series on Testing and Assessment No. 4]*** (1995)

No. 106, *Data Requirements for Registration of Biopesticides in OECD Member Countries: Survey Results* ***[Series on Pesticides No. 3]*** (1996)

No. 107, *Report of the OECD Workshop on the Commercialisation of Agricultural Products Derived through Modern Biotechnology* ***[Series on Harmonization of Regulatory Oversight in Biotechnology No. 3]*** (1995)

No. 108, *Final Report on the OECD Pilot Project to Compare Pesticide Data Reviews* ***[Series on Pesticides No. 2]*** (1995)

No. 110, *Revised Guides for Compliance Monitoring Procedures for Good Laboratory Practice* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 9]*** (1995)[F]

No. 111, *Revised Guidance for the Conduct of Laboratory Inspections and Study Audits* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 10]*** (1995)[F]

No. 115, *Guidance for the Preparation of GLP Inspection Reports* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 11]*** (1995)[F]

No. 116, *The Application of the Principles of GLP to Computerised Systems* ***[Series on Good Laboratory Practice and Compliance Monitoring No. 12]*** (1995)[F]

No. 117, *Industrial Products of Modern Biotechnology Intended for Release to the Environment: The Proceedings of the Fribourg Workshop* ***[Series on Harmonization of Regulatory Oversight in Biotechnology No. 4]*** (1996)

No. 118, *Guidance Concerning Chemical Safety in Port Areas. Guidance for the Establishment of Programmes and Policies Related to Prevention of, Preparedness for, and Response to Accidents Involving Hazardous Substances. Prepared as a Joint Effort of the OECD and the International Maritime Organization (IMO) (1996)*

OCDE/GD(96)104 *Guidance concerning Health Aspects of Chemical Accidents. For Use in the Establishment of Programmes and Policies Related to Prevention of, Preparedness for, and Response to Accidents Involving Hazardous Substances. To Be Read in Conjunction with the OECD Guiding Principles for Chemical Accident Prevention, Preparedness and Response Areas* (1996)

OCDE/GD(96)121 *Activities to Reduce Pesticide Risks in OECD and Selected FAO Countries. Part 1: Summary Report* ***[Series on Pesticides No. 4]*** (1996)[F]

OCDE/GD(96)122 *Activities to Reduce Pesticide Risks in OECD and Selected FAO Countries. Part 2: Survey Responses* ***[Series on Pesticides No. 5]*** (1996)

OCDE/GD(96)163 Methylene Chloride Information Exchange Progamme: Survey Results ***[Series on Risk Management No. 6]*** *(1996)*

Priced Publications:

OECD Guidelines for Testing of Chemicals (updated 1996)[F]
(OECD No. 97 93 50 1) ISBN 92-64-14018-2 992 pages
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[Available in CD-ROM version: for more information, contact the OECD Publications Service]

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Consensus Document on Information Used in the Assessment of Environmental Applications Involving Bacillus