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FIFRA SCIENTIFIC ADVISORY PANEL (SAP)

OPEN MEETING

OCTOBER 13 - 15, 2004

ISSUES ASSOCIATED WITH DEPLOYMENT OF A TYPE OF PLANT-INCORPORATED PROTECTANT (PIP), SPECIFICALLY THOSE BASED ON PLANT VIRAL COAT PROTEINS (PVCP-PIPS)

WEDNESDAY, OCTOBER 13, 2004

VOLUME I OF IV

(Morning session)

Located at: Holiday Inn - National Airport
2650 Jefferson Davis Highway
Arlington, VA 22202

Reported by: Frances M. Freeman, Stenographer

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1 DR. ROBERTS: Good morning. And welcome to the
2 October 13th meeting of the FIFRA Scientific Advisory
3 Panel.
4 The topic that we're going to address in our
5 session over the next couple of days are issues associated
6 with deployment of a type of plant incorporated
7 protectant, specifically those based on plant viral coat
8 proteins.
9 The SAP staff has assembled an outstanding,
10 truly outstanding panel of experts, I think, to address
11 questions that the agency are posing on this topic.
12 I would like to begin today's session by
13 introducing the panel. Let me do so by starting on my
14 left and we'll just kind of go around the table clockwise.
15 Among the panel members I would ask each to state their
16 name, their affiliation and their area of expertise.
17 Beginning with Dr. Melcher.
18 DR. MELCHER: I'm Ulrich Melcher from Oklahoma
19 State University, in biochemistry and molecular biology.
20 I'm a plant virologist with expertise in recombination and
21 bioinformatics.

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1 DR. SHERWOOD: John Sherwood, Department of
2 Plant Pathology, University of Georgia, plant virology,
3 cross protection and epidemiology.
4 DR. ZAITLIN: I'm Milt Zaitlin, professor of
5 Meritis of plant pathology, Cornell University, Meritis
6 director of the Cornell biotechnology program.
7 My research field was plant virology. In the
8 latter parts of my research career we stumbled on another
9 way of making plants resistant to virus, that is, using
10 replicase genes.
11 DR. FALK: I'm Bryce Falk. I'm from the
12 University of California at Davis, a plant virologist, and
13 my primary expertise is in virus biology and epidemiology.
14
15 DR. ALLISON: My name is Richard Allison. I'm
16 from Michigan State University where I'm a plant
17 virologist with an interest in the risk assessment of
18 genetically modified plants and particularly the
19 recombination of RNA viruses.
20 DR. HAMMOND: I'm John Hammond. I'm with USDA
21 agricultural research service. I'm a plant virologist

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1 with expertise in plant virus detection, characterization,
2 transgenic resistance and risk assessment.
3 DR. TEPFER: I'm Mark Tepfer. I work at the
4 National Institute for Agronomic Research in France. It
5 is sort of the
6 French equivalent of USDA. I have worked on virus
7 resistant transgenic plants since the middle of the 1980s
8 and bio safety questions related to that.
9 DR. COOPER: Ian Cooper with Natural Environment
10 Research Council of the United Kingdom concerned with
11 plant viruses, how they spread, what the consequences are
12 and laterally the risks of genetically modified plants.
13 DR. STEWART: Dr. Neal Stewart, University of
14 Tennessee. I work with transgenic plants, mainly looking
15 at gene flow introgression and the consequences in
16 ecological systems.
17 DR. NAGY: My name is Peter Nagy. I'm from
18 University of Kentucky. My major expertise is in
19 mechanism of virus, recombination and replication and the
20 emergence of new viruses.
21 DR. BUJARSKI: I'm Jozef Bujarski from Northern

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1 Illinois University, Department of Biological Sciences.
2 I'm a plant virologist interested in studying bio RNA
3 recombination and replication.
4 DR. STARK: I'm John Stark from Washington State
5 University. I'm an ecotoxicologist and I work in risk
6 assessment, particularly of pesticides.
7 DR. GENDEL: I'm Steve Gendel. I'm with the FDA
8 at the National Center for Food Safety and Technology in
9 Chicago. My expertise is food safety in biotechnology and
10 bioinfomatics.
11 DR. ISOM: I'm Gary Isom, professor of
12 Toxicology at Purdue University. My area is
13 neurotoxicology and research interest in neuro
14 degeneration.
15 DR. PORTIER: I'm Ken Portier, a statistician
16 with the Institute of Food and Agricultural Sciences at
17 the University of Florida with interest in statistical
18 issues of risk assessment.
19 DR. HEERINGA: Steve Heeringa, the University of
20 Michigan. I'm here as a permanent member of the FIFRA SAP
21 panel. I'm a biostatistician.

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1 DR. ROBERTS: I'm Steve Roberts. I'm professor
2 of Toxicology at the University of Florida. It is my
3 pleasure to chair today's session.
4 Our designated federal official for today's
5 session is Mr. Paul Lewis. I would like to welcome Paul
6 and ask him if he has any comments or announcements for
7 today's session.
8 MR. LEWIS: Thank you, Dr. Roberts. And thank
9 you for agreeing to serve as chair for this three day
10 meeting of the FIFRA Scientific Advisory Panel.
11 I also want to again thank all the members of
12 the panel that have spent time preparing for this meeting
13 and looking forward to upcoming deliberations that we're
14 going to have over the next three days and the time they
15 have at this meeting today and the subsequent report
16 writing process taking into account their busy schedules.
17 As the designated federal official for this
18 meeting, I serve as liaison between the panel and the
19 agency. I'm also responsible for ensuring provisions of
20 the Federal Advisory Committee Act are met.
21 The Federal Advisory Committee Act was

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1 established in 1972 for a system of governing the
2 creation, operation, termination of executive branch
3 advisory committees.
4 The FIFRA SAP is subject to all requirements of
5 the Federal Advisory Committee Act. These include open
6 meetings, timely public notice of meetings and docket
7 availability. That's through the Office of Pesticides
8 Program's public docket system and the E docket system.
9 As the designated federal official for this
10 meeting, a critical responsibility is to work with
11 appropriate agency officials to ensure all appropriate
12 ethics regulations are satisfied.
13 In that capacity, panel members are briefed
14 provisions of the federal conflict of interest laws.
15 Each participant has filed a standard
16 government financial disclosure report And I along with
17 our deputy ethics officer for the Office of Prevention of
18 Pesticides and Toxic Substances and in consultation with
19 the Office of the General Counsel at EPA have reviewed the
20 report to ensure all ethics requirements are met.
21 A sample copy of this form is available on the

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1 FIFRA SAP web site.

2 The panel will be reviewing challenging science

3 issues over the next several days. We have a full agenda

4 of topics for discussion. And the meeting times are

5 approximate, thus may not keep to the exact times as noted

6 to panel discussions and public comments.

7 We strive to ensure adequate time for agency

8 presentations, public comments be presented and panel

9 deliberations.

10 For presenters, panel members and public

11 commenters, please identify yourself and speak into the

12 microphones provided since the meeting is being recorded.

13

14 In addition, a transcript will be available

15 for this meeting in approximately 2 weeks.

16 Copies of presentation materials and public

17 comments will be available in the Office of Pesticides

18 Program docket in about two to three days and also

19 available in the E docket system.

20 For members of the public requesting time to

21 make a public comment, please limit your remarks to 5

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1 minutes unless prior arrangements have been made. And for
2 those who have not preregistered, please approach myself
3 or the FIFRA SAP staff sitting behind me to register to
4 speak at the public time this afternoon.
5 As I mentioned previously, there is a public
6 docket for this meeting. All background materials,
7 questions to the panel by the agency and other documents
8 related to the SAP meeting are available on the docket.
9 In addition, the background documents and
10 subsequent materials are available on the EPA SAP web
11 site.
12 The meeting agenda lists the contact information
13 for receiving that material either through the SAP web
14 site or through the Office of Pesticides Program docket.
15 At the conclusion of the meeting, the SAP will
16 prepare a report as response to questions posed to the
17 agency, background materials, presentations and public
18 comments this. This report serves as the meeting minutes
19 basically summarizing the panel's comments during the
20 course of our discussion. We anticipate the
21 meeting minutes will be completed in approximately 6

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1 weeks.

2 I want to again thank the FIFRA SAP members, the

3 ad hoc members, my colleagues at EPA and members of the

4 public for being involved in the upcoming meeting we'll

5 have in the next three days. I'm looking forward to some

6 very interesting and challenging discussion.

7 Thank you, Dr. Roberts.

8 DR. ROBERTS: Thank you, Paul. I would like to

9 welcome to today's session Mr. Joe Merenda, who is the

10 director of the Office of Science Coordination and Policy

11 from EPA. Welcome, Joe.

12 MR. MERENDA: Thank you, Dr. Roberts. It is my

13 pleasure at this point at the beginning of the meeting to

14 welcome all the panel members as well as members of the

15 public to this session. And particularly to thank the

16 panel members for volunteering to take the time from your

17 busy schedules to serve to provide this advice to the

18 Environmental Protection Agency.

19 EPA's commitment to sound science is very

20 heavily based upon getting rigorous independent external

21 peer review of the issues that it has to deal with in

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1 making its regulatory decisions. And among other forums,
2 the FIFRA Scientific Advisory Panel is a key area or key
3 mechanism by which EPA for its pesticide programs in
4 particular obtains that kind of external review.
5 So we very much value your service and we look
6 forward to your participation.
7 In most of these meetings, my office, the Office
8 of Science Coordination and Policy, plays the role of
9 convener and process facilitator for the panel meetings.
10 In this instance, we have a little bit of a
11 broader role because my office also is responsible for
12 some of the activities that the Office of Prevention
13 Pesticides and Toxic Substances carries out in
14 biotechnology.
15 Members of my staff are part of the presentation
16 panel here today. I'm particularly interested not only
17 from the process of having a good panel, but also from the
18 output in this one.
19 Unfortunately, from my schedule, I won't be able
20 to be with you for the entire meeting , but I will try and
21 spend as much of the time over the next 3 days as I can

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1 with you.

2 Again, thank you and welcome.

3 DR. ROBERTS: Thank you very much. We're going

4 to spend much of the morning listening to some

5 presentations by the agency. I would like to welcome Mr.

6 Dennis Szuhay who is the chief of the Microbial Pesticides

7 Branch of the Office of Pesticides Program, and other

8 agency staff will be making some presentations this

9 morning.

10 Welcome.

11 DR. SZUHAY: Thank you, Dr. Roberts. Again, I

12 want to echo Joe Merenda's comments about welcoming you

13 all, thanking you for your service over the next 3 days.

14 Most importantly, I'm looking forward to a very

15 robust discussion of the issues before us this week,

16 because it is my division and my branch, specifically,

17 that will be having to work with your suggestions, deal

18 with our management in terms of figuring out what the best

19 road is should we choose to do some regulatory option with

20 these particular organisms.

21 And it will be a very interesting discussion. I

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1 look forward to all of your participation throughout the
2 week.
3 And I also would like to add that I have had
4 the pleasure of watching this process evolve over the last
5 couple of months as the work group has grappled with the
6 issues, tried to focus on which scientific questions and
7 which issues merited the most of your attention and also
8 in the fine tuning of the presentations that you are about
9 to hear this morning.
10 So with all of this, I thank you again. I look
11 forward to a productive week and also to a final report at
12 the end of the whole process.
13 DR. ROBERTS: Thank you.
14 The first presentation on our agenda is one on
15 PVCP PIPS: The Context, by Dr. Milewski.
16 Good morning, welcome.
17 DR. MILEWSKI: Good morning. It is my pleasure
18 this morning to try to describe to you the context in
19 which we'll be operating today.
20 I will try to cover some of the broader range of
21 context which would include some of the technical things

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1 we're going to be discussing today and also to give you a
2 small sense of the regulatory environment in which we
3 operate.
4 With that, I would like to go to my first slide.
5 Second slide. Thank you.
6 This is the organization of today's
7 presentation. Essentially, we'll be setting the stage for
8 your discussions later today. I will be giving you your
9 charge and discussing your context.
10 Dr. Anne Fairbrother from our Office of Research
11 and Development will be talking about gene flow. Dr.
12 Mellissa Kramer from our Office of Science Coordination
13 and Policy will be talking about viral interactions. And
14 then I will pop up again at the end to talk about some of
15 the other scientific considerations to flow from the
16 earlier presentations and the questions that you will have
17 before you today.
18 I would like to briefly repeat to you the charge
19 to this committee, which is we're asking you to provide
20 scientific advice to assist us in our evaluation of
21 several technical issues associated with PVCP PIPs.

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1 Specifically, we're going to ask you to respond
2 to a series of technical questions related to exposure and
3 hazard considerations for PVCP PIPs.
4 To begin, what is a PVCP PIP. Well, PIP is an
5 acronym for a plant incorporated protectant. That is a
6 type of pesticide.
7 PVCP is an acronym for plant virus coat protein.
8 And because it's kind of a tongue twister, we have agreed
9 amongst ourselves that the first P would be silent. So we
10 would say VCP PIPs. But every once in awhile when you see
11 it up there you put in the P. Sometimes I'll be saying
12 PVCP PIPs and sometimes I'll be saying VCP PIPs, which
13 even as I say it you can hear the second is a little bit
14 easier, at least I think.
15 Hopefully with that explanation let's try to use
16 VCP PIPs.
17 What is a plant incorporated protectant?
18 Essentially, we have defined it as a pesticidal substance
19 that is intended to be produced and used in a living plant
20 or in the produce thereof and the genetic material
21 necessary for production of such a pesticidal substance.

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1

2 I have underlined what I think are the 2 key
3 ideas that we have to carry forward today. One is that
4 when we talk about a PIP, we are talking about the
5 pesticidal substance, which could be a protein produced or
6 it could be another substance such as a messenger RNA and
7 the genetic material that's necessary to produce it. In
8 this case, for example, the DNA.
9 Basically, the definition of a PVCP PIP is a PIP
10 created from the gene or a segment of the gene that codes
11 for coat protein of a virus that naturally infects crop
12 plants. That's the definition we'll be working with
13 today.
14 Because what we'll be talking about for most of
15 this meeting is actually risk considerations, risk issues,
16 I did want to put in one slide that reminded all of us
17 that we see benefits for PVCP PIPs.
18 We see them as an effective means of controlling
19 virus infection. And the consequences of that could be
20 higher yield for the farmer. It could be reduced use of
21 chemical pesticides to control insect vectors which would

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1 be an environmental benefit that the agency would value.
2 In some cases, we see it as being the only
3 option as a means of controlling viral diseases in certain
4 crops.
5 Now, to switch to the technical questions we'll
6 be talking about at this meeting, they are broadly divided
7 into 3 groups. One of them is gene flow. The other,
8 viral interactions. And then the third, these other
9 scientific considerations that kind of tail along from the
10 other two.
11 For gene flow, EPA is seeking your assistance to
12 better understand circumstances in which the flow of PVCP
13 PIPs from transgenic plants to wild or weedy relatives
14 occurs and also the potential for adverse impacts from
15 such gene flow.
16 Obviously, just because gene flow may occur does
17 not automatically follow that there would be an adverse
18 impact associated with that gene flow.
19 Then we're going to ask you to identify and
20 evaluate conditions that might minimize gene flow should
21 such minimization be seen as appropriate.

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1 For viral interactions, we're seeking your
2 assistance to identify and evaluate circumstances wherein
3 interactions between introduced virus sequences and
4 invading viruses might be more frequent than expected to
5 occur in natural mixed virus infections or unlike those
6 expected to occur in such conditions.
7 Similarly, as for gene flow, we will ask you
8 about conditions that might minimize such occurrences
9 should such minimization be seen as appropriate.
10 The other scientific considerations we will be
11 talking about, we're seeking your assistance in evaluating
12 technical issues that might be associated with PVCP PIPs.
13 One of the important things for us is to
14 understand the boundaries of the assumptions under which
15 we would be operating. Then, of course, we would ask you
16 if there are any additional considerations for minimizing
17 risk that we have not put forth in our questions to you.
18 Your role in all of this is to assist EPA in
19 better understanding specifically the degree of risk for
20 each of the issues that we present to you. We would like
21 to have a sense of your degree of certainty of your

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1 estimates.

2 We would like to understand a little bit better

3 what of those estimates of risk are coming from data that

4 you have actually seen generated and which of them are

5 hypothetical.

6 Assuming that there may be things in this

7 meeting that are going to be hypothetical, we would like

8 to get a better sense from you of the direction the

9 science is taking based on these specific issues raised.

10 We would ask you to provide us technical

11 recommendations and advice on the technical questions

12 posed.

13 How will your advice be used? We would then

14 take your advice and we would use it within the parameters

15 established by the statutes under which we operate. I'm

16 going to take a little bit of a detour here, it won't be

17 very long, just to talk a little bit about those statutes.

18

19 Within the legal context, we operate under 2

20 statutes to regulate pesticides.

21 One, you probably are familiar with, the Federal

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1 Insecticide Fungicide and Rodenticide Act. Our
2 responsibilities under that act are to protect both the
3 environment and to protect human health.
4 We also have responsibilities under the Federal
5 Food Drug and Cosmetic Act, Section 408. In that, we're
6 to determine safe levels of pesticide residues in food and
7 feed.
8 So while most of our questions today are going
9 to deal with environmental issues, there is a question in
10 there that goes towards the food safety consideration that
11 might be associated with PVCP PIPs.
12 FIFRA defines a pesticide as any substance or
13 mixture of substances intended for preventing, destroying,
14 repelling or mitigating any pest.
15 As you all know, the PVCP gene sequences can
16 confer resistance to the virus from which it was derived
17 and often to related viruses, to the recipient plant.
18 You can see that PVCP PIP falls within the
19 definition of pesticide under FIFRA.
20 Now, one thing that I also should remind us all
21 is that the United States operates under a coordinated

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1 framework, a framework of laws and regulations that
2 address all products of biotechnology in the United
3 States. So EPA is just one component of a regulatory
4 structure for virus control strategies.
5 We call them PVCP PIPs. That happens to be a
6 term of art for EPA. I'm sure our colleagues from USDA
7 would call them something slightly different. But at any
8 rate, there are 3 regulatory agencies that play
9 complimentary roles in oversight of these types of
10 products.
11 EPA, we regulate the pesticidal functions of the
12 particular product.
13 FDA is responsible for the food safety aspects
14 of a plant that have been modified to express PVCP PIP
15 except for the PVCP PIP itself, which is within EPA's
16 bailiwick.
17 And USDA addresses the plant pest risks
18 presented by these particular types of products.
19 The history of PVCP PIPs at EPA is actually
20 quite a long one. Probably in regulatory terms, not all
21 that long, but I have been involved with this particular

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1 issue since the late 1980s.

2 In 1994, EPA put forth a proposal to exempt PVCP

3 PIPs. We had 2 alternatives in that proposal. We

4 proposed either to exempt all of them or as an alternative

5 to exempt some of them depending upon potential for

6 weediness. We actually put forth some criteria that would

7 help people determine whether their particular PVCP PIP

8 had weediness potential.

9 We did not finalize that in 1997. We went out

10 with a supplemental request for additional comment to

11 exempt PVCP PIPs. Then in 2001, once again we asked the

12 public to help us with this particular issue.

13 So there's been quite a number of times that we

14 have gone to various groups to ask for their assistance.

15 I should say that we actually went to the FIFRA

16 SAP back in 1992 and in 1994 before the proposal came out

17 for their assistance in helping us craft the proposal. So

18 it is actually quite a long history of assistance for us.

19

20 One of the reasons that we have had had to be

21 going back to the public and then back to the SAP is

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1 because we have received such a wide range of comments
2 from the public. Some of them would support the
3 exemptions. Others were opposing it.
4 I have put down some examples of the types of
5 comments that we received. As you can see, if you look
6 under support for exemption proposal, there is one that
7 says, wild species are generally already resistant or
8 exhibit a high degree of tolerance to infection.
9 The second comment was, since viral coat
10 proteins do not act in a toxic manner, all viral coat
11 proteins should be exempt.
12 Then we have comments opposing exemption
13 proposal.
14 The sexual transfer of engineered virus
15 resistance would readily confer an advantage to weedy
16 populations.
17 The second comment. Genetically engineered
18 virus resistant crops present serious ecological risks.
19 One, new viral strains may emerge through recombination
20 and transcapsidation.
21 Now, these comments actually came in response to

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1 a 1994 proposal to exempt. You can see that we're still
2 asking many of those same questions of you today.
3 This has been a fairly long period of discussion
4 of these issues.
5 The next slide shows a more recent set of
6 comments. This is actually from the 2000 National
7 Research Council report entitled Genetically Modified Pest
8 Protected Plants Science and Regulation. This report
9 raised a number of questions which again we're still
10 discussing.
11 For example, they raised questions about the
12 potential for gene flow from transgenic to weedy
13 relatives.
14 They also had suggestions that transgenics could
15 be constructed with mitigated controls to reduce potential
16 for viral interactions. Again, something that we'll be
17 talking about during this meeting.
18 I have given you just a very, very brief
19 overview. Sort of a 70,000 foot overview of the context
20 for this. We have had a very long history involving
21 complicated issues, but I would emphasize that we're

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1 focusing very narrowly in this meeting. We're asking you
2 to provide scientific advice to assist EPA in its
3 evaluation of several technical issues associated with
4 PVCP PIPs. We're asking you to focus on a series of
5 specific questions posed by us.
6 I think that's my last slide.
7 DR. ROBERTS: Thank you. The next presentation
8 is by a veteran of some SAP meetings of recent years, Dr.
9 Fairbrother, on gene flow in viral coat protein transgenic
10 plants.
11 DR. FAIRBROTHER: Thank you very much. The
12 purpose of this presentation and the next one is to give
13 some overviews of the technical details and information
14 that we're going to be discussing over the next 3 days.
15 To kind of get us all on the same playing field,
16 to provide some definitions and get the conversation
17 started.
18 This presentation we're going to be talking
19 about gene flow. For the purposes of our discussion here,
20 we're going to define gene flow as the movement of genes
21 including transgenes, of course, but also natural genes

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1 from crops to weeds and wild relatives or to other crops.
2 This is just the movement of genes. And
3 introgression, then, is when the gene becomes fixed in the
4 recipient population. So two definitions, differences
5 between gene flow and introgression.
6 A lot of the talk in the materials come from
7 several reviews and other papers that were presented to
8 you in the background documents that you were given. The
9 three that you see here in red were also given to you as
10 the entire review material as well and we're lucky enough
11 to have a couple of the authors here with us on the panel
12 to continue with the discussions and provide additional
13 detailed information.
14 We do know that gene flow is something that can
15 occur every year. Every time plants pollinate or produce
16 seeds we can get movement of genes.
17 Introgression on the other hand is something
18 that can happen either quickly or can take multiple years,
19 multiple introductions. And this certainly depends upon
20 the particulars of the plant system that we're looking at.
21 Rates of pollen and seed dispersal are particularly

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1 important as are the relative sizes of the population of
2 the donor plants and the recipient plants.
3 So the relative size in this case of potentially
4 crop systems versus the recipient wild or weedy plants.
5 Selection pressures that are on the recipient
6 plants and also reproduction times. So do plants
7 reproduce every year or is it multiple years for each
8 generation.
9 There has been quite a lot of discussion of this
10 in the literature and many concerns have been raised, some
11 of which have been studied and some of which are still
12 speculative. But some of those that have been talked
13 about the most are here on this slide.
14 And that looks at potential effects on the
15 recipient plants of the movement of genes into those
16 plants, particularly whether this is going to increase or
17 decrease the fitness of those plants.
18 Potential effects on plant communities then can
19 emerge from this as we have changes in competitive
20 advantages of the plants that have received the new genes
21 and changing the potential of extinction risks either of

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1 the plants that have received the genes or those that they
2 interact with.
3 There is also the potential for effects on
4 genetic diversity in the ecosystem. Genetic swamping is
5 one such example of the formation of hybrids that then
6 eliminate a already existing taxon because of the
7 interbreeding that has occurred.
8 There is also the potential for indirect
9 effects, not just within the plant community, but also on
10 other parts of the ecosystem related to herbivore and the
11 food chain.
12 There is a number of lessons that have been
13 learned about the movement of gene flow. We'll review
14 some of those briefly here. Some of this is information
15 that's been learned from looking at the movement of
16 natural genes as well as some studies more recently on
17 transgenes.
18 Allison Snow in her recent discussions in Nature
19 Biotech has suggested that transgenes can act like
20 conventional genes in terms of dispersal and introgression
21 rates. That there may not be anything particular about a

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1 transgene that would change the rate of gene flow.
2 Gene flow can be widespread and can happen
3 regularly year after year. And as a result, you can see
4 either increases, decreases or no changes in fitness of
5 the recipient plant populations depending upon the genes
6 that are transferred.
7 She goes on to say that we need to remember that
8 the first generation hybrids, the F1s, may be sterile, but
9 this does not mean that they cannot propagate. They can
10 continue to transmit genes widely through asexual
11 reproduction, of course, either through vegetative
12 propagation or through development of seeds without
13 pollination.
14 Genes that are not on chromosomes can be
15 transferred, albeit at a much lower rate than the
16 chromosomal genes generally are.
17 She also points out that fitness changes occur
18 only after the plants are released from some type of
19 strong limiting factor that naturally is on that
20 particular plant.
21 I would like to take a moment to define what

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1 we're talking about when we keep using the term fitness.
2 It has come up a number of times already and will continue
3 to do so throughout this talk and I'm sure the rest of
4 today.
5 Ecological fitness is the relative ability to
6 contribute offspring to the next generation. It can be
7 measured either on a population level by looking at the
8 finite rate of increase of that particular population or
9 at the individual level by measuring the number of
10 offspring that individuals produce.
11 Plants have many different kinds of fitness
12 strategies, some of which are increasing number or size of
13 seeds that are produced, perhaps having a faster rate to
14 reach maturity, greater resistance to stress such as
15 draught, temperature extremes, disease, including viral
16 diseases, parasites and so forth and also differences in
17 soil properties.
18 There is probably many more that you can think
19 of to put on this list, but these are some of the things
20 that plants do in order to increase their fitness.
21 What are some of the lessons learned that we

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1 have seen over the years in terms of gene flow first?
2 Ellstrand, et al., reviewed in 1999 13 of the
3 most important conventionally bred crops in the world to
4 see how many of those have demonstrated gene flow to wild
5 relatives. And noted that 12 out of those 13 have shown
6 gene flow, of which 7 can be said to have introgressed
7 into the wild populations.
8 He has since updated that review in his book,
9 Dangerous Liaisons, published last year, and has shown
10 that 22 out of 25 of the largest crops in the world have
11 evidence of gene flow.
12 In the U.S., that would be about 55 percent of
13 our crops. He has pointed out 11 out of 20 of the crops
14 grown here.
15 Now, both Ellstrand in his recent book and also
16 Allison Snow have pointed out that there are many other
17 crops that are of lesser world importance that also have
18 been shown to exhibit gene flow.
19 Along those lines, Dr. Stewart has pointed out
20 that there are tens of thousands of potentially occurring
21 natural hybridizations that can occur amongst plant

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1 communities. But many, many of those have not been well
2 documented.
3 There has been 165 that have been confirmed.
4 And he suggested that of those, 65 have been sufficiently
5 documented to be able to say with certainty that gene flow
6 has occurred.
7 It is difficult at times to separate out
8 hybridizations that are genetically based from
9 evolutionary convergence where you can see plants that
10 look similar, look like they might be hybrids, but are
11 genetically distinct.
12 Over the last 10 years or so with the
13 development of new genetic techniques particularly looking
14 at different types of DNA polymorphisms, we have a much
15 increased ability to be able to determine these degrees
16 and types of relatedness among plants and learning quite a
17 bit more about the relationship of plant genetics to plant
18 morphology.
19 Dr. Stewart in his review also reviews one of
20 the more well known examples of hybridization in a natural
21 system. That's between 2 species of iris they find in the

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1 southeast U.S., iris fulva, which grows in salt marshes,
2 and iris hexagona, which is a related plant and grows
3 nearby, but in more freshwater swamps.
4 These two can exchange genes and have been
5 shown to do so over distances of about 10 to 25
6 kilometers. So a fair distance. You can end up with
7 intermediate hybrids which have different fitness
8 characteristics if you look at their ability to survive in
9 the brackish water swamps.
10 Those that are like the male parent iris fulva
11 tend to have intermediate or higher fitness, whereas those
12 that are like the female parent, the hexagona have
13 intermediate or the same fitness.
14 Also, this system has shown the development of a
15 new taxon that is the hybrid between the two where the
16 genes have become fixed and the hybrids can cross with
17 each other without needing to backcross and produce
18 similar species.
19 So what this has taught us then is that there
20 can be local geographic formation of what are known as
21 hybrid swarms where you have either the plants that can

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1 interbreed in the F1 and F2 generations or interbreed as
2 backcrosses with the parents. The gene flow can occur
3 beyond the range of the original hybridization zone and
4 actually occur over large distances with a formation of a
5 new stabilized taxon.
6 So continuing then, Dr. Stewart has pointed out
7 that introgression of transgenes from GM crops to wild
8 populations can occur, but is more difficult than from
9 wild plants to crops.
10 He also suggests that there may be -- that the
11 linkage to domestication alleles can impose a barrier to
12 gene movement and that domestication genes reduce
13 ecological fitness.
14 So that crops themselves are less fit than their
15 wild relatives.
16 Now, Dr. Norm Ellstrand in his book has also
17 suggested that, with rare exceptions, transgenic traits in
18 plants are almost always dominant traits. And this really
19 makes sense. And if we're gong to all of the effort to
20 develop a particular gene to put into a plant for a
21 reason, you want it to be expressed all the time. So

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1 putting it as a dominant trait would be an appropriate
2 approach to take.
3 However, what this means then is that transgenic
4 hybrids will always express the trait. If you get gene
5 transfer to a wilder weedy relative, they will be
6 immediately subject to selection pressures.
7 Traits that distinguish cultivated plants on the
8 other hand are usually recessive alleles, and so the plant
9 needs to be homozygous for those alleles. Although when
10 they are, they do appear to have a major fitness effect
11 and can be modified by other alleles that have minor
12 fitness effects.
13 So for example, if you have a trait that is
14 looking for development of big seeds, it can also be
15 modified by other traits that will allow those seeds to
16 mature more quickly.
17 There are those that have suggested that the use
18 of mathematical models might be an appropriate way to look
19 at the possibilities and likelihood of gene transfer and
20 introgression and the subsequent effects.
21 Haygood, et al., published a paper last year

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1 that using such a model -- or they argued that at even
2 very low transmission rates of the transgenes to wild
3 populations you will eventually result in fixation of the
4 gene, even if this may take decades that it will happen.
5 Now, Dr. Stewart has argued that Haygood's
6 predictions and his model may be based on some assumptions
7 that one can take, can argue with about the basis of the
8 model. But it does continue to raise the question that we
9 all need to address about what is the ecological
10 significance of even very low levels of gene flow.
11 Dr. Ellstrand has also pointed out that rates of
12 mating of crops with wild relatives are no different for
13 transgenic crops than for conventional crops suggesting
14 that there is nothing about transgenes that would make
15 gene flow be any different with the exception of those
16 crops that are engineered to reduce fertility.
17 However, there are others who are beginning to
18 point out that this may not always be true. And there was
19 a paper published a couple years ago by Joy Bergolson
20 (ph), for example, who has shown that an herbicide
21 resistant transgene was transferred more frequently in a

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1 rabbit opsis than the naturally occurring herbicide
2 resistant mutant.
3 I think that still may be open to question.
4 Taking a look, then, at how we categorize the
5 potential risk of crop to wild introgression by
6 transgenes, these are some suggestions that Dr. Stewart
7 had put forth in his paper. That we need to look
8 particularly at colocation with wild relatives, if there
9 is any evidence for crop to wild gene introgression from
10 normally occurring genes and also the degree of genetic
11 differentiation between the crop and its wild relatives.
12 So looking now more specifically at gene flow
13 concerns for the PVCP PIP crops, Dr. Tepfer has reviewed
14 some studies and has shown that there is a significant
15 negative impact of virus infection on growth, survivorship
16 and reproduction of some plants. As you
17 recall, when we started out this discussion, Dr. Snow's
18 paper had pointed out that in order to have an effect, a
19 transgene must affect something that is a controlling
20 factor on plant populations.
21 So this work is looking at the fact that virus

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1 infection can be a controlling factor on some plant
2 populations.
3 So, therefore, we do need to be concerned with
4 the potential for increased weediness or competitive
5 advantage of plants with virus resistance genes.
6 Here is an example of such a situation with a
7 barley yellow dwarf virus. This is a luteovirus that
8 causes significant amount of crop damage. Conventional
9 breeding has not been able to develop a resistance or
10 tolerance strain.
11 Some virus strains move via aphid transmission
12 into wild hosts such as wild oats, squirrel tail grass and
13 other such species which also have no natural resistance
14 and will show signs of infection.
15 Wild oats are known to be a agronomic weed in
16 many cultivated cereal crops and have been introduced into
17 western United States, particularly in California where
18 it already outcompetes many of the native grasses.
19 Cultivated oats will hybridize really with wild
20 oats at a relatively high rate.
21 The hypothesis then is that fitness,

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1 particularly growth and reproduction, could be enhanced
2 with a PVCP PIP against this virus, and if wild oats were
3 to receive that.
4 If that were the case, that release from the
5 virus infection could increase a competitive advantage of
6 the wild oats species, increasing their weediness in
7 cereal crops and also increasing their invasiveness of
8 grass lands in places such as California.
9 Although this might occur only in the absence of other
10 mitigating environmental factors, it certainly is a
11 possibility.
12 On the other hand, we have an example here of
13 where a virus infection is not a controlling factor in a
14 plant population and transmission of a PVCP PIP to that
15 wild plant may not have an ecological consequence.
16 This is an example presented by Dr. Tepfer of
17 the sea beet which is the progenator of all of our
18 cultivated beets and is susceptible to the beet necrotic
19 yellow vein virus.
20 This virus is absent in brackish water
21 environments where the sea beet naturally occurs, because

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1 the fungal vector that transmit the virus cannot tolerate
2 the salty soils of that environment.
3 Therefore, receipt a transgene that confers
4 resistance to this virus into the sea beet would not have
5 any selective advantage or disadvantage because of the
6 lack of the vector for transmitting the virus.
7 Some of the lessons that we have learned from
8 all of this are that features that increase the likelihood
9 of gene flow are sexual compatibility between the donor
10 and recipient plants, that they are grown in the same
11 vicinity and have overlapping flowering times.
12 If the F1 hybrids persist for more than 1
13 generation, it will significantly increase the likelihood
14 of gene flow, and, particularly, if they are fertile and
15 can also backcross with the parent plants.
16 Features that will increase the likelihood of
17 introgression are dominance of the gene and also that it
18 confers a selective advantage such as we were just talking
19 about.
20 The absence of association with deleterious
21 crop alleles or trait will also increase the likelihood of

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1 introgression, as well location on a shared genome between
2 the donor and recipient plants.
3 Some plants, as you know, have multiple genomes
4 and their close relatives may not have all of the same
5 genomes. So location on a particular genome can be very
6 important as to whether a gene will cross and introgress.
7
8 Similarly, location on a homologous chromosome
9 and particularly on non rearranged chromosome.
10 So taking that, then, some of the approaches to
11 decrease the likelihood of gene flow and introgression,
12 perhaps not to completely eliminate them, but at least to
13 decrease the likelihood of doing so is placement on
14 nontransferred chromosomes, linkage to deleterious crop
15 alleles or traits. So if the gene does get transferred,
16 the fitness of the recipient plant is also decreased.
17 Insertion into maternally transmitted organelle
18 DNA such as in chloroplast. Induced sterility of the
19 transgenic plant to decrease pollen formation and
20 development or germination of seeds. And deployment in
21 areas where crops have no known wild relatives.

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1 In conclusion, then, we have seen that gene flow
2 and gene introgression can occur between crops and their
3 wild and weedy relatives, although the likelihood and
4 consequences can vary greatly depending upon crop species,
5 recipient species and the genes transferred.
6 Questions certainly remain about how to
7 characterize the potential for risks of crops with PVCP
8 PIPs.
9 And that is the topic of much of our discussion
10 for this afternoon.
11 DR. ROBERTS: Thank you, Dr. Fairbrother.
12 I would like to pause for a moment and give the
13 panel the opportunity to ask you any questions they might
14 have, and also I guess extending back into Dr. Milewski's
15 presentation.
16 Are there any questions from panel members?
17 Yes, Dr. Zaitlin.
18 DR. ZAITLIN: I would like to make a point of
19 clarification on the definition of PVCP PIP as given by
20 Dr. Milewski. She says that the definition is it controls
21 virus infection.

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1 I think that's really very narrow. Because what
2 you are really interested in is in the control of virus
3 disease. Because some of these plants actually can
4 support virus replication. They can inhibit the disease.
5 They can prevent movement or suppress the symptoms.
6 DR. ROBERTS: Dr. Stewart.
7 DR. STEWART: I guess I would like to point out
8 that while I agree with many of the points that were in
9 the -- probably most of the points that were in the
10 presentation, many of the citations came from non peer
11 reviewed literature, commentaries and book chapters and
12 books which often express more opinion than material from
13 scientific data.
14 That's just a point of clarification, I think.
15 DR. ROBERTS: Okay. Dr. Gendel.
16 DR. GENDEL: Sort of as a follow up to that,
17 when I was researching the background information for
18 this, I found it difficult to obtain the information on
19 the original SAP panels and the original policies. The
20 background data and the SAP results, I guess they go back
21 to the days before everything was put on the web.

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1 So other than summaries of what all the
2 conclusions were, I couldn't find the data that went into
3 making those conclusions and the deliberations. It would
4 have been interesting to see the discussions that took
5 place at that time and how much was peer reviewed
6 literature and how much was not.
7 DR. ROBERTS: Dr. Tepfer.
8 DR. TEPFER: It's maybe a rather minor sort of
9 clarification, but in one of Dr. Fairbrother's slides,
10 there was one point where she was commenting on the review
11 article I wrote a couple years ago. On the slide it said,
12 viruses are controlling factors for some plant
13 populations. In her oral presentation she said could be.
14
15 I think it is very important that my particular
16 opinion on that is that it could be. It is a purely
17 hypothetical case at the moment. Please don't take what
18 is written on the paper as my point of view on that.
19 It is a very interesting question of science,
20 but we don't know the answers yet.
21 DR. ROBERTS: Dr. Falk.

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1 DR. FALK: Maybe my comment might be similar
2 regarding to one of the slides that you showed regarding
3 the article written by Dr. Tepfer where I think you said
4 that studies have shown significant negative impacts and
5 you mentioned populations and you mentioned a few species.
6 Was that correctly reported in your article, Dr.
7 Tepfer?
8 DR. TEPFER: I think that those are, yes,
9 examples where there were fitness components that were
10 affected by virus infection in those plant species.
11 DR. FALK: In natural population.
12 DR. TEPFER: Not all of those are natural
13 populations. The wild squash was all under experimental
14 conditions. For some of the brassicas -- yes, the others
15 were under natural conditions.
16 DR. ROBERTS: Other questions?
17 If not, let's move ahead to the next
18 presentation. By the way, Dr. Fairbrother, you knew that
19 -- I sympathize with the difficulty of summarizing the
20 results of many of the members of the panel who are here
21 who can always take issue with nuances of your

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1 presentation.

2 That was a tough job. You knew you were going

3 to get a few comments back on that.

4 Let's go ahead and move on with the next

5 presentation.

6 DR. KRAMER: I'm going to follow up Dr.

7 Fairbrother's discussion on gene flow issues to try to set

8 the stage for the questions we're posing on vital

9 interactions.

10 As was the case with Dr. Fairbrother's

11 presentation, I, too, am faced with the task of

12 summarizing many research results from scientists here in

13 the room.

14 I don't think I need to go over this for most

15 people. But just to set the stage, I wanted to provide

16 the basics of virus and the virus infection cycle.

17 First, the virus enters the plant through a

18 mechanical breach of the cell wall. At that point, it may

19 shed its protein coat and be able to replicate within the

20 cell.

21 Movement proteins are needed to modify the

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1 plasmodesmata that would allow the virus to cross the cell
2 wall and then spread throughout the plant, at which time
3 it would become available for a transmission to a new
4 plant.
5 In terms of transgenic virus resistance, many
6 different types of transgenes have been used
7 experimentally to confer resistance. Most notably, of
8 course, plant viral coat proteins, but also viral
9 replicase, genes movement proteins, nuclear inclusion
10 genes as well as a number of non viral sequences.
11 However, plant viral coat proteins are the most
12 common and are the sole topic of the discussion today, I
13 would just like to point out that this has been a topic
14 for discussion for many years now.
15 The first report of a plant viral coat protein
16 transgenic plant was published in 1986.
17 So just again some basic information about plant
18 viral coat proteins. They encapsidate the viral nucleic
19 acid and are important in nearly every stage of virus
20 infection from replication to movement throughout an
21 infected plant and also from transport from plant to

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1 plant.

2 There are a number of mechanisms proposed for

3 how coat protein mediated resistance works. A lot of

4 research is still being conducted in this area.

5 There are basically two major categories of

6 mechanisms, though. One would be protein mediated.

7 That's -- it is believed to be protein mediated because

8 the level of protection correlates strongly with the level

9 of MRNA and protein accumulation within the plant.

10 It is thought that that works through the

11 transgenic coat protein actually blocking the uncoating of

12 virions upon their entry into the cell.

13 However, it was discovered that it is not always

14 the case that there is such a correlation. In fact, at

15 times it was discovered that there was no correlation

16 between the level of MRNA and the level of protection

17 conferred to the plant.

18 Therefore, it was hypothesized that there was a

19 nucleic acid mediated mechanism for virus resistance. And

20 further research has shown that that's likely to be due to

21 post translational transgene silencing which suppresses

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1 expression of the transgene and any accumulating viral RNA
2 that shares sequence homology with the transgene.
3 To set the stage for really what we're
4 considering is the baseline for our discussions today in
5 transgenic plants, I wanted to go over what we know about
6 mixed virus infections.
7 They occur when viral genomes from different
8 strains or species simultaneously infect the same plant.
9 They can be extremely common among certain types of plant
10 and certain plant populations.
11 In rare cases, mixed virus infections have been
12 implicated in adverse agricultural or environmental
13 events. For example there is a case of cassava mosaic
14 disease in Uganda which was thought to be due to either
15 sequential or simultaneous occurrence of recombination,
16 pseudo recombination of synergy.
17 This is worth pointing out because the fact is
18 that in VCP transgenic plants, every infection of a virus
19 other than the one from which the transgene was derived is
20 essentially a mixed infection with respect to the VCP gene
21 itself, which brings us then to really the critical

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1 question that is overarching all of the questions we are
2 asking the panel today in this area.
3 It is, Are the risks associated with virus
4 interactions in VCP transgenic plants greater in degree or
5 different in kind than in natural mixed infections?
6 Here are some issues to consider. This is
7 really an outline of the rest of my talk. For each of the
8 3 types of virus interactions, recombination, heterologous
9 encapsidation and synergy, I want to go through what we
10 know about its occurrence under natural conditions, what
11 we know about its potential to occur in VCP transgenic
12 plants and then ways that have been studied or
13 hypothesized to reduce the frequency of these events if it
14 were gene warranted.
15 Then I want to focus really on the field
16 evaluations that have been done because of their crucial
17 significance for evaluating the ecological significance of
18 these events. And then bring us back to the critical
19 question here, dividing it into 2 parts.
20 The first, is the frequency of virus
21 interactions in PVCP transgenic plants different than in

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1 natural mixed infections, either higher or lower. And
2 second, is the nature of the virus interactions in PVCP
3 transgenic plants different than in natural mixed
4 infections.
5 Starting now with recombination. Recombination
6 means that segments from different parental molecules may
7 form chimeric molecules. The mechanism in RNA viruses is
8 thought to be template switching of the viral replicase
9 during replication.
10 Under natural conditions, recombination very
11 rarely leads to new viable viruses. That's the case
12 because a virus must recombine such that it is competent
13 in all stages of virus infection. That can be very
14 difficult to do.
15 Nevertheless, it is important to point out that
16 recombination has still been thought to play a significant
17 role in virus evolution in a number of virus groups. It
18 is more likely among closely related viruses that can
19 undergo homologous recombination.
20 It's also important that both virus-virus
21 recombination and virus-host recombination can occur.

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1 That is, you can get actual incorporation of host genes
2 into viruses.
3 So recombination in transgenic plants with virus
4 trangenes. When a virus infects a transgenic plant, those
5 nucleic acids may become available for recombination with
6 the host transgenes. And lab experiments have indeed
7 shown that such recombination can occur.
8 These experiments have almost always been done
9 under high selection pressure. That is, the only way that
10 a competent virus could be produced at all is through
11 recombination. Such high selection pressure isn't
12 expected to occur in the field where the recombinant virus
13 would be competing with the parental viruses from which it
14 came.
15 Therefore, the ecological significance of these
16 experiments is unclear beyond showing that such
17 recombination may in fact occur.
18 A number of ways have been investigated for
19 reducing the frequency of recombination. I listed a few
20 here. One, removal of the 3 prime untranslated region
21 necessitating a double crossover to produce a viable

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1 recombinant.

2 Secondly, excluding any replicase recognition

3 sites or other known recombination hot spots in the

4 construct.

5 Third, reducing the extent of shared sequence

6 similarity, for example, through the introduction of point

7 mutations.

8 Fourth, using the smallest viral fragment

9 possible that would give the smallest target for

10 recombination while still allowing virus resistance to be

11 conferred.

12 And finally, the insertion of GC rich sequences

13 downstream of AU rich sequences has been shown to occur in

14 at least one virus system, although its applicability

15 broadly has not been demonstrated yet.

16 Moving now to heterologous encapsidation. This

17 means that the coat protein subunits of one virus may

18 surround the nucleic acid of a different virus.

19 Under natural conditions, it is known that this

20 can affect virus vector interactions, which is perhaps not

21 surprising given that the coat protein does play a

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1 prominent role in interactions with vectors.

2 Among some plant viruses, it can, in fact, be a

3 very regular occurrence. There may indeed be viruses that

4 require heterologous encapsidation for transmission

5 because they don't produce any coat protein of their own

6 at all.

7 Therefore, it can be a very natural part of

8 virus epidemiology. As (ph) recombination, it's more like

9 to occur among closely related viruses.

10 I think it is important to expand on the

11 situation under natural circumstances to point out that

12 there is usually limited environmental concern due to

13 heterologous encapsidation for a number of reasons that I

14 would like to go through.

15 First, vector specificity is often determined by

16 the coat protein, but often only partially determined.

17 Therefore, the encapsidation by an unrelated coat protein

18 may not be sufficient to allow a new vector to transmit

19 it.

20 Secondly, vectors may carry a heterologously

21 encapsidated virus only to plants it already infects.

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1 You could imagine if you had a large
2 monoculture of a plant growing in a field, a new vector
3 that may be able to pick it up because it contains a novel
4 coat protein may very well be likely to only transmit it
5 to other plants of the same type within the field.
6 Therefore, although heterologous encapsidation
7 occurred, it would only be transmitted to a plant that the
8 virus is able to infect anyway.
9 Finally, perhaps most importantly, once the
10 virus replicates in a novel host, if it is able to
11 replicate, it then becomes reencapsidated in its own coat
12 protein and, therefore, it will not be competent to be
13 transmitted by novel vector that put it there in the first
14 place.
15 However, I think it is important to point out
16 that one could imagine certain limited circumstances under
17 which you might expect that there could be some
18 environmental concern due to heterologous encapsidation.
19 One, a high enough frequency of heterologous
20 encapsidation. Even if the virus then becomes
21 encapsidated in its own coat protein in those new plants

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1 may mean that you don't necessarily require a secondary
2 transmission of new host plants for impact. Particularly,
3 if you are thinking about a rare susceptible population.
4 Secondly, viruses are thought to exist as quasi
5 species in which the many different types of viruses
6 differ by few nucleotides from a consensus sequence. And
7 the most best adapted variance may be able to rapidly
8 evolve in a new host.
9 Thirdly, once in a novel host, there may be
10 potential for exposure to new vectors that it didn't have
11 interaction with in the plant that it came from.
12 So what do we know about heterologous
13 encapsidation in transgenic plants with viral transgenes?
14
15 Laboratory experiments have been done to show
16 that protein from VCP transgenes can encapsidate infecting
17 viruses, even unrelated infecting viruses.
18 I have there, protein, when it is produced.
19 Because as I mentioned before, the mechanism of resistance
20 in some cases is nucleic acid mediated. In those cases,
21 no protein may be produced, in which case heterologous

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1 encapsidation would obviously not be a concern.

2 A number of ways have been hypothesized and

3 investigated for reducing the impact of heterologous

4 encapsidation. I say the impact because that can be done

5 in two different ways.

6 One would be by reducing the frequency of

7 heterologous encapsidation itself. The other would be by

8 reducing the frequency of vector transmission per se.

9 That is the heterologous encapsidation may still

10 occur, but that heterologously encapsidated virus would be

11 expected to remain in the plant where it was originally

12 infected.

13 Certain regions are known to affect aphid

14 transmission specificity. A few are listed here on the

15 slide. These have been hypothesized as good candidates

16 that one might target in the design of a construct to

17 eliminate them or mutate them and thereby affect aphid

18 transmission.

19 A number of experiments have been done looking

20 at PVCP gene modifications that have been shown to reduce

21 the frequency of either heterologous encapsidation or

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1 vector transmission. A few of those are listed there on
2 the slide as well.
3 Moving then to the third type of virus
4 interaction. Synergy. Synergy is when the disease
5 severity of 2 viruses together infecting a plant is
6 greater than expected based on the severity of each alone.
7
8 Under natural conditions, there are many known
9 viral synergisms. They are more common among some viruses
10 than others. Important for our context here today, the
11 coat protein is less likely to be responsible for viral
12 synergisms than other regions of the virus genome.
13 In transgenic plants with viral transgenes,
14 synergy is largely an agroeconomic concern. That is that
15 the impacts are expected to most directly affect the
16 transgenic plant itself. Therefore, there is a high
17 incentive for a developer to evaluate synergy before
18 deployment because it's the efficacy of the product itself
19 that is at stake.
20 If by chance there were, in fact, a product that
21 were able to be deployed and a synergy were discovered

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1 after deployment, farmers would likely quickly abandon the
2 product because it would not only not achieve the goal for
3 which it was purchased, but the farmer would in fact be
4 worse off than before deploying this product.
5 A number of ways have been investigated or
6 hypothesized for reducing the frequency of synergy.
7 Again, constructs may be engineered to reduce the
8 likelihood of synergy by avoiding particular transgenes
9 known to be involved or using defective copies of genes.
10 Another strategy may be to stack multiple
11 resistances within the same plant, thereby reducing the
12 frequency of mixed virus infections and, therefore, the
13 potential for synergy between different types of viruses
14 to occur.
15 So now I want to really move from what I have
16 been talking about which is basically what we know from
17 laboratory experiments about these viral interactions to
18 talking about field evaluations that are really critical
19 for assessing what the impacts and likelihood of these
20 types of events are.
21 There have been really a relatively small number

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1 of published studies that have been done in this field,
2 but they to this point seem to provide no evidence of
3 adverse effects. I actually because of the small number I
4 want to run through the major ones here.
5 The first is Thomas and others in 1998. This is
6 a relatively long and large experiment looking at 25,000
7 potato plants, 442 lines transformed with 16 potato leaf
8 role (ph) virus coat protein constructs.
9 They were exposed to field infection over 6
10 years. At the end of that time, they looked and found
11 there were no new viruses or viruses with altered
12 transmission or disease characteristics detected as you
13 might expect if any of these virus interactions had
14 occurred and led to a significant impact.
15 Fuchs and others in 1998 looked at transgenic
16 melon and squash containing the coat protein from an aphid
17 transmissible strain of cucumber mosaic virus. They
18 infected the plants with an aphid non transmissible strain
19 of cucumber mosaic virus, the idea being to see if the
20 transgenic coat protein would be able to heterologously
21 encapsidate this other virus strain and therefore enable

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1 it to be transmitted by aphids.

2 At the end of this experiment they found there

3 was no aphid vectored spread of the non transmissible

4 strain.

5 Fuchs and others in 1999 did a very similar

6 experiment this time looking at transgenic squash

7 containing the coat protein from an aphid transmissible

8 strain of watermelon mosaic virus.

9 Plants were infected with an aphid non

10 transmissible strain of zucchini yellow mosaic virus. The

11 results were a little bit different in this experiment.

12 In nontransgenic fields, they found there was no

13 transmission of the zucchini yellow mosaic virus. However

14 in transgenic plants, there actually was transmission to 2

15 percent of the plants in the transgenic fields.

16 It was thought this was likely due to

17 heterologous encapsidation. However, it is important to

18 point out that no epidemic of the disease developed.

19 Lin and others in 2003 did a little bit type of

20 different experiment looking really at the potential for

21 resistance to virus infection to evolve. They estimated

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1 the biological and genetic diversity of cucumber mosaic
2 virus isolates before and after development of transgenic
3 squash containing the coat protein from 3 different
4 viruses.
5 What they found is that most cucumber mosaic
6 virus isolates showed no significant sequence changes
7 between those infecting the transgenic squash and those
8 infecting the non transgenic squash.
9 There was one isolate that did differ, but it
10 was not due to recombination or selection.
11 Finally, Vigne and others in 2004 looked at
12 transgenic grape vines containing the coat protein of
13 grape family virus.
14 Non transgenic scions were grafted onto
15 transgenic and non transgenic rootstocks. They were
16 exposed over three years to grape family virus infection.
17
18 The transgenic grapevines were found not to
19 assist in the emergence of viable grape family virus
20 recombinants or to affect the molecular diversity of
21 indigenous population.

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1 So now I want to return back to what the
2 critical questions were that I posed at the beginning.
3 The first being does the frequency of interactions change
4 in viral coat protein transgenic plants?
5 I point out at first that there is really as far
6 as I know essentially no data on this topic. That's
7 because it is very difficult to measure these events due
8 to their rarity in any case.
9 There are some factors that suggest there may be
10 a decrease in the frequency in transgenic systems. That
11 is that there is generally going to be a lower
12 concentration of cellular RNA transcripts from a transgene
13 than there would be from an infecting virus that would
14 reach a very high titer (ph) in an infected plant.
15 Secondly, assuming that the virus resistance is,
16 in fact, working, there would be a lower concentration of
17 infecting virus in that transgenic plant.
18 However, there are some factors that suggest you
19 may in fact get the opposite case and that there could be
20 an increase in frequency in transgenic systems. One would
21 be usually these transgenic plants are constructed with

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1 constitutive promoters. The cauliflower mosaic virus
2 promotor that would cause expression to occur in all
3 developmental stages and all tissues of the plant.
4 That may not necessarily be the case in all
5 viruses that could be cell or stage specific.
6 Secondly, there are thought to be natural,
7 temporal or spatial expression patterns that could be
8 obscured in a transgenic plant. That is that in natural
9 systems a virus may enter a plant cell wall after uncoats
10 and replicates and moves to other cells within the plant.
11 That virus may become reencapsidated and be unavailable
12 for interactions with other infecting viruses.
13 Second part of the question is whether PVCP
14 transgenic plants might lead to novel viral interactions.
15 I think it is here important to point out what we really
16 mean by novel viral interactions.
17 Prior to this, I had been talking about viral
18 interactions per se. When I say novel what I mean are
19 interactions that we would not expect to occur in a
20 natural system. That is this particular sequences that
21 are interacting do not exist in nature in that

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1 combination.

2 Here are some examples that one might imagine

3 could lead to such an interaction. The first one would

4 be transgenic multi resistances. That is if you stack

5 multiple virus resistances within the same plant or you

6 stack virus resistance with some other trait, say an

7 herbicide tolerance trait, you might expect that you would

8 increase the likelihood that that product could be

9 deployed in an area where you are not actually attempting

10 to control the virus.

11 Perhaps an area where the virus is not actually

12 infecting you may be introducing virus sequences into a

13 system where they weren't previously.

14 Second would be heterologous resistance. It is

15 known that in some cases you can, in fact, get resistance

16 to a certain virus through incorporation of a similar

17 type, but a different type of virus coat protein into a

18 plant.

19 If any such product were ever developed, you

20 might obviously expect you could deploy that in an area

21 where the virus from which the coat protein was derived

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1 did not actually infect those plants in that area.
2 The third would be you could imagine the use of
3 an exotic strains coat protein. Perhaps this could be
4 intentionally done to try to stave off the infection
5 that's known to exist in other parts of the world in a
6 region where that virus does not currently exist.
7 But doing so may, in fact, be introducing virus
8 sequences that would be competent for recombination with a
9 similar infecting virus that is in the area. You could
10 have new types of novel interactions through that
11 mechanism.
12 The fourth I have actually already touched upon.
13 That is you can get expression in new cells or tissues
14 through the use of a constitutive promoter and may leave a
15 virus available for interaction with viruses that don't
16 normally infect the type of cell that it does naturally
17 infect.
18 Finally, this is getting at a question we'll be
19 talking about later. It is possible to alter a coat
20 protein gene within a construct in a myriad of ways. Our
21 question really is how much can that be done. At what

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1 point do you achieve something that is so unlike anything
2 that exists in nature that you would have what you might
3 call a novel viral interaction occurring.
4 Just in summary, I want to put forward now what
5 our overarching issues are for the panel to consider. The
6 first is are viral interactions in PVCP transgenic plants
7 an environmental concern above and beyond what occurs
8 naturally in mixed virus infections.
9 First, is there a potential for increased
10 frequency of interactions and, second, are novel
11 interactions, again, I want to emphasize novel, are novel
12 interactions likely to occur and have any adverse
13 environmental impacts.
14 Secondly, really what would the value be of any
15 mechanism designed to reduce the likelihood of some
16 interactions.
17 Thank you for your attention.
18 DR. ROBERTS: Thank you, Dr. Kramer. Let me ask
19 the panel if they have any questions or any gentle
20 corrections they might want to offer.
21 Dr. Hammond.

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1 DR. HAMMOND: I would like to ask whether you
2 have any examples where a coat protein is responsible for
3 a synergistic reaction. Because I can't think of any.
4 DR. KRAMER: No. Not specifically.
5 DR. NAGY: Actually, that is -- turnip clinco (ph) virus
6 coat protein is a suppressor of gene silencer.
7 DR. ROBERTS: Dr. Allison.
8 DR. ALLISON: I just would like to point out
9 concerning mixed infections that it's generally thought
10 that mixed infections had to do with simultaneous
11 infections.
12 But just a point of clarification. The
13 infections don't have to occur simultaneously, but,
14 rather, mixed infections represent an accumulation of
15 different plant viruses in the same plant, and those
16 infections, the actual introduction may have occurred at
17 different points in time, which may provide different
18 opportunities for recombination.
19 DR. ROBERTS: Anyone else.
20 We're a little ahead of schedule, but we'll go
21 ahead and do the last presentation and then take our

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1 break.

2 So with regard to the third set of issues then.

3

4 DR. MILEWSKI: Thank you. These are the other

5 scientific considerations that I mentioned earlier in my

6 presentation. They are in 2 broad categories, the first

7 one dealing with boundaries of assumptions, and the second

8 one, additional considerations for minimizing risk.

9 In terms of the boundaries of assumptions, we're

10 seeking your assistance in examining how far VCP PIP can

11 be modified while still supporting assumptions of dietary

12 safety for humans. No new effects on non target species

13 and no potential for novel viral interactions.

14 In terms of dietary safety, the assumption

15 under which we have been operating is that humans have

16 consumed viral coat proteins for generations as part of

17 the food supply.

18 Our question would be to what degree and in what

19 ways might a PVCP gene be modified and the PVCP PIP still

20 present no new human dietary exposures. We know that

21 genes can be modified in small ways and large and, for

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1 example, you might even wind up with a gene that is
2 expressing a totally new function.
3 For us, it is very important to get an
4 understanding of how much modification can occur before
5 you really start seeing something as Mellissa had
6 mentioned earlier, for example, a novel gene, something
7 that is not likely to have occurred in nature before.
8 And then can the SAP help us with providing a
9 succinct statement describing that boundary?
10 On the question of no new effects on nontargets,
11 our assumption is that species that interact with non
12 transgenic comparator plants have been exposed to viral
13 coat proteins for generations.
14 In other words, there would be no new novel
15 exposures. But to what degree and in what ways might a
16 PVCP gene be modified and the PVCP PIP still present no
17 new effects on non target species. Again, we would ask
18 you if you could help us by providing a sussinct statement
19 describing the boundary of that.
20 Finally, no potential for novel viral
21 interactions. To what degree and in what ways might a

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1 PVCP gene be modified before it becomes a concern that
2 novel viral interactions could occur because the gene
3 could be significantly different from any existing in
4 nature.
5 And again, can you help us by providing a
6 succinct statement describing that boundary.
7 Then we're going to ask you if there are any
8 additional risk considerations that we have not touched on
9 in our major group of questions.
10 For example, are there any considerations
11 related to the PVCP PIP construct that might be considered
12 when attempting to identify risk. For example, does the
13 insertion site have any relevance in considering risk.
14 Secondly, are there any scientific
15 considerations beyond gene flow, recombination and
16 heterologous encapsidation as posed in the EPA's
17 questions.
18 Once again, the charge that we're giving to the
19 SAP is to provide scientific advice to assist EPA in its
20 evaluation of several technical issues associated with
21 PVCP PIPs.

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1 Specifically, we're asking you to respond to a
2 series of technical questions related to exposure and
3 hazard considerations for PVCP PIPs.
4 Those revolve around gene flow, viral
5 interactions and the other scientific considerations which
6 we have just covered.
7 Now, if you can bear with me, I would like to
8 read into the recorded record the questions that we're
9 going to be posing for you.
10 The first question is what scientific evidence
11 supports or refutes the idea that plant viruses have
12 significant effects on reproduction, survival and growth
13 of plant populations in natural settings?
14 Is there scientific evidence that plant
15 populations freed from viral pressure could have increased
16 competitive ability leading to changes in plant population
17 dynamics.
18 Second question. Please comment on the validity
19 of the agency list of crops that have no wild or weedy
20 relatives in the United States with which they can produce
21 viable hybrids in nature. That is, tomato, potato soybean

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1 and corn.

2 Question 3. Please identify other crops that

3 have no wild or weedy relatives in the United States with

4 which they can produce viable hybrids in nature, for

5 example, papaya, peanut and/or chick pea.

6 Question 4. What laboratory techniques used to

7 achieve genetic exchange between species. For example,

8 embryo rescue, use of intermediate bridging crosses,

9 protoplast fusion are not indicative of possible genetic

10 exchange between these species in the field.

11 Conversely, what techniques, if any, used in

12 laboratory or greenhouse experiments provide the most

13 reliable indication of ability to hybridize in the field.

14

15 Question 5. Given that current bioconfinement

16 techniques are not 100 percent effective, what would the

17 environmental implications be of extremely low transfer

18 rates of virus resistance genes over time.

19 Question 6. Please comment on the prevalence of

20 tolerance and/or resistance to viruses in wild relatives

21 of crops.

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1 Question 7. Please specify techniques that do
2 not or do provide measures of tolerance and/or resistance
3 that are relevant to field conditions.
4 Question 8. How do environmental or other
5 factors, for example, temporal variations affect tolerance
6 and/or resistance. Given the expected variability, what
7 measures of tolerance and/or resistance would be reliable?
8
9 Question 9. What would be the ecological
10 significance if a plant population acquired a small
11 increase in viral tolerance and/or resistance above a
12 naturally occurring level.
13 Question 10. Please comment on how necessary
14 and/or sufficient these conditions are to minimize the
15 potential for the PVCP PIP to harm the environment through
16 gene flow from the plant containing the PVCP PIP to wild
17 or weedy relatives.
18 Would any other conditions work as well or
19 better.
20 Question 11. To what extent are novel viral
21 interactions, for example, recombination, heterologous

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1 encapsidation, involving a viral transgene an
2 environmental concern.
3 Question 12. What conclusions can be drawn as
4 to whether the likelihood of recombination and/or
5 heterologous encapsidation would be increased or decreased
6 in a transgenic plant compared to its non bioengineered
7 counterpart.
8 Question 13. How effective is deleting the
9 three prime untranslated region of the PVCP gene as a
10 method for reducing the frequency of recombination in the
11 region of the PVCP gene.
12 Is this method universally applicable to all
13 potential PVCP PIP constructs.
14 Would any other methods work as well or better.
15
16 Which methods are sufficiently effective and
17 reproducible such that actual measurement of rates to
18 verify rate reduction would be unnecessary.
19 Question 14. Are any methods for inhibiting
20 heterologous encapsidation or transmission by insect
21 vectors universally applicable to all PVCP PIPs.

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1 Which methods are sufficiently effective and
2 reproducible such that actual measurement of rates to
3 verify rate reduction would be unnecessary.
4 Question 15. How technically feasible would it
5 be to measure rates of recombination, heterologous
6 encapsidation and vector transmission in PVCP PIP
7 transgenic plants in order to show that rates are reduced.
8
9 Question 16. Please comment on how necessary
10 and/or sufficient each of these conditions is to minimize
11 the potential for novel viral interactions.
12 Please address specifically what combination
13 would be most effective or what conditions could be
14 modified, added, or deleted to ensure that potential
15 consequences of novel viral interactions in PVCP PIP
16 transgenic plants are minimized.
17 Question 17. To what degree and in what ways
18 might a PVCP gene be modified. For example, through
19 truncations, deletions, insertions or point mutations,
20 while still retaining scientific support for the idea that
21 humans have consumed the products of such genes for

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1 generations and that such products therefore present no
2 new dietary exposures.
3 Question 18. What are the potential adverse
4 effects, if any, of such modifications on nontarget
5 species. For example, wildlife and insects that consume
6 the PVCP PIP.
7 Question 19. To what degree and in what ways
8 might a PVCP gene be modified, for example, through
9 truncations, deletions, insertions or point mutations,
10 before it would be a concern that novel viral interactions
11 due to the modifications could occur because the PVCP gene
12 would be significantly different from any existing in
13 nature.
14 Question 20. Would any additional requirements
15 related to PVCP PIP identity and composition, for example,
16 demonstration that the transgene has been stably inserted,
17 be needed for significant reduction of risks associated
18 with PVCP PIPs.
19 Question 21. Are there any considerations
20 beyond gene flow, recombination, and heterologous
21 encapsidation as posed in the preceding questions that the

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1 agency should consider in evaluating the risk potential of
2 PVCP PIPs, for example, synergy. Thank you.
3 DR. ROBERTS: Thank you. Are there any
4 questions for Dr. Milewski on this last set of technical
5 issues of the overall charge to the panel?
6 Dr. Gendel.
7 DR. GENDEL: I'm not quite sure how to phrase
8 the question. I'm curious having been on some other SAPs
9 that have considered other PIPs. In question seventeen,
10 you phrase it with the assumption that history of
11 consumption shows that these are safe. How much
12 modification would be necessary to validate that
13 assumption.
14 In previous cases, we have discussed proteins
15 which also were consumed under various circumstances such
16 as by deliberate application or as contaminants for many
17 generations.
18 But the agency never phrased the safety
19 questions related to those proteins in the same way. Why
20 are virus proteins being approached differently than other
21 PIPs have been in the past?

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1 DR. MILEWSKI: I'm not sure that we're actually
2 asking -- even though the phraseology may be different, we
3 may not actually be asking a different question.
4 What we're looking for is the safety
5 consideration in terms of dietary safety of these
6 particular proteins.
7 DR. GENDEL: You understood my question. In
8 previous SAPs, the question has been actually worded
9 essentially from the other side.
10 The assumption of safety was not explicit and it
11 was asked how do we establish safety rather than making it
12 explicit here. I just wondered if it was a stylistic
13 thing or there was a reason why this was approached from a
14 different angle.
15 DR. MILEWSKI: It was approached from a
16 different angle simply because we have a history of
17 comments on the docket which support that assumption.
18 Now, I don't know that I have seen any data in the docket
19 that support the assumption.
20 DR. GENDEL: Which was the question I asked
21 before. Right?

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1 DR. MILEWSKI: Yes.

2 DR. GENDEL: Thank you.

3 DR. ROBERTS: Anything else? If not, let's take

4 a 15 minute break or so then reconvene.

5 (Thereupon, a brief recess was taken.)

6 DR. ROBERTS: Let's go ahead and reconvene.

7 Before we move to the public comments section of

8 the agenda, I would like to give the panel one more

9 opportunity if there are any questions or clarifications

10 they would like to pose to the agency presenters before

11 they move on.

12 Let me just say to preface that there may be

13 some situations where interpretations of the literature by

14 panel members differ from the interpretation perhaps the

15 agency has.

16 I think the best mechanism to articulate that

17 would be in the context of the questions, the responses to

18 the questions.

19 So in responding to the question, if part of

20 that involves a different interpretation the agency has

21 taken in the literature, let's highlight it then.

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1 If we get through the questions and there is
2 some literature that we haven't touched upon and
3 individual panel members feel that that it would be
4 important to share a differing interpretation of the
5 literature, let's get to that at the end of the session.
6 With that in mind, let me ask the panel if there
7 are any clarifications. Other than that, are there any
8 clarifications for the presenters before we move on to the
9 public comment session?
10 I see none. Before we move on to the public
11 comment session, I would like to thank Dr. Fairbrother,
12 Dr. Milewski, and Dr. Kramer for their presentation. I
13 think that was very useful in terms of helping the panel
14 understand the thinking in the agency and how it has led
15 to the questions that are being posed to the panel. That
16 was very informative for us.
17 Let's now take public comment. I'm fumbling
18 around to see who the first public commenter is. Dr. John
19 Turner from the United States Department of Agriculture.
20 And the Animal Plant Health and Inspection
21 Service has requested the opportunity to address the

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1 panel.

2 Welcome, Dr. Turner.

3 DR. TURNER: Thank you. I assume my mic is on

4 and you can all hear me.

5 I am, as you said, with the U.S. Department of

6 Agriculture and Biotechnology Regulatory Services, one of

7 EPA's sister regulatory agencies. But I'm speaking today

8 in the public comment period.

9 And I thought because this is one of those areas

10 of overlapping authority, one where we have been very

11 active, I offer my comments today just as context. Maybe

12 helpful to know what the other agency, USDA, is doing with

13 respect to virus resistant plants.

14 We have been regulating virus resistant plants

15 really since the coordinated framework back in 1986. Our

16 original authorities at that time were the Federal Plant

17 Pest Act and the Plant Quarantine Act. Those have since

18 been rolled into one authority, The Plant Protection Act

19 in the year 2000.

20 That's our authority, the Plant Protection Act.

21 We at APHIS protect plants and animals against all

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1 pathogens and pests. And it is under that
2 authority that we regulate genetically engineered plants.
3 And under the original coordinated framework policy in
4 '86, we had the lead responsible for genetically
5 engineered plants.
6 There was also some language in 1986 about
7 overlapping authority. When possible, to avoid confusion,
8 it is best when responsibility lies with 1 single agency.
9 But, of course, that's often not the case. And
10 overlap is always better than gaps. So when more than one
11 agency are involved, there should be a lead agency with
12 coordinated reviews.
13 This was reaffirmed actually by the NRC, this
14 idea in the 2002 report on genetically modified pest
15 protected plants.
16 They also said it is good. If there is a lead
17 agency, they stress effective communication and encourage
18 MOUs between agencies to provide guidance for reviews and
19 encourage coordination when there are more than one agency
20 involved.
21 Under the Plant Protection Act under which we

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1 regulate, the very first piece of text in there really
2 gives you a flavor of our charge from Congress. Congress
3 finds that the detection, control, eradication,
4 suppression, prevention or retardation of the spread of
5 plant pests or obnoxious weeds is necessary for the
6 protection of agriculture, environment and the economy of
7 the United States, and places that responsibility with the
8 Secretary of Agriculture.
9 And so our regulations under the Plant
10 Protection Act at 7 CFR 340, we have designated
11 genetically engineered plants, certain genetically
12 engineered plants as regulated articles.
13 And regulated articles are any plants in which
14 genetically engineered plants in which plant pests, any
15 sequences from plant pests are used in the creation of the
16 organism or any transgenic organism where there is a
17 reason to believe that it might pose a plant pest risk.
18 So clearly, transgenic virus protected plants
19 given issues of recombination, synergy. There are plant
20 pest implications that fit readily into our definition.
21 So under our authority, we're responsible for

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1 field testing. Virtually any genetically engineered
2 plants, you have to come to us for a permanent or
3 notification. Importation or state movement, same thing,
4 we permit these.
5 And then after the field testing stage when
6 things are ready for commercial application, one must
7 submit a petition for a determination of non regulated
8 status.
9 That's where we give our intense scientific
10 review to determine if something is safe for unconfined
11 release.
12 APHIS has deregulated more than 60 crops
13 representing 14 crop species. Where applicable, EPA and
14 FDA have completed most of these reviews. And many of
15 these have entered commercial production.
16 This is a list of some of the crops that have
17 been deregulated. You see VR stands for virus resistance.
18 We have had virus resistant potato lines, squash and, of
19 course, papaya.
20 When we consider something for deregulation, we
21 ask does the organism pose a plant pest risk. Which means

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1 can it cause harm, injury or disease to plants or plant
2 parts.
3 Also, we ask will the decision to grant non
4 regulated status present a significant impact on the
5 environment. That's to fulfill our responsibilities under
6 NEPA and will the decision have an impact on threatened
7 and endangered species.
8 In order to make those determinations, we ask
9 for several types of data. We need data on the crop
10 biology itself. Much of the risk assessment relies on
11 understanding the crop biology and especially the
12 reproductive biology of the non transform plant.
13 Then we look at the genetic differences, the
14 inserted genetic material and its characterization, and we
15 have provided a very detailed guidance on molecular
16 characterization.
17 We look at any phenotypic differences between
18 the transform plant and a comparator plant. We look at
19 the reports from all of the field tests, any relevant
20 experimental data. And also, applicants are required to
21 give us any unfavorable data or information.

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1 And at the bottom there is a web site, you
2 should have gotten this handout, where you can see all of
3 the data requirements that we have for petitions.
4 And then analyzing those data we look at plant
5 pest characteristics, generally, can the plant itself
6 cause harm or injury to other plants or the environment,
7 disease and pest susceptibilities, expression of gene
8 products, new enzymes or changes to plant metabolism,
9 weediness and impact on sexually compatible plants,
10 agricultural or cultivation practices, effects on
11 nontarget organisms including humans, effects on other
12 agricultural products and gene transfers to organisms with
13 which it cannot interbreed.
14 Those are the issues we look at for any crop
15 that comes through deregulation. At this point I'm going
16 to focus a little more tightly on virus resistant plants
17 so you can get an idea of how those have been handled
18 through our system.
19 First of all, in terms of permits there have
20 been over 850 permits and notifications for field test of
21 virus resistant plants. And because notifications usually

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1 include several sites, you can say that there probably
2 have been well over 1,000 if not a few thousand field
3 tests of virus resistant plants. And there have been 5
4 virus resistant plants deregulated, starting in December
5 1994 and the last one in December of '98.
6 And USDA has also played a leadership role in
7 the organization of meetings to address issues related to
8 virus resistant plants.
9 I'm not going to dwell on this, but this is a
10 list of the plants that have been deregulated. 2 squash
11 lines there with the virus resistance. The papaya, of
12 course, developed by Dennis Gonzales, and two potato
13 lines.
14 I'll mention at this point, by the way, I'm not
15 a virologist. If you ask me heavy technical questions
16 about these, I may not know the answer. But my purpose is
17 to give you an overview of flavor for the types of issues
18 that we look at.
19 So we submitted into the docket for this our
20 decision document an environmental assessment for petition
21 number 9733901, which is a PVY resistant potato. And if

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1 you look through that document, you can see the issues
2 that we addressed in detail.
3 We looked at plant pest risks posed by these
4 virus resistant plants themselves. Could they somehow
5 cause harm. Plant pest risks posed by the potential
6 appearance of new plant viruses.
7 In this section we looked at recombination,
8 transencapsidation and synergy. We looked at the
9 potential for changes in weediness or invasiveness of the
10 transformed plant, changes in weediness or invasiveness of
11 the wild relatives, impacts on nontarget organisms
12 including threatened and endangered species and impact on
13 raw and unprocessed commodities.
14 In addition, we have held several meetings on
15 virus resistant plants. We feel we have played an
16 important role in getting some of these same questions
17 that you are talking about today into the public arena and
18 getting public input and science input into our
19 decisionmaking.
20 In 1995, in conjunction with the American
21 Institute of Biological Sciences, we at USDA APHIS had a

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1 meeting called Transgenic Virus Resistant Plants and New
2 Plant Viruses. It was actually a workshop. Some of you
3 on the panel were in attendance.
4 These are the first few questions just to give
5 you a flavor of the types of things, very similar to some
6 of the questions that you are asking, what are the
7 propensities of various taxa to recombine that we consider
8 both between taxa and within taxa.
9 What are the characteristics of RNA sequences
10 that combine. What data are available on the frequency of
11 mixed viral infections. Is there a difference between the
12 rate of recombination in virus resistant plants expressing
13 a virus transgene compared to plants that express a virus
14 transgene, but are not resistant or compared to plants
15 naturally infected with multiple viruses, getting at that
16 issue of the titer, how important that is, that Melissa
17 Kramer touched on earlier, and how do plant mechanisms
18 such as co suppression that alter the expression of
19 transgene affect the risk of recombination between
20 infected virus and the viral transgene.
21 I didn't want to make an exhaustive list. Those

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1 are the first questions. There are more questions. They
2 deal with effects of the transgene expression levels on
3 recombination, effect of location of the expression and
4 compartmentalization where you may get expression where a
5 naturally occurring virus wouldn't occur, the likelihood
6 of recombination as a function of the scale of transgene
7 deployment, genomic masking and phenotype mixing, which of
8 course you can get with trans encapsidation, synergy,
9 experimental design and benefits and post
10 commercialization monitoring.
11 And you can read this report at this particular
12 place on our web. It is still posted.
13 We held a follow up meeting in 1997 for some new
14 issues. And these are the 4 major areas, not to get into
15 questions.
16 Recommendations for design of transgenes to
17 minimize recombination concern. There was a section on
18 luteo viruses which really focused more on replicase genes
19 as opposed to virus coat proteins.
20 Gemini viruses, this was a forward looking
21 section thinking about DNA viruses if they were to come on

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1 and how they might differ from the RNA viruses, and
2 stacking of virus resistance genes.
3 And that report is also available currently and
4 has been on our web site.
5 Finally, in 1999, we held with Virginia Tech's
6 information systems for biotechnology a workshop on the
7 ecological effects of pest resistance genes and managed
8 eco systems. So it wasn't specific to viruses, but there
9 are many instances in there where we did discuss viral
10 implications.
11 That's when we really got into weediness,
12 fitness characteristics and gene escape. And we looked at
13 the potential impacts of the weediness of these crops,
14 gene escape, potential weediness of wild relatives, and,
15 then, of course, the important question is the role of
16 pathogens in limiting weed populations.
17 Gene flow per se is not a risk if there is no
18 impact. But if it gave resistance to a pathogen which was
19 limiting a population, that would be significant. And we
20 talked about gene stacking and crop specific parameters,
21 which could affect impacts.

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1 And so these types of meetings are an ongoing
2 event for USDA APHIS.
3 Finally, in summary to pull all this together,
4 transgenic virus resistant plants clearly meet the
5 definition of regulated articles based on being derived
6 from plant pests and their potential to pose a plant pest
7 risk.
8 It will continue to be a central activity for
9 USDA APHIS as is our charge from Congress under the Plant
10 Protection Act.
11 We have a long history of regulating transgenic
12 virus resistant plants through the permitting of field
13 tests. The first one was in 1988 with the virus resistant
14 plants.
15 And we consider many of the big issues that have
16 been discussed at these previous workshops and are on your
17 agenda of questions today, virus recombination, trans
18 encapsidation, synergy and weediness and fitness of the
19 transgenic crop and wild relatives.
20 And we will continue to be on the forefront in
21 raising questions for virus resistant plants and gaining

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1 outside input to enhance the effectiveness of our
2 regulation.
3 I hope this has given you -- your charge is with
4 EPA. This is purely what we at USDA do. I hope it has
5 been helpful for you. Thank you.
6 DR. ROBERTS: Thank you, Dr. Turner. Are there
7 any questions from panel members regarding Dr. Turner's
8 presentation, the regulatory role of USDA?
9 Dr. Hammond.
10 DR. HAMMOND: Yes. How does APHIS view the
11 regulation of cross protection the deliberate inoculation
12 of crops with a mild isolate virus to protect against the
13 effects of a severe isolate compared to the use of
14 transgenic plants expressing coat proteins?
15 DR. TURNER: Well, with the isolate, the mild
16 isolate, if it were genetically engineered, it could
17 likely be a regulated article. And if it were, then we
18 would be regulating that.
19 Can you give me a little more feel for in terms
20 of how you regard it? I mean, it is our role, of course,
21 to see that field tests are safe and deregulations are

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1 safe and not really move one technology or the other
2 forward.
3 DR. HAMMOND: I was essentially getting at
4 whether there is concern about the use of cross protection
5 as compared to transgenic plants expressing coat protein
6 because both are doing the same thing to a large extent.
7 You have a deliberate presence of virus coat
8 protein in the crop plant.
9 DR. TURNER: Right. And I don't know if I'm
10 prepared to answer that in that I'm not familiar with any
11 review that we have done of the cross protection in terms
12 of the issues that would be raised versus transgenic
13 plants.
14 So maybe I don't have a good answer for that.
15 But certainly, if it were using viruses which occurred in
16 that area and didn't pose a new risk and it provided some
17 sort of protection, it would be something which it seems
18 as though the risk issues could be addressed in some
19 suitable way.
20 DR. ROBERTS: Any other questions?
21 Thank you very much, Dr. Turner, for joining us in

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1 clarifying those issues.

2 The next public presenter would be Dr. Susan

3 Tolin.

4 Welcome, Dr. Tolin. Could you introduce

5 yourself for the record.

6 DR. TOLIN: Yes. I'm Dr. Sue Tolin. I'm

7 commenting today on the behalf of the American

8 Phytopathological Society or APS.

9 In that capacity, I represent our approximately

10 5,000 members who work with plant pathogens and the

11 diseases they cause. In that work, they device ways of

12 managing losses that are caused by plant pathogens.

13 I have been active in the society serving as

14 president, about 10 years ago, and as a member of the

15 public policy board until just 2 years ago. And in this

16 capacity I have had experience in addressing the issues

17 that are important to plant pathology, including the

18 regulation of biotechnology.

19 I'm also a plant virologist by profession and a

20 professor at Virginia Tech. What I will comment

21 on today, though, is APS's activities, because we have

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1 gone on record as supporting the use of biotechnology as a
2 means of improving plant health, food safety and
3 sustainable growth in plant productivity.
4 In 2001, APS issued a position statement on
5 biotechnology, and I have given reference in the written
6 text to the APS web site where you can see this complete
7 statement. But I'll pull out a couple things that are
8 relevant to today's discussion.
9 The first is that we stated that insertion of
10 viral sequences into the plant genome to cause plants to
11 resist virus infection provides a new and effective
12 genetic approach for managing plant viruses.
13 Secondly, future environmental benefits of biotechnology
14 for improved management of plant diseases are enormous.
15 Particularly, the potential to reduce the dependency of
16 growers on synthetic pesticides and to enhance approaches
17 that minimize adverse effects to the environment.
18 And then the concerns that are being raised of
19 environmental and food safety risks of biotechnology
20 through, first, gene exchange or, second, evolution of
21 plant pathogens, or from putative increased or unexpected

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1 allergenicity are legitimate risks that will be addressed
2 as have similar potential risks with any new plant or
3 plant product.
4 Assessment and management of these risks and
5 other risks of new technologies in the form of process is
6 appropriate and must be conducted in a science based
7 manner, and
8 also consider economic, human and animal health and
9 ecological consequences.
10 The statement went on to say that the risks and
11 concerns of plants modified through biotechnology must be
12 viewed in perspective relative to other genetic
13 modification methods and that the consequences of not
14 using biotechnology as an augmentation over a controlled
15 methodology must also be considered.
16 For many years, APS has followed the issue of
17 regulation of biotechnology by federal agencies. During
18 my presidency of APS, EPA first proposed its policy to
19 define substances produced in plants that play a role in
20 resistance to pathogens as plant pesticides. And
21 therefore, is subject to regulation under FIFRA.

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1 At that time APS provided comments to the 1994
2 Federal Register on this proposal, and included our
3 support of the exemption of coat proteins from plant
4 viruses that was proposed at that time.
5 APS was instrumental in developing a report in
6 1996 from a coalition of 11 scientific societies, which
7 examined the scientific basis for EPA's proposed policy,
8 and actually prepared a report that was called Appropriate
9 Oversight for Plants With Inherited Traits for Resistance
10 to Plant Pests.
11 In this, there were provided principles for
12 oversight that are currently used in plant breeding and
13 cultivar release that is done in a non regulatory fashion
14 and has been used for plants prepared by conventional
15 methodology.
16 And this is available on a web site which is
17 included in the written document.
18 We continue to dialogue with EPA ultimately
19 leading to a change in the name from Plant Pesticides to
20 Plant Incorporated Protectants which is currently used.
21 APS also provided extensive comments to EPA on

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1 the 2001 proposal for PIPs, and these are posted on our
2 web site. At that time we continued our support for the
3 full categorical exemption of the plant virus coat
4 protein.
5 Relative to the specific comments and the charge
6 to the panel, we feel that the charge that we have heard
7 this morning has been derived from comments received in
8 these prior publications in 1994 and 2001 that have
9 highlighted the areas of greatest scientific uncertainty.
10
11 The questions asked of the panel should enable
12 the panel to discuss the deployment of coat protein to
13 protect plants from viruses and to explore whether
14 scientific information gained in recent years can be used
15 to decrease the level of uncertainty of environmental
16 impacts.
17 The background material and literature review
18 provided are adequate, but there are still many unanswered
19 questions, simply because the research has not been done
20 and little research funding has been directed to these
21 areas.

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1 The panelists for this meeting, many of whom are
2 APS members, certainly have the accumulated expertise to
3 provide an excellent review for EPA. Thus I will not
4 attempt to provide any additional indepth response from
5 APS to all the questions that are answered, but simply to
6 bring up a few points that were made in our prior
7 comments. Regarding the question of gene flow
8 as an environmental hazard and its possible mitigation, we
9 concur with the conclusion that gene flow can occur from
10 plants containing PVCP PIPs to wild or weedy relatives.
11 Molecular and genomic approaches have provided
12 the tools to demonstrate that gene flow is probably much
13 more extensive than we previously realized. But the
14 interpretation of the consequence of this is still in its
15 infancy.
16 Gene transfer alone, however, should not
17 categorically be considered an environmental hazard, but a
18 natural process. There is little information on flow of
19 resistance genes from crop plants to wild or weedy species
20 and whether or not that has ecological implications.
21 Specifically, could virus resistance confer a

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1 selective advantage on wild or weedy plants.

2 Many weeds are symptomless carriers of viruses

3 that commonly infect crop plants and do not appear, at

4 least in my observations, to be adversely affected

5 relative to their population and geographic range. But

6 I'm sure we'll hear more on this from the panel.

7 As I said, weeds are reservoirs for virus

8 inoculum. Thus, if we transferred natural resistance

9 gene to the weeds, this could actually help reduce virus

10 reservoirs while having little or no effect on the weed.

11 Crop plants are often developed by conventional

12 breeding to be resistant to viruses because this is a

13 major constraint in productivity.

14 Yet, there is no evidence that I'm aware of that

15 such resistance genes have moved from the crop plants to

16 wild species. In some cases, but not as often for viruses

17 as for other pathogens, wild species have been the source

18 of resistance genes.

19 Many of these points were discussed in the 1999

20 workshop on ecological effects of past resistance genes

21 and managed ecosystems that Dr. Turner just mentioned.

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1 And we suggest that EPA look at this document.

2 Mitigation of gene flow could be accomplished

3 simply by spatial and temporal separation of the species.

4 The species have to be together and they have to flower

5 at the same time for gene flow to occur.

6 Concerning mitigation by risk management, in our

7 2001 comments to EPA, APS strongly supported the position

8 that the review by USDA that Dr. Turner has just

9 described, concerning gene flow, that this was sufficient

10 regulatory oversight of this potential risk.

11 We trust that EPA as it explores this area

12 further or takes further action on it will continue to

13 work cooperatively with USDA.

14 On the second charge, do viral interactions pose

15 environmental hazards and could they be mitigated, we

16 recognize that the potential exists for any viral

17 transgene to recombine with viruses infecting the

18 transgenic plant and that recombination to form new

19 viruses or virus strains can occur in certain

20 circumstances.

21 New virus emergence per se does not pose an

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1 environmental hazard. The phenomenon of new virus
2 appearance during mixed infections or increased virus
3 diversity as influenced by its host or vectors is known
4 to occur in nature.
5 Such phenomena are much more readily
6 demonstrated today with the increased knowledge of viral
7 sequences and the tools of viral genomics and
8 bioinformatics. The significance of this emergence could
9 now be explored if more funding were available.
10 The panel will undoubtedly bring up many
11 specific examples. I look forward to listening to their
12 discussion during the course of this meeting.
13 With regard to the other questions, in its 1994
14 comments, as I said, APS supported the exemption of viral
15 coat proteins and the tolerance, level in the tolerance
16 level.
17 As of that time, there was no known toxicity or
18 allergenicity of coat proteins to humans. We were
19 concerned at that time of possible modifications to the
20 proteins and made the statement that APS suggests
21 additionally that the language in the exemption to

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1 tolerance requirements should be made perfectly clear to
2 refer only to those viruses normally infecting plants.
3 At that time, we were aware that research had
4 just begun to modify coat proteins to express, for
5 example, epitopes from animal or human viruses which
6 specifically we believe should not be covered by this
7 exemption, but should be examined more completely.
8 The mechanism of synergy between viruses was
9 largely unknown in 1994, but today it is quite well
10 understood as a function of certain portions of the viral
11 genome in gene silencing and silencing suppression.
12 To summarize, APS supports the exemption of the
13 application of plant virus coat proteins incorporated as
14 protectants for the control of plant virus diseases.
15 Assessment and management of risk must be
16 conducted in a science based manner and should also
17 consider the benefits resulting from deploying these
18 resistant plants.
19 Risks should be viewed in perspective relative
20 to other genetic modifications and virus control methods.
21 Thank you for the opportunity to present these

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1 written and oral comments on behalf of APS, and I will be
2 pleased to answer any questions.
3 DR. ROBERTS: Thank you, Dr. Tolin. Do any
4 panel members have any questions for Dr. Tolin, her
5 presentation? I don't see any. Thanks very much.
6 I would like to also point out for the audience
7 that there have been a number of written comments provided
8 by interested parties for public comment regarding today's
9 session.
10 Those written comments have been copied and
11 distributed to the panel members and they are also
12 available for public review on the docket.
13 At this time I would like to ask if there are
14 any members of the audience who would like to make
15 comments to the panel on this topic.
16 In other words, is there anyone who has not
17 previously indicated a desire to address the panel on this
18 but would like to do so now? I would point out that this
19 is really the only opportunity in the agenda for this
20 meeting for public comment.
21 I don't see anyone. In that case, let me thank,

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1 then, Drs. Turner and Dr. Tolin for coming here, making
2 presentations to the panel. We appreciate that.
3 Appreciate the information that you have provided.
4 And also thank the other folks who were not able
5 to make presentations, but provided written comments for
6 the panel. The panel takes very seriously input from
7 stakeholders and the public in our deliberations on these
8 issues. And we appreciate the effort that was expended to
9 make that information available to us.
10 This, then, closes the public comments section
11 portion of the agenda. It is 11:15. I think that we have
12 time for the panel to maybe tackle the first question on
13 our list. Get one under our belts before we go to lunch.
14 So let me suggest that we do that.
15 Can I ask the agency to pose the first question
16 to the panel.
17 DR. KRAMER: What scientific evidence supports
18 or refutes the idea that plant viruses have significant
19 effects on reproduction, survival and growth of plant
20 populations in natural settings? Is there scientific
21 evidence that plant populations freed from viral pressure

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1 could have increased competitive ability leading to
2 changes in plant population dynamics?
3 DR. ROBERTS: Dr. Sherwood, would you lead off
4 our discussion in response to this question.
5 DR. SHERWOOD: We have been asked to begin this
6 session on gene flow with a discussion of what scientific
7 evidence supports or refutes the idea that plant viruses
8 have significant effects on reproduction survival and
9 growth of plant populations in natural settings. Is there
10 scientific evidence that plant populations freed from
11 biopressure could have increased competitive ability
12 leading to changes in plant population dynamics.
13 Agro ecosystems are not natural settings. Even
14 if we look at our production areas at a larger scale,
15 beyond the borders of a field or fields, we are still
16 examining an environment that has long been disturbed.
17 If we were to examine natural settings, I'm not
18 aware of any extensive inventories of plant virus in an
19 undisturbed ecosystem.
20 As viruses are obligate parasites, it would be
21 an evolutionary dead end if they impacted their host or

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1 vectors too significantly. Thus for the sake of the
2 initial comment for this session, I'm considering the
3 natural setting as those areas adjacent to production
4 areas.
5 Our primarily knowledge about the effects of
6 virus on plants come from cultivated plants. And a trite
7 but truthful answer to the effect of plant viruses on
8 reproduction, survival and growth of plants is it depends.
9
10 The obvious goal with cultivated plants is to
11 lessen the impact of virus infection on plant grown and
12 subsequent yield of the plant part of commercial interest.
13 The effect of virus infection on cultivated
14 plants is quite variable and dependent on a specific host
15 and specific virus.
16 Microscopic symptoms can include reduction in
17 growth, reduction in vigor, reduction in quality or the
18 infection may be masked.
19 While we do know something about the impact of
20 virus on cultivated plants, our knowledge about the effect
21 on virus infection on non crop plants is quite limited

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1 and, again, could be succinctly answered, it depends.

2 Most virus epidemics result from the virus and

3 or vectors coming from non crop plants adjacent to

4 production areas. If the host from these natural settings

5 are too adversely affected by the virus or vector, the

6 epidemic cycle would be broken as the plant reservoir for

7 virus and vector would no longer be present.

8 As summarized by Duffus, from the standpoint of

9 control of virus diseases, there is perhaps no phase of

10 virology more important than epidemiology.

11 The role of weeds in the occurrence and spread

12 of plant virus disease is an integral part of the

13 ecological aspect of virus transmission.

14 So the question now becomes what is the impact

15 of viruses on weeds. The literatures is filled with

16 reports of different viruses on different plant hosts

17 either found in natural infections or purposely inoculated

18 as plant host strains has long been a method to

19 differentiate viruses and virus strains.

20 What is lacking is a significant body of

21 literature on the effect of viruses on weed species.

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1 Freiss and Maillet found that in cucumber mosaic
2 cucumo virus infected chick weed plants, stellaria media,
3 grown in a monoculture had similar vegetative production
4 to a monoculture of control healthy plants.
5 However, when healthy and infected plants were
6 grown together, as the density of the healthy plants grown
7 with infected plants increased, infected plants were not
8 as vegetatively productive or as reproductive.
9 Work from this lab on nitrogen partitioning and
10 CMV infected versus healthy weeds found no difference in
11 virus infected and healthy chick weed plants, but nitrogen
12 partitioning to shoots and roots was different in CMV
13 infected and healthy purslane, portulaca oleacea.
14 Romold examined the incidence of barley yellow
15 dwarf luteo virus in 3 grass hosts, soft brome grass,
16 green foxtail and yellow foxtail.
17 Using panicle length as a measure of fitness,
18 soft brome grass was not affected by virus infection.
19 Fitness of green foxtail was about half of
20 uninfected plants. And infected yellow foxtail had about
21 25 percent greater fitness than uninfected plants.

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1 Maskell, et al., found that wild cabbage
2 inoculated with either turnip mosaic poty virus or turnip
3 yellow mosaic tymo virus had significantly reduced
4 survival, growth and reproduction.
5 In a recent 3 year study of CMV in central
6 Spain, Sacristan found that the incidence in CMV in weeds
7 fluctuated in various habitats such as fallow fields,
8 edges and waste lands through the growing season with a
9 maximum incidence of 20 to 30 percent in summer and
10 autumn. The greater amount of biomass and soil coverage
11 was correlated with a greater incidence of CMV.
12 Thus, there is quite a bit of variation in the
13 impact of viruses on plant growth. Virus infection,
14 regardless of the plant being a crop or a non crop plant,
15 will likely negatively impact some aspect of plant
16 development and reproductive capacity.
17 Is there scientific evidence that plant
18 populations freed from viral pressure could have increased
19 competitive ability leading to changes in plant population
20 dynamics as the second question posed.
21 I'm not aware of any study that has purposely

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1 freed a weed species of a known virus and determined its
2 competitive ability or looked at the population dynamics
3 of virus infected versus uninfected plants in a multiple
4 species ecosystems.
5 However, if we were to use the definition of a
6 weed as a plant out of place, our agro ecosystems provide
7 many good examples as most crop plants grown in tempered
8 ecosystems do not originate in those ecosystems.
9 The classic example is running out in potato
10 that results from accumulation of pathogens, particularly
11 viruses, in the vegetatively reproduced seed material
12 versus the use of true seed that is commonly used for most
13 crops.
14 Virus free seed potatoes, developed through
15 certification programs, are far more vigorous than virus
16 infected seed potatoes.
17 For viruses that are transmitted through true
18 seed, greater vigor and reproductive capacity is also
19 commonly observed for plants originating from virus free
20 seed.
21 Starting virus free, regardless of the plant

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being a crop or non crop species does not give a plant immunity.

Weeds or crop plants that are susceptible to viruses in that environment can be infected.

We see from the work of Remold cited above that viruses have variable effects on non crop plants.

In regards to plant population dynamics, the effect of tomato spotted wilt tospo virus on peanut plant production is a good demonstration of the effect virus can have on a population dynamics.

Fields of peanut plants that have no or little virus are more competitive in that the vines lap earlier, blocking the sun between rows, thus reducing weed pressure.

The significant study of Jones and Nicholas in self regenerating pasture in Australia over a four year period provides a good look at the introduction of virus in a multiple species complex environment.

They sowed seeds of burr medic that was either free or infected with alfalfa mosaic alfamo virus in mixed species pastures and followed the effect on proportionate

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1 of species over time.

2 Generally, less desirable species became

3 established as the virus became established, but the

4 effect varied with medic cultivar.

5 Difficulty in determining the effect was

6 compounded with the extent of aphid abundance that

7 transmits AMV which was variable. I'm not aware of any

8 reports on the impact of virus infection on plant

9 competition in non agricultural settings.

10 DR. ROBERTS: Thank you, Dr. Sherwood.

11 Dr. Cooper, would you like to contribute to the

12 response?

13 DR. COOPER: I will add only a few things to

14 what John said. Of course, the impact is strongly

15 suspected, but not proven. We have quite a lot of

16 evidence of impact upon wild plants, but many of those

17 wild plants are not known to be crucial to the survival of

18 the virus in its evolutionary sense.

19 It may have many alternate hosts. So we can't

20 make definitive judgments about whether devastating effect

21 of one particular species would really have any serious

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1 impact. It may have several alternative ways of using it.
2 All virus isolates are not equal. And all, of
3 course, of the plants that we group together as species
4 are not equal in terms of the reactions to the viruses.
5 Fundamentally, we don't have very much
6 information, if any, about the whole life cycle impact of
7 any of these viruses.
8 Dramatic impact on seed production, growth,
9 vigor, which we can recognize, is not felt to be an
10 adequate description of what the impact would be on an
11 evolutionary sense on the species.
12 And so what we ideally seek is some life cycle
13 assessment over the stages which are crucial to the
14 survival of the species from seed back to seedling,
15 seedling to flowering plant, flowering plant to seed,
16 including what proportion of the seeds are lost through
17 whatever cause.
18 And those together make the population dynamic
19 of a wild species, which is perhaps a somewhat different
20 sort of approach to that which might be considered by in
21 an agricultural context.

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1 Because those pieces of data are absent, it is
2 really difficult to prove the concept which is implicit in
3 the question. So that we have differential virus patho
4 (ph) types with different effects and we don't know the
5 full life cycle implications of any of them that we have
6 observed so far.
7 DR. ROBERTS: Dr. Zaitlin.
8 DR. ZAITLIN: I think my colleagues have stated
9 the case.
10 DR. ROBERTS: Let me then open the question up
11 to other members of the panel, their viewpoints that they
12 want to contribute, agree with, disagree with, so forth.
13 DR. FALK: I agree with the comments that have
14 been made previously. I think one of the things that
15 we're hearing in regards to these questions is the
16 assumption that or it is based on the assumption that
17 viruses are pathogens.
18 I think it is legitimate questions (ph). They
19 are viruses (ph). Are they naturally pathogens with their
20 natural host plants or even host animals.
21 If they are not pathogens, then in a natural

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1 system, then, some of these questions are not that
2 relevant.
3 I too am not aware of data that shows that plant
4 viruses have significant effects on characters in plants
5 in natural settings.
6 There are some reports, however, that show the
7 opposite. For example, a report by Adrian Gibbs shows
8 that virus infection in wild legum host actually protects
9 that plant against herbivore by some animals that eat that
10 plant.
11 In some cases, virus infection in their natural
12 host can actually confer advantages. So they are not
13 always pathogens.
14 I think if viruses had obvious negative effects
15 on wild hosts, this could have been noted already or we
16 should have noted this.
17 I think that plant viruses and all viruses do
18 not necessarily kill their host plants. We see serious
19 effects on our cultivated crops and the losses that we see
20 are those that Professor Sherwood mentioned. I
21 think that if we think of virus disease, we have to

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1 consider population and inoculum pressure. In the past we
2 have controlled viruses through many means.
3 And when we control the viruses in agricultural
4 settings, if those viruses were important in affecting
5 weeds in a natural setting, we have already reduced
6 inoculum and should have seen some effects.
7 So I don't think or my point is I'm trying to
8 bring up the or have us think about whether, in fact,
9 viruses actually are controlling weeds in natural
10 settings.
11 DR. ROBERTS: Other viewpoints. Dr. Tepfer.
12 DR. TEPFER: I just wanted to sort of propose a
13 type of clarification, which is quite in the same lines of
14 what the other panel members have said.
15 I think that it is very important to make the
16 distinction between the effect of a fitness advantage in
17 which case you could expect that a virus resistance
18 transgene would become more and more frequent within a
19 population of a wild or weedy species and that is a quite
20 different situation from actually having effect on the
21 size or distribution of the populations of the plant

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1 species in question.

2 It is only the latter situation which would

3 constitute ecological release and could increase

4 weediness.

5 As the other speakers have said, there are

6 numerous reports of changes in fitness effects and because

7 these are experimentally relatively manageable.

8 But I think that doing an experimental study on

9 ecological release is remarkably difficult because in many

10 cases you can have a fitness effect which may not be

11 limiting to population size.

12 DR. ROBERTS: Any other comments from panel

13 members. Yes, Dr. Hammond.

14 DR. HAMMOND: I just would like to say I pretty

15 much agree with what has been said so far, but I would

16 like to further go and document the fact that there are

17 frequent occurrence of mixed infections of viruses in wild

18 plants without obvious evidence of any symptoms or

19 deleterious effects.

20 In a survey that I carried out myself as part of

21 my doctoral research, I looked at viruses infecting the

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1 common weed plantago lanceolata.

2 And randomly collected plants from wild settings

3 around the country without regard to any symptom

4 expression and found that seventeen percent of these

5 randomly selected plants had, in fact, multiple infections

6 with as many as 4 viruses present in a single plant

7 without any obvious deleterious effects.

8 This has been documented in other species as

9 well. Alan Dodds carried out a study in nicotiania glauca

10 and found that infections of 5 to 7 viruses in single

11 plants were common again without significant apparent

12 obvious effects on the plant.

13 And Jim Duffus had found up to 9 viruses in

14 individual plants of spinach. There are also interactions

15 between viruses and other pathogens, in some cases

16 positive effect and in some cases negative effects.

17 One that comes to mind is an effect between

18 barley yellow dwarf infection in some grass populations

19 having a protective effect on a fungal disease that

20 otherwise infects those plants.

21 But there are also cases where virus infection

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1 increases susceptibility to fungal diseases.

2 So there are many cases when wild living plants

3 are infected with more than one virus without any apparent

4 detrimental effect.

5 DR. ROBERTS: Other questions from panel

6 members.

7 DR. KRAMER: Can I ask for one clarification.

8 What I have heard from a lot of the panelists is that

9 there is basically a lack of evidence suggesting this

10 changes in plant population dynamics.

11 Would you consider that sufficient to conclude

12 that it does not occur or are you saying that there simply

13 is no evidence to conclude either way?

14 DR. ROBERTS: Dr. Cooper and then Dr. Sherwood.

15

16 DR. COOPER: If there is no evidence, then there

17 is no evidence on which you can make an assumption. At

18 the moment it's being investigated. One specific example,

19 brassica rapa and now it's called compestris. That's been

20 initially resulting in evidence that suggests the impact

21 on seed production is not crucial to the survival of the

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1 species.

2 But there are several more years worth of

3 investigations even in that species in relation to one

4 virus, turnip mosaic virus. And even that might not be

5 generally applicable, but it would at least answer all the

6 points which seem relevant to the survival of the species,

7 its persistence and it's dynamic.

8 In the absence of those data at the moment, I

9 would strongly recommend that we shouldn't rush to

10 judgment.

11 DR. ROBERTS: Dr. Sherwood, do you want to add

12 anything?

13 DR. SHERWOOD: I think he said it far more

14 eloquently than I could have.

15 DR. ROBERTS: Any other follow-up questions or

16 clarifications?

17 Let me poll the panel members. Do you want to

18 go ahead and take the second one or do you want to break a

19 little bit early for lunch and then come back?

20 DR. STEWART: Take the second.

21 DR. ROBERTS: We have a vote to take the second

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1 from the lead discussant on this. Let's go ahead and
2 take the second question.
3 DR. KRAMER: Number 2. Please comment on the
4 validity of the agency list of crops that have no wild or
5 weedy relatives in the United States with which they can
6 produce viable hybrids in nature. That is, tomato,
7 potato, soybean and corn.
8 DR. ROBERTS: Dr. Stewart, would you lead off
9 our discussion on this one.
10 DR. STEWART: This list is adequate insofar that
11 it lists crops of large acreage in the U.S. I think it
12 is dandy.
13 DR. ROBERTS: Well and concisely stated. Dr.
14 Cooper.
15 DR. COOPER: I would question one of the species
16 in this list, is tomato. To my mind, it has a strong
17 potential at least to be a weed.
18 In U.K. conditions, it is manifestly a nuisance
19 plant in the vicinity of sewage treatment works because
20 the seed of tomato grows readily through the human and
21 indeed the rodent elementary canal.

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1 So the transmission of the seeds of that species
2 into places of accumulation and disturbance results in
3 lots of opportunities for tomato.
4 Tomato doesn't invade agricultural land, but it
5 is certainly a nuisance plant which at least in British
6 conditions is recognized as such with the potential.
7 Perhaps in other parts of the world.
8 I don't know about the American experience, but
9 it does seem to me worth flagging that difference.
10 I think cuba bearing salinums (ph) are well
11 established as being rigorously isolated from one another,
12 and, therefore, even if any of them were nuisance plants,
13 as sometimes they can become, the risks of moving genes
14 between them would be minimal. Perhaps even non existent.
15 But some of the others I don't know about. I
16 can defer only to the local expert. DR.
17 ROBERTS: Dr. Hammond.
18 DR. HAMMOND: I have nothing to add. I agree
19 with Dr. Stewart.
20 DR. STEWART: I don't think tomatoes is
21 naturalized in the U.S. I think the U.K. experience is a

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1 bit different there. DR. ROBERTS: For the
2 record, that was Dr. Stewart.
3 Dr. Tepfer.
4 DR. TEPFER: I just have a question, in fact, of
5 clarification regarding this list. Does this include also
6 territories associated with United States that are not the
7 50 states, some of the tropical territories as well?
8 DR. KRAMER: Yes. If I remember correctly, I
9 think there is a footnote in the background paper that we
10 handed out that would list all of the included territories
11 in the statement.
12 DR. ROBERTS: Other comments or comments from
13 other panel members on this?
14 Was the response from the panel concise and
15 clear?
16 DR. KRAMER: Yes.
17 DR. ROBERTS: Taking the direction from Dr.
18 Stewart, I suspect we could probably take number 3.
19 DR. STEWART: Number 3 might take a little bit
20 longer, but I'm willing to go at it if you are.
21 DR. ROBERTS: Let's do number 3. We're on a

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1 role.

2 DR. KRAMER: Please identify other crops that

3 have no wild or weedy relatives in the United States with

4 which they can produce viable hybrids in nature, for

5 example, papaya, peanut and/or chickpea.

6 DR. ROBERTS: Dr. Stewart.

7 DR. STEWART: This question depends on which

8 crops are grown, where they are grown in the U.S. Annual

9 crops can go year to year, deployed in time, whereas

10 perineals can be long lived. Crops can be also

11 naturalized and considered wild or feral at some point.

12 So the question pertains to wild relatives per

13 se and not whether the crop will hybridize or introgress

14 with them. We're looking at this pretty broadly. And

15 we're looking to exclude plants that we can maybe move up

16 into question number 2. The list in number 2.

17 And I think these could be considered candidates

18 (ph).

19 And I'll be interested in hearing what you all

20 think of my list anyway. This is the one time which I had

21 my university ovarium curator in my back pocket.

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1 So the list of crops without wild relatives in
2 the U.S. that I'm aware of would be papaya, peanut,
3 chickpea, bean, pea, black eyed pea as we say in the
4 south, cow pea other places, lima bean, cucumber, sugar
5 cane, onion, pepper, spinach, barley, peach, almond,
6 citrus, sweet potato, daffodil, olive, and I have question
7 marks beside chrysanthemum, tobacco and apple, the last 3.
8 Tobacco is an American -- it is indigenous to
9 the Americas. I'm not sure how many wild tobacco there
10 actually is left in the U.S. I don't know.
11 You know, there are new crops coming up every
12 year. I guess this would be the larger crops and no one
13 really would consider daffodil to be a large crop. So
14 there is that caveat.
15 DR. ROBERTS: Let me just ask did everybody get
16 a chance, since this is a good starting place for
17 discussion, do you need Dr. Stewart to go through the list
18 again or did everybody get them down?
19 Do them one more time.
20 DR. STEWART: Papaya, peanut, chickpea, bean,
21 pea, black eyed pea or cow pea, lima bean, cucumber, sugar

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1 cane, onion, pepper, spinach, barley, peach, almond,
2 citrus, sweet potato, daffodil, olive, and chrysanthemum,
3 tobacco and apple.
4 One of the commenters also included brassica
5 oleracea vegetable such as cabbage, cauliflower, broccoli,
6 those types of things. And I wasn't as
7 comfortable with that one because there are wild relatives
8 that share a genome from brassica oleracea even though
9 they don't cross very easily. DR. ROBERTS:
10 Dr. Cooper, what do you think about list.
11 DR. COOPER: It is very long. At least the
12 brassica having seen wild or perhaps feral brassica
13 oleracea types in San Francisco just across the Bay
14 reasonably abundant, I'm surprised -- they may not be a
15 weed situation, but they are reasonably abundant in the
16 wild.
17 So at least they may be isolated physically from
18 many potential transgenic crops, but nevertheless I
19 consider even in my limited experience that they are
20 there.
21 I really can't comment about most of the other

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1 things. The tobacco is such a variety of different
2 types. When we looked at the risks of gene flow into
3 tobacco, many of the ornamental tobaccos were actually
4 thought to be on limited experience genetically isolated
5 from the nicotiania tobacum types we were using. But the
6 evidence was not a complete basis for making a safety
7 judgment.
8 I won't comment on anymore at the moment.
9 DR. ROBERTS: Dr. Hammond.
10 DR. HAMMOND: I'm not aware of any wild
11 relatives. I don't consider myself qualified to judge in
12 this area.
13 DR. ROBERTS: Let me ask other panels members if
14 they want to weigh in. Dr. Melcher.
15 DR. MELCHER: I would like to have it clarified
16 for me not being a taxonomist what is meant by a relative
17 in this case. Because I can recognize that some of these
18 are legums and I know that there are wild legums and some
19 of them are rosacea. There are wild rosacea and so forth.
20
21 DR. STEWART: My interpretation of this question

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1 takes it to the species level in many instances, the
2 general level in some instances. That is my criteria for
3 not including some things on this list. And then
4 relative abundance.
5 So there is a lot of interpretation here. If we
6 get right down to brass tax, all the plants are related at
7 some level. So you would have nothing on the list.
8 DR. ROBERTS: For the record, that was Dr.
9 Stewart responding. Let me go ahead and ask the agency to
10 clarify that for us.
11 DR. KRAMER: I want to clarify. I think what
12 we're concerned with is the latter part of that sentence
13 where we're saying can produce viable hybrids in nature.
14 So how ever you would define relative that would encompass
15 such plants would be fine.
16 DR. ROBERTS: With that in mind, you're still
17 comfortable with your list, Dr. Stewart?
18 DR. STEWART: Yes. That's how I interpreted
19 this list. These would have an extremely low chance of
20 forming viable hybrids.
21 Now, you could really add more plants to this

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1 list where the hybrids would be really low fertility or
2 the hybridization rates would be extremely low.
3 Especially, when you consider where the wild and
4 the weedy relatives might be compared with where a crop is
5 grown.
6 Here again with annual plants, the crop can be
7 grown in different places each year. With perennials,
8 they are a little bit longer term.
9 DR. ROBERTS: Dr. Zaitlin and then Dr. Tepfer.
10 DR. ZAITLIN: I was going to say with tobacco
11 there are a number of nicotiania species that do grow wild
12 in the south western United States, but I think genetic
13 incompatibility with nicotiania tobacum.
14 DR. ROBERTS: Dr. Tepfer.
15 DR. TEPFER: I want to confirm and remind you
16 that the commercial tobacco, nicotiania tobacum, is an
17 allotetraploid. And is in fact genetically quite
18 completely isolated from anything that grows in the United
19 States.
20 And the tetraploid form does not grow in nature.
21 It has never been described, and the two parental species

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1 come from very obscure places in South America. I think
2 tobacco is one we could definitely add to the list.
3 In contrast I'm a little bit concerned about
4 pepper, which is capsicum because there are feral
5 populations of capsicum in Caribbean Islands.
6 So that might be one that we might put a
7 question mark behind at least. I suspect that you could
8 get gene flow in places like Puerto Rico and things like
9 that, virgin islands.
10 DR. STEWART: When it comes down to I guess the
11 really tropical locations, territories, Hawaii, granted I
12 think I need to do some more study there, the list is a
13 bit shaky.
14 My list is mainly pertains to continental U.S.
15 DR. ROBERTS: Dr. Kramer.
16 DR. KRAMER: I just wanted to apologize. The
17 footnote I was referring to disappeared in a draft. It
18 was unintentional. I wanted to read into the record what
19 exactly we mean by the United States in this context.
20 That would mean a state, the District of
21 Columbia, the Commonwealth of Puerto Rico, the Virgin

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1 Islands, Guam, the Trust Territory of the Pacific Islands,
2 and America Samoa.
3 DR. STEWART: What was the last 3?
4 DR. KRAMER: American Samoa.
5 DR. STEWART: What was before that?
6 DR. KRAMER: Let me read the whole list again.
7 A state, the District of Columbia, the
8 Commonwealth of Puerto Rico, the Virgin Islands, Guam, the
9 Trust Territory of the Pacific Islands and American Samoa.
10
11 DR. ROBERTS: Dr. Tepfer.
12 DR. TEPFER: I would just raise the question
13 about sugar cane because again there is a lots of feral
14 sugar cane in many tropical islands. I'm not sure how
15 sexy it is. I think that a lot of sugar cane is rather
16 sterile. I think you can make crosses.
17 DR. STEWART: There is a shatter cane that's
18 actually compatible. I was not considering the Caribbean
19 when this came up. That one should perhaps be removed
20 from my list anyway.
21 DR. ROBERTS: When we write up our minutes,

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1 maybe we can sort of clarify which ones we have high
2 confidence in and which ones given the other territories
3 that we're talking about a little more far flung that we
4 might have some reservations about.
5 Any other comments, edits to the list?
6 Dr. Kramer, is that response do you think going
7 to meet the needs of the agency?
8 DR. KRAMER: I would ask when you are writing up
9 the final minutes to indicate whether this is a consensus
10 view. I know you had expressed some reservations about
11 the list at this point. We ask in the final write up to
12 be sure that you are comfortable with the list that you
13 actually put in the minutes.
14 DR. ROBERTS: Let me ask right now while we're
15 in session. Is everyone pretty comfortable? Let me ask
16 it this way. Is there anyone that is uncomfortable with
17 the list, with the caveats that we anticipate about some
18 of the territories?
19 DR. COOPER: I'm not comfortable with the
20 brassica.
21 DR. STEWART: Brassica wasn't in there. As I

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1 noted, a couple of the commenters included brassica
2 oleracea vegetables. My list did not have them for the
3 very reasons that you mentioned.
4 DR. ROBERTS: So there seems to be agreement on
5 that. That that probably shouldn't be on the list. Dr.
6 Tepfer.
7 DR. TEPFER: I don't seem to have the list, the
8 preexisting list. Could we leave this open until after
9 lunch so I can have a look at the previous list to see
10 whether there are things that strike me on it before we
11 come to a final conclusion on this point?
12 DR. ROBERTS: Sure. There is no problem with
13 that.
14 Then we are getting kind of close to lunchtime.
15 Let me then suggest that we take a break now. We will
16 close out this question when we return from lunch. Let me
17 suggest that we do that at 1 o'clock.
18 So I'll give you guys a chance to sort of take a
19 look at that list. We'll finish up question 3 immediately
20 when we convene at 1 and then we'll proceed on with
21 question 4.

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1 Let's plan on getting together back here at 1

2 o'clock.

3 (Thereupon, a luncheon recess was taken.)

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2 I, Frances M. Freeman, Stenotype Reporter, do
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4 FRANCES M. FREEMAN
5
6 TODAY'S DATE: 102704
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8 DATE TAKEN: 101304
9
10 CASE NAME: epa sap
11
12 DEPONENTS:
13
14 **TOTAL: -- PAGES:** 165 plus sitting fee split with
15 monica
16
17 ATTORNEY TAKING DEPO:
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19 COPY SALES To: Mr.
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21 DELIVERY: 10
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23 COMPRESSED:
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25 DISK:
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27 E-MAIL: no
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29 EXHIBITS: none
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