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)

THURSDAY, OCTOBER 14, 2004

VOLUME II OF IV

(Morning session)

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

Reported by: Frances M. Freeman, Stenographer

1 **C O N T E N T S**

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1 DR. ROBERTS: Good morning and welcome to the
2 second day of our session on issues associated with
3 deployment of a type of plant incorporated protectant.
4 Specifically those based on plant viral coat proteins
5 We need to take care of a little bit of business
6 before we resume our discussions. And the first thing we
7 need to do in case we have any new members to the audience
8 joining us today is to reintroduce the panel.
9 So let's take a few minutes to do as we did
10 yesterday. Go around the table and ask each individual to
11 state their name affiliation and expertise. As yesterday,
12 let's start with Dr. Melcher.
13 DR. MELCHER: I'm Ulrich Melcher from Oklahoma
14 State University in biochemistry and molecular biology.
15 I'm a plant virologist interested in recombination,
16 evolution, bioinformatics.
17 DR. SHERWOOD: John Sherwood
18 Department of plant pathology, University of Georgia,
19 interested in viral cross protection and epidemiology.
20 DR. ZAITLIN: I'm Milton Zaitlin,
21 professor, emeritus plant pathology, Cornell University.

1 My specialty is virus replication.

2 DR. FALK: Bryce Falk, University of California

3 at Davis. I'm a plant virologist. Interested in all

4 aspects of viruses.

5 DR. ALLISON: My name is Richard Allison. I'm

6 in the plant biology department at Michigan State

7 University where I'm a plant virologist. I'm particularly

8 interested in plant virus recombination.

9 DR. TEPFER: Mark Tepfer, I'm a research

10 scientist at INRA which is a French government research

11 agency. I worked on virus resistant transgenic plants for

12 many years, bio safety questions, in particular,

13 recombination as a special issue.

14 I also want to mention that I'm editor of a

15 journal devoted entirely to gemo bio safety research. If

16 you are interested, here are a few blurbs and you can find

17 more about the journal.

18 DR. COOPER: Ian Cooper, Natural Environment

19 Research Council of the United Kingdom. Research

20 scientist concerned with virus ecology and the risks of

21 genetically modified plants.

1 DR. HAMMOND: John Hammond, U.S. Department of
2 Agriculture, Agricultural Research Service. I work with
3 ornamental plants primarily, virus identification,
4 characterization and virus resistance in
5 transgenic plants and risk assessment
6 DR. STEWART: Neal Stewart, University of
7 Tennessee. I work with transgenic plants, study their
8 introgression and the ecological consequences of
9 introgression.
10 DR. NAGY: My name is Peter Nagy from
11 University of Kentucky. My specialty is mechanism of
12 virus replication and recombination.
13 DR. BUJARSKI: I'm Jozef Bujarski from Northern
14 Illinois University. I'm a molecular virologist
15 interested in virus evolution, RNA recombination and
16 replication. DR. STARK: I'm John Stark from
17 Washington University. I work in ecotoxicology and risk
18 assessment.
19 DR. GENDEL: Steve Gendel from the U.S. FDA at
20 the National Center for Food Safety and Technology,
21 interested in biotechnology, food safety and

1 bioinformatics.

2 DR. ISOM: I'm Gary Isom from Purdue University.

3 I'm a neuro toxicologist with research interest in neuro

4 degeneration. DR. PORTIER: Ken Portier, a

5 statistician at the University of Florida, Institute of

6 Food and Agricultural Sciences, with interests in

7 statistical issues of risk assessment.

8 DR. HEERINGA: Steve Heeringa, University of

9 Michigan. I'm a research scientist and director of the

10 Statistical Design Group at the Institute for Social

11 Research. I'm here as a permanent member of the FIFRA

12 Science Advisory Panel.

13 DR. ROBERTS: I'm Steve Roberts. I am a

14 toxicologist, Director of the Center for Environmental and

15 Human Toxicology at the University of Florida.

16 We also need to cover a few announcements from

17 our designated federal official, Mr. Paul Lewis.

18 MR. LEWIS: Thank you, Dr. Roberts. And, again,

19 everyone welcome back to our second day of our FIFRA

20 scientific advisory panel meeting and members of the

21 audience for participating again, being observers for our

1 discussion over the course of today.

2 As I mentioned yesterday, the FIFRA scientific

3 advisory panel operates under the guidance of the Federal

4 Advisory Committee Act. This is an open meeting.

5 All materials that were shared with the panel

6 are available in the docket. In addition following this

7 meeting the panel will write its report summarizing its

8 position serving as meeting minutes based on the

9 discussion that occurred over yesterday and today and any

10 discussion we have tomorrow in the public meeting.

11 The report will be available in approximately

12 six weeks after the meeting. It will be available in the

13 OPP docket. In addition, will be published on the EPA SAP

14 web site. Thank you.

15 DR. ROBERTS: Thank you, Paul. I think we would

16 like to start today by sort of recapping a little bit from

17 some of our discussions yesterday. I'm going to turn it

18 over to Dr. Kramer to maybe cover a few things.

19 DR. KRAMER: Thank you. I think we just wanted

20 to start again by reviewing the charge to the SAP. That's

21 to provide scientific advice to assist EPA in its

1 evaluation of several technical issues associated with
2 PVCP-PIPs. Specifically to respond to a series of
3 technical questions related to exposure and hazard
4 considerations for PVCP-PIPs.
5 If we can switch to the slide of the role of the
6 SAP.
7 I wanted to pull this slide up again because I
8 think this was something as we were going through the
9 questions yesterday that we were trying to make sure we
10 got this information from you for each question.
11 But just to make sure that you understand that
12 this is really the type of information we're looking for
13 as we go through the questions today.
14 We would like for each question to understand
15 the degree of risk for each issue, also the degree of
16 certainty of the estimates that you are giving us.
17 Then the relevance of data versus hypothetically
18 supported information and then also your opinion of the
19 direction that the science is taking for the specific
20 issues that are raised.
21 DR. ROBERTS: Thank you, Dr. Kramer. I think

1 this is going to come up in the context of revisiting
2 question 10. We have been asked to revisit question 10 a
3 little bit to clarify some things and I would like to do
4 that.
5 Before we do that, first thing I want to do is
6 we had a very useful discussion, I think, in terms of
7 terminology yesterday.
8 And I think it became clear in that discussion
9 of terminology that in terms of resistance and immunity
10 and so forth what the agency was using and the questions
11 as they were posed to us was resistance and had a
12 definition I think that was different in the minds of some
13 of the members of the panel. And I want to
14 be sure to take just a little bit of time this morning and
15 be sure that our responses to the agency's questions when
16 they talk about resistance and tolerance, which they do in
17 many of the questions, that we are using their definition
18 of resistance as it was articulated in the documentation
19 provided to us and not another definition of resistance
20 that we might have as a panel.
21 I'm not sure about how to do that, but I want to

1 be sure. Can you sort of --

2 DR. KRAMER: As you are answering the questions,

3 if you are uncomfortable using definition that we have and

4 you would rather use the term immunity, that would be

5 fine, but please don't change the definition of resistance

6 and use that term while you are answering the questions.

7 DR. ROBERTS: Is that clear to everyone on the

8 panel? The agency wants us to use their definition.

9 DR. KRAMER: The definition is more important

10 than the term. We want to stick with the definition that

11 we have which ever term is used.

12 DR. ROBERTS: Dr. Melcher.

13 DR. MELCHER: I was thinking about this also. I

14 have, I suppose, answers to questions 6, 7, 8 and maybe

15 even 9 that maybe somewhat are different than what we

16 heard yesterday.

17 I'm not sure. Should I go through each of

18 those?

19 DR. ROBERTS: Sure.

20 DR. MELCHER: Question 6 regarded the prevalence

21 of immunity in wild relatives of crops. We heard from Dr.

1 Cooper about experiments indicating that undetectable
2 levels of viruses are found in some such plants.
3 I think they were relatives of the brassica. It
4 became clear in the discussion that the possibility that
5 the assays used were not sensitive enough to distinguish
6 between subliminal infection and immunity does exist.
7 Further, I believe that it is not common
8 practice to attempt to make this distinction between
9 immunity and some other level of resistance.
10 So unless others know that that's not true, I
11 would conclude that the prevalence of immunity as opposed
12 to other levels of resistance in wild relatives of crops
13 is fairly unknown.
14 But I would suspect that it exists. But I don't
15 think we have any degree of certainty on that.
16 So that's on 6. Should I go on to others?
17 DR. ROBERTS: Let's see if other members of the
18 panel have a differing take on that. Dr. Falk.
19 DR. FALK: I'm sorry, Ulrich. I was reading
20 while you were talking, but according to the definition in
21 your appendix, we don't need to use the term immunity.

1 Basically, we just use resistance and tolerances. Is that
2 right?
3 DR. KRAMER: We want to stick with a term,
4 whichever term you would like, that means the plant is not
5 infected by or is a nonhost of the virus concerned.
6 DR. ROBERTS: Dr. Cooper.
7 DR. COOPER: All I would say if you are trying
8 to achieve an absolute limit on the detectable level of
9 virus to prove immunity, it becomes technically very
10 difficult to apply.
11 You have to apply a realistic level of detection
12 using the most sensitive available tools on that
13 particular occasion. And having regarded the fact that I
14 think the viruses I was talking about were very high
15 concentration, readily detectable viruses, the concept of
16 immunity as an absolute is difficult to apply in practice.
17 Because there is a possibility mentioned by Dr.
18 Zaitlin that single cells might be infected and the rest
19 of the plant escapes.
20 Some aspect of that could be recognized by using
21 the word field immunity which I think plant pathologists

1 and plant breeders use to describe the fact that a plant
2 can be infected under rigorous inoculation challenge.
3 But in the field, remains free. And that's a
4 practical use of that term with that qualification. But
5 with immunity being an absolute term as one definition, it
6 becomes impossible to measure, because there are always
7 changes in technology which might move the barrier a
8 little bit more.
9 DR. ROBERTS: Dr. Hammond.
10 DR. HAMMOND: I think that to all practical
11 purposes a subliminal infection is irrelevant because it
12 is unlikely to serve as a source of infection for another
13 plant. And so a plant which is subliminally infected is
14 effectively resistant and not of an epidemiological
15 concern.
16 DR. ROBERTS: Mr. Zaitlin.
17 DR. ZAITLIN: I'm still thrashing my dead horse.
18 I still think that we're worried about disease, not
19 whether or not there is a subliminal infection or a very
20 low level of infection that doesn't result in symptoms. I
21 mean the real issue here is why do you want to protect the

1 plant. To protect it against disease.

2 DR. ROBERTS: Dr. Melcher.

3 DR. MELCHER: Dr. Zaitlin, the question is about

4 the infection in wild relatives of the crop plants. And I

5 think the reason for asking it is whether the virus could

6 survive in the wild relatives whether or not the wild

7 relatives are sick or not. It really doesn't matter.

8 DR. ZAITLIN: I would agree with that exception,

9 but if we're talking about the crop plant itself.

10 DR. KRAMER: We're talking about the wild

11 relatives here.

12 DR. ROBERTS: Dr. Falk. You didn't even have to

13 signal. Your body language told me you wanted to speak.

14 DR. FALK: Because I'm so confused. Here is what

15 I wrote for number 6 in trying to put together what people

16 said. Most plants are resistant to most pathogens and

17 viruses, in parentheses. Resistance is the norm and

18 susceptibility is the exception. We had that in our

19 discussion.

20 Tolerance resistance and immunity to indigenous

21 pathogens, viruses are all present in wild populations in

1 many plants. The phenotypes of these range from
2 supporting virus replication with no symptoms, being
3 tolerance, to limited infection foci, being subliminal
4 infections in a single cell, that is in parentheses, to no
5 infection, immunity.
6 So that was --
7 DR. KRAMER: I think that gives us the
8 information that we need with the understanding from your
9 -- the latter half of that answer.
10 At the beginning you were using resistance with
11 a different definition that we had, which was clarified
12 later by essentially defining it as immunity.
13 DR. ROBERTS: Dr. Melcher.
14 DR. MELCHER: I am still confused now what level
15 you would like us to look at. Would you include now the
16 subliminal infections as something that you are interested
17 in?
18 DR. KRAMER: Well, actually I --
19 DR. MELCHER: Or field immunity that Dr. Cooper
20 mentioned?
21 DR. KRAMER: If what you are telling us is that

1 that is relevant for us to look at as well, then that is
2 useful information for us to know. Our initial questions
3 were not based on that.
4 DR. ROBERTS: Dr. Nagy.
5 DR. NAGY: I don't know whether this is actually
6 the subliminal infection is not irrelevant because
7 recombination can take place in single cells. So one
8 other possibility is that use Bocom's (ph) definition,
9 which instead of using immunity he likes to use extreme
10 resistance.
11 In this case it just would mean that we have
12 different levels of resistance and that might be helpful.
13 DR. ROBERTS: Dr. Melcher.
14 DR. MELCHER: Again, we're talking here about
15 the wild relatives and in the wild relatives -- well,
16 maybe the recombination is important. I'm sorry. It
17 probably is.
18 DR. ROBERTS: I think that it is clearly in the
19 minds of some of us on the panel or some on the panel that
20 may have been thinking again about a different definition
21 of resistance in crafting our answers than the definition

1 that the agency has.

2 We can make as a separate comment that in

3 addition to what I think in the minds of the panel members

4 is immunity, in addition to immunity they ought to also be

5 thinking about resistance or other forms, other levels.

6 But nevertheless, their question was asked with

7 resistance as some of us would define as immunity.

8 We need to be able to answer that question with

9 that definition and we need to maybe look back at our

10 response and make sure that our response is based on

11 mentally substituting the word immunity for resistance in

12 our response.

13 Dr. Sherwood.

14 DR. SHERWOOD: I have to reiterate what has been

15 said earlier, that most plants are not host of most

16 viruses. So I guess in answer to the question, the

17 prevalence, I would say the prevalence is probably high.

18 DR. ROBERTS: Dr. Tepfer.

19 DR. TEPFER: Maybe we're talking -- with wild

20 plants there are sort of two

21 types of phenomena that maybe we're mixing a little bit.

1 One is indeed most plants are non host for most viruses
2 and the other is that, in fact, expression of symptoms in
3 wild plants seems to be a rare fact.
4 So one can if you simply go and analyze wild
5 plants you can find viruses. Most often you had not seen
6 any symptoms. So that's a different type of behavior
7 completely. The plants are infectable, but simply they
8 are tolerant in the usual virologist terminology.
9 DR. ROBERTS: Let me leave it to the lead
10 discussants on the questions where tolerances and
11 resistance are mentioned in the question to think about
12 the panel's response and whether or not the panel response
13 makes sense if the word immunity were substituted for
14 resistance.
15 And if it doesn't or if you think the discussion
16 really was based on some other understanding of the
17 question, to let me know and we will reopen that question.
18 Does that sound reasonable?
19 Please let me do so at some point today before
20 we get to the end.
21 The next thing is I would like to revisit

1 question number 10.

2 In responding to question 10 toward the end of

3 our discussion, some members of the panel expressed their

4 opinion as to whether gene flow or introgression was

5 really something that we needed to be worried about.

6 I think what the agency needs from us to extend that

7 discussion is whether or not that is based on, those

8 opinions are based on speculation or whether there is

9 some sort of data or objective evidence to support that.

10 Because in order to make regulatory decisions,

11 they have to be able to point to some sort of scientific

12 finding as opposed to merely expert opinion.

13 So I would like to revisit this question. And

14 I'm going to give Dr. Kramer the opportunity also to sort

15 of articulate and perhaps Dr. Milewski to give us the EPA

16 perspective in terms of asking the questions about these

17 conditions and sort of what they need to use these

18 conditions in the way of support. Dr. Kramer.

19 DR. KRAMER: So yesterday I felt that the panel

20 was really answering whether these conditions are

21 sufficient and I pushed back a little bit to ask whether

1 they are actually necessary. And we got the answer from a
2 few people that made me think they are not necessary.
3 I wanted to push back on that answer yet again.
4 And just to say from our perspective, in order to
5 conclude that they are not necessary, we would like to
6 have you believe that gene flow is not a concern for any
7 virus in any plant under any circumstances.
8 Short of that, what are the conditions. And we
9 listed three possibilities that would help one to
10 differentiate between those PVCPs that might be a concern
11 and those that might not.
12 DR. ROBERTS: So with that clarification, Dr.
13 Stewart?
14 DR. STEWART: I would say that I would not even
15 go so far as to say that introgression is not a -- we
16 can't completely rule it out.
17 In my review, I brought up the case of sorghum
18 and Johnson grass as an example where I simply don't think
19 we should produce transgenic sorghum because of how nasty
20 weed johnson grass is.
21 Yesterday we heard about oat and wild oat.

1 That's another example where the wild host is a noxious
2 weed and it can -- viruses do play on it. So that's
3 another example. In my review in Nature Reviews Genetics,
4 I only looked at the top 10, I think, top 10 or 12 crops.
5
6 Oat is one of those crops that is being
7 cultivated less and less every year in the U.S., but still
8 a case that we wouldn't want to go there.
9 So the new plant list that we'll show expands
10 that top 12 or so many crops another notch. But you could
11 imagine all types of situations where gene flow or
12 introgression would be something that we would definitely
13 want to avoid. So I prefer to think about your conditions
14 as being sufficient or not.
15 DR. ROBERTS: Other opinions?
16 DR. KRAMER: Could I also interject again. If
17 there is any way to stimulate some discussion on those
18 exact conditions, maybe we got it exactly right the first
19 try, but there hasn't been a lot of discussion on how
20 those conditions might be edited. If there is any
21 thoughts on that, we would appreciate hearing them.

1 DR. ROBERTS: Sure. Could you go ahead and
2 project the conditions?
3 DR. KRAMER: I think that's the next slide.
4 Should I read them again.
5 DR. ROBERTS: Yes, just to give people a little
6 chance to think about them a little bit more while you are
7 reading them
8 DR. KRAMER: The conditions again.
9 Number 1, the plant into which the PVCP-PIP has
10 been inserted has no wild or weedy relatives in the United
11 States with which it can produce viable hybrids in nature.
12 For example corn, tomato, potato, or soybean.
13 Number 2, genetic exchange between the plant
14 into which the PVCP-PIP has been inserted and any existing
15 wild or weedy relatives is substantially reduced by
16 modifying the plant with a scientifically documented
17 method, for example, through male sterility.
18 Or number 3, it has been empirically
19 demonstrated that all existing wild or weedy relatives in
20 the United States with which the plant can produce a
21 viable hybrid are tolerant or resistant to the virus from

1 which the coat protein is derived.

2 DR. ROBERTS: Dr. Tepfer.

3 DR. TEPFER: In rereading the three conditions,

4 it seems to me that the weak link is the second of the

5 three. I think that the other two are relatively solid

6 because present technologies for preventing gene flow or

7 reducing gene flow are not 100 percent effective.

8 So there is a point of where you have to make

9 some sort of judgment as to whether the -- if the gene

10 flow does occur at a low level, what is the likelihood of

11 this having a severe impact. So your question in the end

12 of the day in asking what is acceptable risk, which is a

13 very difficult one that sort of escapes from the

14 scientific domain to some extent. But definitely the

15 second one is a leaky one.

16 DR. ROBERTS: Is there a way to fix that?

17 DR. TEPFER: There is a lot of research going --

18 this is Mark Tepfer again -going on to increase the

19 effectiveness of gene containment strategies. But as

20 Neal Stewart mentioned yesterday, these truly, you know --

21 really resistant and proven strategies are several years

1 down the pipeline probably.

2 So that is the weak point, but it is being

3 worked on.

4 DR. KRAMER: Do you think that Number 3 can

5 stand alone? Is there a way to maybe combine Number 2

6 with some other condition that would enable it to stand?

7 DR. TEPFER: Well, again, it becomes -- it's

8 not as absolute, because the third one is -- again, you

9 know, you are talking about tolerant or resistant.

10 Sometimes you can have a small degree of

11 additional resistance that can be added that could at

12 least in theory have an additional fitness effect. But

13 again, are we hair splitting.

14 Again, it becomes a question of well is this

15 worth talking about. And that's the kind of terrain I

16 don't feel happy on. Resistances may not be absolute.

17 If resistance in the wild relative is truly

18 immunity, to get back into that more as definitions, in

19 which the virus simply does not replicate and nothing

20 happens ever, then, I would feel fairly comfortable.

21 But that remains to be demonstrated in nearly all

1 the weeds I know of.

2 DR. ROBERTS: Dr. Stewart and then Dr. Sherwood.

3

4 DR. STEWART: I think Dr. Tepfer has -- that's a

5 great point and that we need to know more about what the

6 virus is actually doing in the wild relative before you

7 can make that call.

8 That's my only problem with Number 3. But just

9 to illustrate the bit of problem in Number 2 and why it is

10 really the weak link, and so if we're talking about a 1

11 percent introgression rate, which would be pretty high,

12 but a 1 percent introgression rate from sorghum to Johnson

13 grass or oat to wild oat and we can decrease that by 1,000

14 fold, well, your typical field in an acre is probably

15 going to have 60 thousand Johnson grass plants.

16 So in your 1 acre, you are still going to have

17 probably six hybridization introgression effects per acre.

18

19 If the virus is important for the wild host, if

20 virus resistance is important, with those types of numbers

21 you have to be more leak proof than what our current

1 technology can give us, and probably what the technology
2 will be able to give us in five years. I say I think 10,
3 20 years we'll be able to do much better by combining
4 mitigation strategies.
5 So to say that a terminator technology is going
6 to solve everything I think is a bit misleading for the
7 riskiest crops.
8 For crops that are less risky, that might be
9 enough just to put you over the top as far as on a
10 regulatory side.
11 DR. SHERWOOD: I think, though, that one can
12 empirically conclude that if the virus has not been
13 recently introduced or the weed has not been introduced,
14 and those two have shared an ecosystem for a while, that
15 that weed is either tolerant or resistant to the virus.
16 Because it is there.
17 And as viruses are obligate parasites, they
18 have to have a living host.
19 DR. ROBERTS: Dr. Hammond.
20 DR. HAMMOND: One of the problems that I
21 continue to have with this is that we seem to be

1 differentiating between resistance that is derived from a
2 transgene and any resistance that is naturally present in
3 the plant in either in the weed or in the crop and has the
4 potential to introgress from the crop to the weed.
5 Disease resistance have been used in crop plants
6 for a very long time. And have the opportunity to
7 introgress into the wild species.
8 And there is no good evidence that I'm aware of
9 that anybody has even looked for the introgression of
10 natural resistance genes from the crop to the host or been
11 concerned about possibility of that occurring.
12 But it is out there in many crops, and many of
13 them do have related wild and weedy species and there has
14 not appeared to be any concern in the past.
15 Why is there a concern now over resistance
16 induced by a transgene? I don't see the difference.
17 DR. ROBERTS: Dr. Cooper.
18 DR. COOPER: Could I make a suggestion. Most of
19 the movement of transgenes, it would be from the crop into
20 the wild plant, the wild plant potentially has a very long
21 life in the field.

1 The movement of genes from the wild plant into
2 the crop is likely to be hard to find because that crop is
3 very often an annual and you wouldn't need to think about
4 subsequent generations.
5 Any transfer would be hard to find. Of course
6 you could find it using modern techniques. And I proposed
7 yesterday that the brassica system allows that surrogate
8 system to be annualized.
9 We have specific genes in brassicas that have
10 been defined in molecular terms as to their chromosomal
11 positions, and those could be easily be monitored.
12 And they bring in another point which is
13 relevant to 3 here. The concept of a pathotype, where
14 they are usually pathotype specific, so they only work for
15 pathotype 3 or pathotype 1 of turnip mosaic.
16 And that, therefore, means that you have to have
17 regard not to the virus, but to a subset of that virus
18 which has particular pathological attributes, in
19 particular, genotype of hosts.
20 DR. ROBERTS: Dr. Stewart.
21 DR. STEWART: I think Dr. Hammond makes a really

1 good point on the difference between the transgene and
2 natural resistance. And I think there are a couple fine
3 points. I'm not sure that I agree with Dr.
4 Cooper on the wild relative being longer lived than the
5 crop.
6 They are probably going to be both annuals in
7 that case. The difference is that hybrids must -- if you
8 have hybrids, they must mate with the wild relatives every
9 year or every cycle in order for the transgene to persist.
10
11 Now, there is a lot of -- well, go back to what
12 is the difference. Well, much, I think, natural host
13 resistance is multigenic. And this is unigenic in many
14 cases.
15 It could be easier for the single gene to be
16 transferred rather than a number of genes in a number of
17 different loci. I think there has been a lot of work on
18 introgression.
19 But mainly on the other side from introgressing
20 natural plant resistance to crops. And that's very
21 difficult that takes an intensive breeding effort. And it

1 is usually regarded that going that way is easier than
2 going from crop to wild.
3 I don't know that I really answered anything at
4 all. But just as some background there. A lot of work
5 has been done on introgression.
6 DR. ROBERTS: Dr. Tepfer, but first let me
7 before we get too far, I think in order to answer this
8 question we just have to preface it by given that there is
9 interest in controlling gene movement and regulating that
10 for transgenic crops, to what extent do these conditions
11 allow the agency to exempt a particular material,
12 particular product?
13 What they are asking is: On a scientific basis,
14 how strong are these conditions. Are these conditions
15 going to be useful for them. And if not -- or if they
16 could be improved, how would we improve them.
17 DR. KRAMER: It is really to the point where we
18 can conclude that the product or the PVCP is of extremely
19 low risk.
20 DR. ROBERTS: Dr. Tepfer.
21 DR. TEPFER: I guess I was going a bit farther

1 down the same trail that Dr. Stewart was. So maybe I
2 should sort of defer those sorts of comments as being not
3 necessary for the present questions.
4 DR. ROBERTS: Are there any additional comments
5 on these conditions or suggestions on these conditions?
6 DR. KRAMER: Can I try to reiterate what I think
7 we have heard, not just today, but yesterday too.
8 DR. ROBERTS: Please.
9 DR. KRAMER: And that is for
10 condition number 1, that list can be expanded quite
11 considerably. I think we're going to talk later on today
12 about how exactly to expand that list.
13 But that condition as it stands would be a
14 necessary condition to have.
15 For condition number 2, that's perhaps not a
16 condition to have at this point at all. Perhaps something
17 anticipating future techniques that might be closer to 100
18 percent would be a possibility.
19 And the third condition would be necessary to
20 have. However, in light of the conversation yesterday,
21 probably very few things would actually qualify for this

1 given that from yesterday the way that this would have to
2 be demonstrated most likely would be through a manual
3 inoculation in which you are setting essentially a very
4 high bar for a product to be shown to be resistant.
5 DR. ROBERTS: Dr. Sherwood. Does that sound
6 correct?
7 DR. SHERWOOD: To add to the last one, that
8 known host range of all viruses is not known, so you would
9 be putting a person in a situation that they would have to
10 go and inoculate every plant in that environment to find
11 out whether it is or is not a host.
12 DR. ROBERTS: Dr. Hammond.
13 DR. HAMMOND: I think that the third condition
14 is too extreme because there is variability within
15 populations and susceptibility in some individuals and
16 resistance in others.
17 And you cannot prove that every individual or
18 every population has resistance. If resistance is
19 prevalent, that should be sufficient to meet condition 3
20 rather than every individual or every population of the
21 weedy or wild species having resistance.

1 DR. ROBERTS: Dr. Allison.

2 DR. ALLISON: May I just add on point number 2,
3 that while it sounds like a very good idea and we can look
4 forward to theoretically this barrier can be established,
5 that each one of the new techniques under consideration
6 has its own regulatory hurdles to pass.

7 So to be able to speculate that 5 years from now
8 or 10 years from now we'll be able to use these to curtail
9 any gene flow is not necessarily -- that time span is not
10 necessarily real.

11 DR. ROBERTS: Let's get back to the third
12 condition. Is the third condition impractical or is there
13 suggested, consensus suggestion that it should be
14 redefined or maybe both?

15 I was following up on Dr. Hammond's suggestion.

16

17 DR. HAMMOND: I would suggest that rather saying
18 all existing wild or weedy relatives to that resistance is
19 prevalent in the wild and weedy relatives. Because there
20 is never going to be a case where every individual or
21 every population has a high level of resistance.

1 Dr. Cooper talked yesterday about variability in
2 the populations of brassica with some individuals being
3 immune and others having tolerance and others being
4 susceptible. That is the case in most populations.
5 DR. ROBERTS: Then as a practical matter, do you
6 have to define an acceptable level of prevalence?
7 PANEL MEMBER: No, you can just delete the word
8 all from that sentence.
9 DR. HAMMOND: I'm not an ecologist of a
10 population geneticist. I don't know what would be a
11 reasonable level to define as prevalent. A population
12 geneticist could probably come up with a reasonable
13 definition of prevalent for that.
14 DR. STEWART: I guess I would add that this is
15 going to be pretty easy to define empirically because the
16 population genetics would favor tolerance or resistance if
17 it were available. Right?
18 Because this would be selected on. And
19 furthermore, if a species population, whatever taxon you
20 want to look at does have tolerance, you will also have a
21 good idea about the ecological niche that it is occupying.

1

2 So if you had nine plants out of 10 that were
3 tolerant, making that one out of 10 also tolerant by the
4 PVCP-PIP, it wouldn't have an effect on the plants, on the
5 plant population.
6 I think that's a great idea, just deleting the
7 all. I think that would be pretty easy to define. Any
8 company that wants to actually look at this is going to do
9 the field experiments anyway.
10 DR. ROBERTS: Are you interpreting all as
11 meaning all species or all individuals?
12 DR. STEWART: The way it is written now it is
13 all individuals.
14 What you are really talking about is the
15 species. Whether the species or the populations within
16 the species are mostly tolerant or not. Do they have it
17 or don't they.
18 DR. ROBERTS: Actually, I thought it was a
19 little ambiguous on that point.
20 DR. STEWART: Well, it is. I'm saying the way I
21 read it. You could include all existing wild or weedy

1 species, wild relatives or weedy species.

2 DR. KRAMER: If I could clarify. When we wrote

3 this we meant that the trait existed within the

4 population. Not that every individual within every

5 population.

6 Maybe you could tell us how we might clarify

7 that in the language there.

8 DR. ROBERTS: Dr. Hammond.

9 DR. HAMMOND: I would rewrite that to say that

10 tolerance or resistance to the virus is prevalent in the

11 populations of all wild and weedy relatives.

12 DR. ROBERTS: Dr. Sherwood.

13 DR. SHERWOOD: I would like to change prevalent

14 to just found. That way you don't have to worry about

15 getting into the population genetics and percentage.

16 DR. ROBERTS: Dr. Tepfer.

17 DR. TEPFER: I suggest adding sexually

18 compatible wild or weedy relatives. Because you will have

19 -- the others you are not concerned about, the more

20 distant ones.

21 DR. KRAMER: We have, for which the plant can

1 produce a viable hybrid. Is that something different from
2 what you are saying? Okay.
3 Do I understand is there agreement to use to the
4 term found and would that -- can that be then defined? Is
5 that one instance?
6 DR. ROBERTS: They are sort of nodding. But
7 let's see if we can get some verbal responses to your
8 questions.
9 DR. STEWART: Found or how about observed?
10 DR. SHERWOOD: Observed is quite acceptable.
11 DR. ROBERTS: Dr. Sherwood says that's
12 acceptable.
13 DR. KRAMER: In this case we are talking about
14 immunity.
15 DR. SHERWOOD: We're talking about tolerance.
16 DR. KRAMER: Tolerance and/or immunity. Right.
17
18 DR. ROBERTS: Is there a different opinion among
19 panel members on that?
20 DR. KRAMER: Just to clarify. If we're talking
21 about finding tolerance and/or immunity within a

1 population, is that within all populations of the species
2 or anywhere within the species?
3 DR. ROBERTS: Dr. Sherwood.
4 DR. SHERWOOD: Anywhere within the species. I
5 thought the kind of consensus was that we're looking now
6 at -- you are going to have to look at individual plants.
7 And if you see an individual plant that has tolerance or
8 immunity, it would then pass this bar.
9 DR. ROBERTS: Dr. Cooper.
10 DR. COOPER: Could I contribute the fact that if
11 you have tolerance or immunity detected in one of the
12 southern islands on the extremes of the continental USA,
13 it may be very geographically and practically isolated in
14 a genetic sense from the mainland of the USA. I think
15 that is a real constraint on decisionmaking.
16 DR. ROBERTS: Not to answer for the agency, but
17 presumably they would take that into consideration in
18 making a management decision on that, but Dr. Hammond and
19 then Dr. Tepfer.
20 DR. HAMMOND: I think that a single population
21 wherever it is would not be sufficient. But it is not

1 realistic to examine all populations because for one thing
2 you would have to do an extensive survey to find out where
3 all the populations are.
4 It would be reasonable to survey populations
5 scattered in the areas where the crop is expected to be
6 grown.
7 The populations at the extremes are by
8 themselves unlikely to be informative or useful. It is
9 where the crop will be grown. But it needs to be more
10 than one population. DR. STEWART: And fewer
11 than all.
12 DR. HAMMOND: Many fewer than all.
13 DR. ROBERTS: That certainly narrows it down.
14 Dr. Tepfer.
15 DR. TEPFER: So we are going someplace where it
16 is more than a single observation of tolerance or
17 immunity.
18 So we are having to observe it in several
19 populations. Is that where we seem to be going -- in the
20 area where the crop is going to be cultivated. It sounds
21 like that's the sort of direction we're going in.

1 DR. ROBERTS: Dr. Sherwood.

2 DR. SHERWOOD: I have a real problem with a

3 pragmatic implementation of such a guideline. How far in

4 terms of miles or kilometers are you going to find putting

5 a crop in a new area. There is constant shifting of what

6 crops are grown in the United States and parts of the

7 United States. Certainly one thing we encourage

8 our producers to do is rotate crops around different

9 portions of their farms which may be scattered over

10 several different miles.

11 DR. ROBERTS: Dr. Hammond.

12 DR. HAMMOND: I would think a practical solution

13 would be to suggest within the state multiple populations

14 from within any state where the crop is expected to be

15 grown. But certainly not to go down to the county level

16 or the farm level.

17 That would be ridiculous.

18 DR. ROBERTS: Dr. Sherwood.

19 DR. SHERWOOD: Again, I would have problems with

20 that. Just you have shifting agricultural practices. For

21 me, for example, it's north Florida and south Georgia.

1 If they are a farmer producing in north Florida
2 one season and going to their farm in south Georgia the
3 next season, they are going to have to go through and do a
4 survey in order to produce the same crop that they just
5 produced across the state line the last year.
6 DR. ROBERTS: Dr. Stark.
7 DR. STARK: I have another problem with this
8 statement, number 3. It says, it has been empirically
9 demonstrated. Who does this demonstration? Where is the
10 onus? Who is responsible for demonstrating that wild or
11 weedy species have this tolerance?
12 DR. ROBERTS: Dr. Kramer.
13 DR. KRAMER: It would be the product developer.
14
15 DR. STARK: Again, you get to the point how do
16 you define wild and weedy populations and their extent and
17 range and all this.
18 In other words, if I was producing a genetically
19 engineered crop and I had to come to the EPA and
20 demonstrate that wild and weedy relatives have this
21 tolerance, what are the limitations of this?

1 If I came and said, well, we went into Alabama
2 and we are in a specific area we plan to grow this crop
3 and we have sampled around the crop borders, is that
4 sufficient? The way this is worded to me is is
5 an impossibility.
6 I don't see how anyone could ever come to you
7 with the right amount of data to meet that statement,
8 empirically demonstrated tolerance in these species.
9 DR. KRAMER: Are you disagreeing then with Dr.
10 Hammond's suggestion of how to sample a population of few
11 populations within each state where the crop is grown?
12 DR. STARK: I'm not saying that. I'm saying the
13 way it is written doesn't define anything. It is very
14 open ended.
15 DR. KRAMER: I think it is helpful to have the
16 suggestions from the panel on how it might be defined in a
17 practical and meaningful way.
18 I actually think that we have gotten an answer
19 from Dr. Hammond if everyone is in agreement with that
20 suggestion.
21 DR. ROBERTS: I'm not sure that's the case.

1 DR. SHERWOOD: I'm not in agreement with the
2 statement.
3 DR. KRAMER: And your suggestion would be that a
4 single occurrence in any population would be sufficient?
5 DR. SHERWOOD: Yes.
6 DR. ROBERTS: Dr. Allison.
7 DR. ALLISON: With the variations in geography
8 of different states, I think to be able to pick a number
9 or something for a particular state is unreasonable.
10 Some states are -- the populations may be quite
11 uniform, while in others the population is going to be
12 tremendously variable.
13 And to make a decision on tolerance or immunity
14 or resistance, it is going to be different -- the sampling
15 would be different in different states.
16 DR. ROBERTS: Dr. Stewart.
17 DR. STEWART: You could take it away from the
18 state distinction to a more of a regional distinction and
19 leave that with the agency to define further. I would
20 hate to define something as it has been said so
21 stringently that no one can meet even though it is

1 reasonable.

2 DR. ROBERTS: Dr. Hammond --

3 DR. STEWART: I would also say that a situation

4 in Virgin Islands might not be indicative of south Georgia

5 also as far as population biology.

6 So I think a regional definition might get

7 around that.

8 DR. ROBERTS: Presumably, the product can be

9 registered in different regions. Is that right? You can

10 specify or is it when it is registered it is registered

11 everywhere?

12 DR. KRAMER: There are certain restrictions that

13 can be placed on a product registration and exemption, but

14 likely not have such restrictions.

15 DR. ROBERTS: Good point. Dr. Hammond.

16 DR. HAMMOND: I think a regionally

17 representative population is probably the most practical

18 way to go. For many of these species, just getting hold

19 of seed from different populations is going to be a major

20 effort.

21 There are not seed banks for weed populations

1 that I'm aware of that are held in many places. I think
2 that John Sherwood is right and it does have to be
3 restricted to what is reasonable and not to have to send
4 somebody out at various times of year to collect seed
5 populations from different places. That poses an
6 unreasonable burden.
7 I think it would have to be a limited number of
8 populations from a limited number of regions to be
9 actually a practical solution to be able to achieve.
10 The burden could otherwise be easily pushed way
11 too high to allow anybody to reach it.
12 DR. ROBERTS: Dr. Kramer, I think all the
13 concept is clear, crafting the language for this condition
14 is challenging.
15 DR. KRAMER: I think we have gotten a lot of
16 helpful information from the panel for this question.
17 DR. ROBERTS: Thank you. Let's then move -- Dr.
18 Sherwood.
19 DR. SHERWOOD: Something you might want to look
20 into is find out how weed populations differ with the USDA
21 climate zones for plants. That might be a way to going

1 about finding regions, is use of climate zones.

2 DR. ROBERTS: Thank you, Dr. Sherwood, for that

3 suggestion. Let's go to question 11. Before we start

4 that, I just want to point out that Dr. Gendel mentioned

5 yesterday about previous SAP deliberation on issues

6 associated with a similar issue.

7 And what has been distributed to you is the

8 report from the previous SAP. So that may provide some

9 helpful foundation for our discussions today.

10 Dr. KRAMER: So question number 11 --

11 DR. ROBERTS: Let's go to question number 11.

12 DR. KRAMER: To what extent are novel viral

13 interactions, for example, recombination, heterologous

14 encapsidation involving a viral transgene an environmental

15 concern.

16 I would like here to also read the definition of

17 novel viral interaction.

18 This is found in the appendix. Novel viral interaction

19 means an interaction between portions of two or more

20 different viruses. For example, through

21 recombination or heterologous encapsidation, not expected

1 to occur in a mixed viral infection found in nature.
2 DR. ROBERTS: Dr. Tepfer, could you lead off our
3 discussion on this.
4 DR. TEPFER: I have written a little bit of
5 text. I will read it into the record to start. It
6 doesn't really cover all the questions that are raised,
7 but I wanted to start with this at least.
8 The degree of potential risks associated with
9 heterologous encapsidation are quite different from those
10 associated with recombination. The former being of less
11 concern for several reasons.
12 First, in many cases, viruses that are closely
13 enough related for heterologous encapsidation to occur are
14 transmitted by the same vectors and thus the phenomenon
15 would have no effect on vector transmission.
16 Second, if unexpected unwanted effects due to
17 heterologous encapsidation were observed, damage could be
18 limited by simply ceasing to grow the plant variety in
19 question. The problem is reversible.
20 Third, there are excellent means of mitigation
21 which make it possible to simply render the question moot.

1 For these reasons, the comparison or the aptitude for
2 heterologous encapsidation to occur in transgenic and
3 nontransgenic plants is of little importance.
4 In contrast, at least in theory, impact of
5 recombination could be much greater since there is no
6 abundant bioinformatic evidence that recombination has
7 indeed as had been long suspected played a key role in the
8 emergence of new viruses.
9 In the world of human health, for analogous
10 reasons, there is great concern about the potential
11 outcome of recombination between human and avian influenza
12 viruses.
13 However, it can be argued that the important
14 point is not the relative likelihood for recombination to
15 occur, but rather whether recombinants in transgenic
16 plants are different from those in nontransgenic plants.
17 In order to determine if novel events could
18 occur, the situation in nontransgenic plants must be
19 studied carefully, which has not been done very often.
20 We are currently addressing this by comparing
21 recombinants in transgenic and nontransgenic plants. This

1 is actually getting redundant.

2 One part of this has involved mapping several

3 hundred recombination sites between CMV and relative TAV

4 across the viral RNA 3 which includes the coat protein

5 gene in nontransgenic plants. And we have an article in

6 press on this subject.

7 As expected, there were hot spots for

8 recombination. At the majority of sites, precise

9 homologous recombination was observed. However, hot spots

10 for other types of recombination, homologous, imprecise,

11 and aberrant recombination were also observed.

12 Similar experiments are underway using transgenic

13 plants, but is too soon to present the results.

14 However, it is striking to note when the same

15 templates and same primers were used with the risk

16 transcriptase invitro, very similar assortment of

17 homologous precise recombinants was observed, but none of

18 the imprecise or aberrant sort. And here also there is an

19 article in press.

20 Considering the great biochemical differences

21 between the RNA viral replicase and a risk transcriptase,

1 this suggests that the determinance for homologous precise
2 recombination would apply to a very wide range of
3 situations.
4 And that at least for this type of recombination
5 nothing novel would be expected in transgenic plants. The
6 probable outcome of these experiments is likely to be that
7 the recombinants in transgenic plants would be similar to
8 those in non transgenic plants. Nonetheless,
9 it has been noted that at least in the biological system
10 that we are using, which is the cucumo viruses,
11 recombinants that have a selective advantage relative to
12 the parental strains have been observed.
13 Can be obtained. This is article by Fernandez-
14 Quartero, et al., and there is also one from, I think,
15 Ding's group. In addition in unpublished work from Zak
16 Mol's (ph) lab, it was shown that one could obtain cucumo
17 viral RNA through recombinants that induced worse symptoms
18 than the parental viruses.
19 If you look at those two sorts of features, it
20 would seem in this particular case -- these recombinants,
21 however, were outcompeted by the parentals in

1 coinoculation experiments. So they would not be expected
2 to persist.
3 If you look at sort of these two type of
4 results, one, that cucumo virus with recombinants with
5 selected advantage can be produced and that we can also
6 observe viruses with worse symptoms, this does sort of
7 suggest there is an evolutionary space for recombinant
8 cucumo viruses with properties at least somewhat worse
9 than those presently known.
10 DR. ROBERTS: Thank you, Dr. Tepfer. Dr.
11 Melcher, what do you have to add?
12 DR. MELCHER: Probably not much. But I could
13 underscore the comment of Dr. Tepfer that recombination is
14 not likely to be a novel event in the case of a transgenic
15 plant being infected by a virus, that recombination events
16 have happened throughout the evolution of viral history.
17 You just have to consider what the structure of
18 viral genomes are. They are a assemblage of different
19 modules.
20 A protein -- a gene that would coat, for
21 example, a movement protein could be next to a variety of

1 different kinds of proteins involved in DNA replication.
2 Could be involved next to a gene flow reverse
3 transcription for RNA polimeraeces (ph) from different
4 super families of RNA polimaraeces, next to a replication
5 protein case of the gemini viruses. This movement protein
6 could be on the other hand also associated with a variety
7 of different types of coat proteins. Coat
8 proteins for isometric viruses and coat proteins for
9 helical viruses, filamentous and rod shaped viruses.
10 There has been scrambling through evolution of these genes
11 all the time.
12 And anything that could happen in a transgenic
13 plant is probably very unusual and unlikely. Of course,
14 there are time scales here involved.
15 The evolution that I have just been talking
16 about is probably over many, many years. And you may be
17 worried about more limited time scales.
18 The only case where this might be a problem, I
19 think, and Dr. Kramer introduced to us yesterday already
20 is i fa transgene containing plant is planted in an area
21 where it encounters a virus that the virus that is part of

1 the transgene never encounters in nature, in different
2 region or if that transgene is expressed in a part of a
3 plant that the virus from which it comes is not normally
4 expressed.
5 So those are possibilities. If they are there,
6 then I'm sure recombination will happen with some
7 frequency. It can happen frequently even without
8 selection. So that's relative to the recombination issue.
9 And trans encapsidation is pretty much a dead
10 end like you said. So I guess maybe I should quit there.
11
12 DR. ROBERTS: Thanks, Dr. Melcher. Dr.
13 Sherwood.
14 DR. SHERWOOD: Really not much to add. Just to
15 reemphasize Rick's comment about recombination occurring
16 on a regular basis. And the viruses we detect today are
17 probably the most fittest. That's why we detect those
18 particular species.
19 And they do exist. As was said earlier, most
20 viruses exist as a quasi species, so there is always a
21 certain amount of variation in the population.

1 DR. ROBERTS: Comments from other panel members.
2 Dr. Hammond.
3 DR. HAMMOND: Neither recombination,
4 heterologous encapsidation or synergy are novel virus
5 interactions unless viral genes are deployed in plant
6 species which are not susceptible to the virus.
7 There is little justification for the use of
8 virus constructs in crops in which the virus does not
9 infect. Constructs from the cognate virus should be used
10 instead. Viruses do and will evolve by
11 recombination most typically between isolates of the same
12 virus. However, recombination between viruses and
13 different taxonomic groups also occurs and plays a role in
14 virus evolution.
15 Recombination has been demonstrated between
16 viruses and related transgenes, and most recombinants will
17 be similar to the parental viruses whether resulting from
18 recombination between two viruses in a mixed infection or
19 between a virus and a transgene.
20 There is little a priori reason to believe that
21 recombinants between viruses and transgenes will be more

1 of a problem than recombinants between two viruses
2 infecting the same plant unless transgenes are derived
3 from severe or exotic isolates.
4 As the general recommendation has been to use
5 mild endemic isolates as the source of the transgene, this
6 should minimize any potential for creation of novel
7 isolates that would not equally or easily arise from
8 naturally mixed infections. And mixed infections are
9 common in both wild plants and in agricultural situations.
10
11 Heterologous encapsidation also occurs naturally
12 in mixed virus infections as well as in coat protein
13 expressing plants. Heterologous encapsidation in
14 transgenic plants has very limited probability of course
15 in interactions different from those that will occur
16 naturally and will typically be limited to a single
17 passage.
18 In contrast, when heterologous encapsidation
19 results from a mixed infection, it is highly likely that
20 both viral genomes will be transmitted to the new host
21 allowing further interactions.

1 Indeed this is probably the only significant way
2 in which potato virus C, an aphid non transmissible
3 derivative of potato virus Y, can spread within a crop.
4 Use of viral genes that are responsible for
5 synergy with other viruses is not recommended. But in any
6 event, farmers will soon abandon any transgenic variety
7 which was more susceptible to other varieties.
8 Synergy would also be restricted to the
9 transgenic plant and is unlikely to have a significantly
10 greater effect than in a mixed infection.
11 DR. ROBERTS: Dr. Zaitlin.
12 DR. ZAITLIN: In order for interactions to take
13 place, the two viruses must be in the same cell. And it
14 is known that in many cases of mixed infections the
15 viruses exclude themselves. They are not in the same
16 cell.
17 However, if you had a transgene and a super
18 infecting virus, you then would have the possibility that
19 they would, in fact, be in the same cell. But as
20 enumerated here, that probably doesn't make this more of a
21 concern.

1 DR. ROBERTS: Dr. Falk.

2 DR. FALK: In response to Professor Zaitlin.

3 When viruses are excluding each other, are you talking

4 about related viruses as opposed to unrelated viruses?

5 DR. ZAITLIN: The cases which have been

6 demonstrated for mutual exclusion were done a long time

7 ago in protoplast usually with related viruses. If they

8 are put into the cell at the same time, they can both

9 replicate.

10 But if you have one in there and then try to

11 infect with another, these are protoplast experiments, you

12 then cannot get virus -- you can get the virus in

13 together. But not subsequent -- unrelated viruses it

14 doesn't apply.

15 DR. ROBERTS: Dr. Tepfer.

16 DR. TEPFER: I wanted to say there is more

17 recent evidence that a similar phenomenon exists between

18 related viruses in whole plants. There have been two

19 papers, one that came out last year with poty viruses, one

20 that has just come out cucumo viruses showing that in non

21 transgenic plants when you have these two related viruses

1 together they do tend to exclude each other.
2 For instance, particularly with the poty virus, it very
3 beautifully shown with flourescently marked, viruses that
4 coat for GFP or DS red, that, in fact, the two viruses
5 only existed in a single sort of layer of cells between
6 the patches were singly infected.
7 So this phenomenon is apparently quite real in
8 plants as well.
9 DR. ROBERTS: Other comments or areas of
10 agreement or disagreement. Dr. Melcher.
11 DR. MELCHER: Just for historical record, I
12 think this is not new information. The whole basis for
13 the technology we're looking at is viral cross protection,
14 which is inoculation of a plant with one isolate of the
15 virus and that protects it against infection by related
16 isolate of the virus that's usually more severe.
17 So that's old news, I think.
18 DR. ROBERTS: Any other comments on this
19 question? Dr. Kramer. Do you want a clarification or a
20 follow up question.
21 DR. KRAMER: I had a couple things. I wanted to

1 go back to Dr. Tepfer's original response in which you
2 mention the idea that mitigating factors can be
3 incorporated into the construction of a construct.
4 And I wanted to say that this question is really
5 asking absent any type of mitigating factors. So I want
6 to make sure that your answer for heterologous
7 encapsidation would stand even given that.
8 DR. TEPFER: In the absence of mitigating
9 factors which are commonly built into transgenes, then, of
10 course, I think that there are a few cases where you
11 wouldn't want to perhaps use full length coat proteins in
12 certain luteo viruses that can be health risk for umber
13 (ph) viruses, for instance.
14 Again, it is a case by case sort of a situation.
15 There are a few situations where without mitigation I
16 would feel a bit uncomfortable.
17 The reason I mentioned that usually this is not
18 a problem is that often when you have heterologous
19 encapsidation it is between closely related viruses that
20 are transmitted by the same vectors anyhow.
21 Again, John Hammond mentioned the case of potato

1 virus c. It is another situation where the virus
2 apparently requires assistance from a second virus. But
3 those are rather rare circumstances. But those would be
4 ones where mitigation would be required.
5 Whereas, the others, if you are just talking
6 about two related viruses that are transmitted by the same
7 aphids, then really there is no problem that is really
8 perceived.
9 DR. FALK: I think that in the specific argument
10 about heterologous encapsidation that you just asked Dr.
11 Tepfer, I don't think that there are going to be novel
12 interactions resulting from the transgene that would be an
13 environmental concern.
14 DR. ROBERTS: Dr. Melcher.
15 DR. MELCHER: I have the same opinion. But I
16 think I want to address what some people argue as a hazard
17 of the trans encapsidation.
18 That is when this trans encapsidated virus gets
19 into a host species that it normally doesn't, it may then
20 be transmitted by another vector that has a specificity
21 for that second plant and thus it could be propagated into

1 several different species that it normally would not have
2 been. I think this is not a novel interaction
3 because I think this has happened. There are some viruses
4 that have very wide plant host ranges and probably the
5 reason they do is they are transmitted by multiple vectors
6 and it has happened before. I don't think it is
7 a novel interaction, but it is something that has been
8 brought up, I think, at least in some of the reading that
9 I was given.
10 DR. ROBERTS: Dr. Kramer, do you have some
11 follow up.
12 DR. KRAMER: I had another question. I have the
13 sense that the panel isn't quite answering this question
14 and that is that what I'm hearing from a number of people
15 is saying that in most circumstances you are not going to
16 get a novel viral interaction.
17 And therefore it isn't a concern except -- and
18 then there is the list of a couple factors that I laid out
19 yesterday that people are bringing up, if you plant the
20 plant in an area where the virus doesn't exist.
21 And that's really where this question starts

1 given that you have that circumstance. To what extent do
2 you have an environmental concern. If the answer is, but
3 that's so rare, you have no environmental concern because
4 novel interactions never arise even in those
5 circumstances, I want that to be on the record.
6 But barring that, the question really is
7 beginning, assuming that you do have a novel viral
8 interaction through one of these routes that have been
9 discussed, what concern do we have then?
10 DR. ROBERTS: Dr. Sherwood.
11 DR. SHERWOOD: I do not think you would have any
12 more concern than deploying traditionally bred virus
13 resistant plants in an area where the disease does not
14 occur and you have a strain of virus that overcomes that
15 resistance.
16 So any time you put resistance out there you
17 begin selecting for the virus to overcome it.
18 So I don't see why that has any more
19 environmental impact than does the use of transgenes.
20 DR. KRAMER: I guess because there isn't the
21 opportunity in natural bred virus resistance to have these

1 sort of interactions between two different viral genomes.
2
3 That's what this question is getting at. Not
4 the opportunity to overcome resistance, but the
5 opportunity for recombination and heterologous
6 encapsidation. DR. ROBERTS: Dr. Falk.
7 DR. FALK: You mentioned specific examples, but
8 I would say that those specific examples that have been
9 mentioned still do not provide any novel possibilities.
10 And the specific examples you mentioned, I think
11 -- the only one I remember is that the triple gene
12 resistance in squash basically and then --
13 DR. KRAMER: I could go through the list again
14 if that would be helpful.
15 DR. FALK: Okay.
16 DR. ROBERTS: Would you please.
17 DR. KRAMER: We can flip up the slide. It is
18 one of the last ones in my discussion from yesterday.
19 DR. ROBERTS: Dr. Zaitlin.
20 DR. ZAITLIN: I think the one example that has
21 been already mentioned here is the study of Thomas with

1 potatoes in which they took resistant potatoes, transgene
2 resistant potatoes, tried to super infect with other types
3 of viruses to see whether novel ones would arise.
4 And I think they found in that study, which is
5 limited, that they couldn't detect any.
6 DR. KRAMER: Let me just go through them. The
7 first example was transgenic multi resistances. This
8 wouldn't necessarily lead to novel viral interaction, but
9 the idea being if you had, for example, a virus resistance
10 trait stacked with an herbicide tolerant trait, that crop
11 could be planted in an area for the herbicide tolerance
12 trait, but may not necessarily be actually infected by the
13 virus.
14 The second example is if you had heterologous
15 resistance, which I think Dr. Hammond mentioned again
16 today, that you actually were conferring resistance to a
17 virus by using the coat protein from a different virus and
18 you may not actually have infection by that virus in the
19 plant.
20 The third was the use of an exotic strains coat
21 protein. Actually perhaps intentionally in order to try

1 to stave off a new infection from that virus from outside
2 if you are concerned about it spreading to a Hawaiian
3 Island, for example.
4 The fourth would be expression of new cells or
5 tissues by the use of different promoter that could allow
6 a virus to interact with viruses that don't normally
7 infect the cells that that virus naturally infects.
8 And the final one would be through just simple
9 alterations in the coat protein such that you have created
10 something that actually doesn't exist in nature at all.
11 Really, the question is getting at, I mean, I
12 guess there has to be two parts. If there is no consensus
13 that these circumstances could lead to novel interactions
14 or that these circumstances are so rare that they would
15 actually never occur, then that should be put into the
16 record.
17 But if there is any agreement that through any
18 of these you could get a novel interaction, the question
19 is really asking in those circumstances is there an
20 environmental concern.
21 DR. ROBERTS: With that clarification, Dr. Falk.

1

2 DR. FALK: Then I stand by my statement, which,
3 no, I don't think there is any opportunity for any real
4 world opportunity for novel interactions to occur that
5 would be of any significance.
6 DR. ROBERTS: Dr. Melcher, do you want to weigh
7 in on this?
8 DR. MELCHER: I guess so. Yes. These
9 situations will occur and there may be a recombination
10 event that. That recombination event will very likely
11 either produce a viral genome that does not function and
12 will not go anywhere or it will produce a viral genome
13 that has already been explored during evolution sometime,
14 somewhere.
15 And therefore is not likely to be a novel
16 product. The event may be somewhat novel that doesn't
17 usually occur, but somewhere or other it probably has
18 occurred.
19 DR. ROBERTS: Dr. Sherwood.
20 DR. SHERWOOD: I would like to agree with Dr. Melcher.
21 DR. MELCHER: I should mention that Dr. Sherwood

1 works on viruses that are a very good example of this. It
2 is got one of these movement protein genes, but all the
3 rest of the genes are genes that you find normally in
4 animal viruses.
5 The bunya viruses.
6 DR. SHERWOOD: People have tried using cross
7 protection with tospo viruses. And Dennis Gonzales had
8 very little luck.
9 But there is an example where you look at the
10 genome of tospo viruses. It is in bunya verity that are
11 primarily animal or people infecting viruses.
12 But somewhere along the line it picked up a
13 movement -- a protein that allows it to move from cell to
14 cell while all the other bunya verity do not have an
15 analogous protein.
16 DR. ROBERTS: Dr. Tepfer.
17 DR. TEPFER: I want to object a little bit. I
18 think that I never feel very comfortable with the idea
19 that all the possible combinations have been tested in
20 nature.
21 It is based on the assumption that we're at some

1 sort of evolutionary equilibrium. I think human activity
2 in terms of moving plants, moving viruses, moving vectors
3 from one location to the next has been extremely important
4 in bringing things in contact which had not occurred
5 previously. I would suspect if we really were
6 at evolutionary equilibrium there would be no emergent
7 viruses in any case because they would all have been
8 tested.
9 So I think we just need -- I would prefer to
10 back off a little bit from this idea that everything has
11 already occurred in nature.
12 DR. ROBERTS: Any other viewpoints? Dr. Falk.
13 DR. FALK: I would still argue that it doesn't
14 present any novel risk.
15 DR. KRAMER: The question is, is there any
16 environmental concern?
17 DR. ROBERTS: Dr. Falk
18 DR. FALK: Novel viral interactions that would
19 present an environmental concern and I would say no.
20 DR. ROBERTS: Dr. Cooper.
21 DR. COOPER: At the risk of being provocative, I

1 would say it is premature to make that assessment. We
2 really don't have any evidence on which to make it yet.
3 At least that would be my opinion. You may be
4 right, but you may be wrong.
5 DR. ROBERTS: Dr. Kramer, it appears the panel
6 is divided on this.
7 DR. KRAMER: It does. Maybe if Dr. Falk could
8 expand a little bit on what he is basing that decision on.
9 I would also like to push back on a couple of the people
10 before who were caveating earlier statements that under
11 certain circumstances as long as you plant the product in
12 the area where the virus naturally infects.
13 It seems people have backed off of that. I want
14 to clarify whether they have or not.
15 DR. ROBERTS: I think Dr. Falk you were asked to
16 clarify.
17 DR. FALK: Of the examples you mentioned, I
18 don't see that those represent novel situations where we
19 will get significant new effects.
20 DR. ROBERTS: Dr. Zaitlin.
21 DR. ZAITLIN: I would like to support that.

1 Because once again it is this idea of fitness. Novel
2 virus would have to be more fit than the one from which it
3 is derived or ones from which it is derived.
4 There are many examples as pointed out from Dr.
5 Sherwood that these viruses are really populations of
6 viruses. There are many strains contained within them.
7 They are lurking around, but they don't get to express
8 themselves because the most fit strain is the one that
9 predominates.
10 DR. ROBERTS: Dr. Sherwood.
11 DR. SHERWOOD: I hate to say this, but my
12 colleagues on this side of the isle the major impact, I
13 think, it's going to be on increasing the diversity of
14 viruses is just continuation of modern agricultural
15 practices and that this is not going to pose any greater
16 risk than the deployment of any other management practice
17 or resistance genes or cross protection or any other thing
18 that we have done in trying to prevent disease in crops.
19 DR. ROBERTS: Dr. Falk.
20 DR. FALK: I agree with what Dr. Sherwood said.
21 And I think that Dr. Tepfer also pointed out the biggest

1 problem, I think, we have in new viruses is the activity
2 of human beings and what we have moved around.
3 DR. ROBERTS: Dr. Hammond.
4 DR. HAMMOND: I essentially concur with what has
5 recently been said. I think that there is little
6 probability of novel interactions. The interactions can
7 occur readily in mixed infections and any possible novel
8 interactions I think are as minimal significance.
9 DR. KRAMER: For the second part of my question
10 that would be under all circumstances. There is no caveat
11 to that statement anymore about planting the product in
12 the area where the virus infects?
13 DR. ROBERTS: Dr. Sherwood.
14 DR. SHERWOOD: I would say no more greater risk
15 to the general population as getting in a car and driving.
16
17 DR. ROBERTS: I'm not sure that's very
18 reassuring, Dr. Sherwood.
19 Dr. Melcher.
20 DR. MELCHER: I guess I should compromise a
21 little bit and say yes there should be some caveats that

1 can be very easily put in, which I guess we'll be talking
2 about later.
3 That is if you are using a transgene from a
4 virus that is limited to certain tissues, when you make
5 this transgenic plant you should not use a promoter that
6 will express it all over.
7 You should have it just in being expressed in
8 the tissues in which it exists.
9 DR. KRAMER: I realize we're going to get to
10 that a little bit later, but the question is why would we
11 bother doing that if nobody is suggesting that there is
12 any environmental concern at all. So when we get to that
13 question, are we going to be jumping back to this question
14 here to try to understand why any of those mitigating
15 things might be suggested.
16 DR. MELCHER: We're finding that not everybody
17 is in agreement with this. So -- alright --
18 DR. ROBERTS: Dr. Kramer, I think -- Dr. Hammond
19 can speak in just a minute. But this is probably going to
20 be one of those things where there is not a consensus
21 among the panel and there are going to be some differences

1 of opinion.

2 DR. HAMMOND: I think we do those things that

3 Dr. Melcher was just addressing to stave off perceived

4 risks not because we consider that they are real risks.

5 DR. ROBERTS: Dr. Zaitlin.

6 DR. ZAITLIN: Putting an impossible burden to

7 say there is no possibility at all, none of us here would

8 say that that's -- that there is no possibility at all.

9 But as enumerated here, the probability of this happening

10 is very very low.

11 DR. ROBERTS: I think Dr. Kramer -- it wasn't so

12 much no possibility, but in sort of -- that the answers

13 seem to be conditional. Under certain sets of conditions

14 we're not concerned. And I think what she was trying to

15 elicit if you throw out all the conditions and just in

16 principle would you be concerned about this.

17 We seem to be getting -- the panel seems to not

18 have a consensus answer on that.

19 Let's then move to question 12.

20 DR. KRAMER: What conclusions can be drawn as to

21 whether the likelihood of recombination and/or

1 heterologous encapsidation would be increased or decreased
2 in a transgenic plant compared to its non bioengineered
3 counterpart.
4 DR. ROBERTS: Dr. Allison.
5 DR. ALLISON: Thank you. If I may read this
6 into the record, please.
7 Viruses have gone through years of evolutionary
8 refinement and adaptation to their host. During this time
9 they have maintained evolutionary flexibility by
10 preserving their capacity for RNA recombination, high
11 mutation rates through an error prone replicase and
12 reassortment in the case of multi component viruses.
13 Comparisons of viral nucleotide sequences of
14 different viruses have identified blocks of similar
15 sequences. These blocks are considered evidence of
16 previous recombination events. Such recombination events
17 most likely occurred during mixed infections.
18 While mixed infections are frequently viewed as
19 providing extensive recombination opportunities, viral
20 reproductive isolation maybe greater than envisioned.
21 Viruses do not constantly replicate within an

1 infected plant cell. Rather, the infection spreads from
2 cell to cell.
3 As replication in one cell is completed, some of
4 the newly replicated RNA is warehoused in a form of
5 virions and the active infection spreads to adjacent cells
6 where the process is repeated and the infection spreads
7 throughout the plant.
8 Trans encapsidation and recombination events
9 recorded in nucleotide sequences of different viruses
10 suggest that two or more viruses replicate within the same
11 cell simultaneously.
12 However, this may not be the norm. Finding two
13 or more viruses in a plant or plant cell does not mean
14 that they were introduced to that cell and replicated
15 simultaneously.
16 In fact, this may be the exception.
17 Consequently, a mixed infection may represent a collection
18 of different viruses that were introduced independently
19 over a span of time and never replicated simultaneously in
20 the same cell.
21 In my own lab and as indicated by others that

1 there are other evidence of this, inoculations leading to
2 mixed infections of brome mosaic virus and cowpea
3 chlorotic mottle bromo virus were most successful when the
4 inoculations were separated by two weeks.
5 Simultaneous inoculations led to the recovery of only one
6 of the two viruses. Thus mixed infections may not provide
7 the unlimited RNA recombination opportunities envisioned
8 when mixed infections are sorted. In contrast,
9 a constitutive promoter ensures that the transcript of the
10 viral transgene is available to a replicating virus in
11 each newly infected cell. Consequently, recombination
12 opportunities are constantly available to a replicating
13 virus.
14 And this distinguishes recombination
15 opportunities in PVCP PIPs from those of mixed infections.
16 Therefore the likelihood of recombination appears to be
17 increased in transgenic plants as compared to their non
18 bioengineered counterparts.
19 Now, for trans encapsidation, encapsidation of
20 viral genome depends on specific protein RNA interactions.
21 The constitutive promoter ensures the availability of

1 coat protein, but reports suggest that the quantity is
2 limited as compared to the coat protein translated
3 directly from the viral genome.
4 If the viral genome is capable of trans
5 encapsidation in mixed infections, it should also occur in
6 transgenic plants. Theoretically, trans encapsidation in
7 non bioengineered plants is dependent on the availability
8 of the coat protein of one virus to the genome of the
9 other, a situation dependent on the simultaneous
10 replication and translation of the two viruses in the same
11 cell.
12 Trans encapsidation is now a recorded event in
13 both PVCPs and non bioengineered plants. Situations would
14 be different for different plant species and transgenic
15 lines. Therefore I find it difficult to clearly predict
16 which situation will provide the greater number of trans
17 encapsidation events.
18 DR. ROBERTS: Thank you, Dr. Allison. Dr. Falk.
19
20 DR. FALK: I believe that heterologous
21 encapsidation events almost certainly will be less or

1 decreased in transgenic or non transgenic plants -- Or in
2 transgenic versus non transgenic plants. I also believe
3 that the biological significance of those will be even
4 greater reduced.
5 Now, and I think we'll talk later about ways
6 that we could modify them if they were biologically
7 relevant, I don't think that new or greater possibilities
8 for trans encapsidation and resulting biological effects
9 will result even though some have said that all of the
10 plants now express the transgene in all tissue types.
11 I still don't think that that's of biological
12 relevance. So I think that the results will be decreased.
13
14 I also believe that many of these arguments
15 could also be applied to recombination events. I think
16 that one of the things that Professor Allison did not
17 mention in regards to recombination is that certainly
18 tighter of RNA molecules in mixed infections will be much
19 greater than say the level of the transgene MRNA that's
20 available for recombination events.
21 Also, I think several people have mentioned

1 things like virus exclusion. That is not -- when
2 unrelated viruses coinfect the cell, that they set up
3 their own little replication factories and sort of exclude
4 the other virus, well, I think if that is true and there
5 is lot of evidence then, in fact, that is not completely
6 true.
7 But if that is true, then they would just as
8 likely exclude host MRNAs. In fact, in our lab we have
9 done experiments with mixed infections of unrelated
10 viruses and show that we can get recombinant RNAs from the
11 unrelated and coinfecting viruses.
12 And it is not dependent upon the time of
13 inoculation. It doesn't matter whether you co inoculate
14 or inoculate one before the other. So opportunities for
15 recombination or RNA interaction events to occur in mixed
16 infections are very great.
17 DR. ROBERTS: Dr. Bujarski, do you want to
18 resolve this?
19 DR. BUJARSKI: It would be difficult to resolve,
20 but I would like to add what I wrote on that question as
21 regarding to RNA recombination. Theoretically, there

1 should be no difference in recombination possibilities for
2 the transgenic RNA as compared to the non bioengineered
3 viral RNA. However, several factors can
4 contribute to recombination activity and thus the
5 differences among them may define the final figures.
6 They include first the expression in different
7 tissues of transgenic RNA as compared to non transgenic
8 environment. Second, going deeper to the
9 subcellular level the expression of transgene versus non
10 engineered RNA may occur within different intracellular
11 compartments.
12 Three, different levels of expression might
13 occur. Usually they are lower for transgenic RNA for
14 transgene viral RNA.
15 Four, replication and encapsidation might also
16 differ in transgenic and non transgenic situations.
17 And five, possible molecular differences may
18 exist between transgene and non transgene RNA.
19 So to answer the question I would say that there
20 might be -- that there is a possibility that the
21 probability or likelihood of recombination in both

1 situations might be different.

2 DR. ROBERTS: If I heard correctly, we have

3 increased, decreased and might be different.

4 I think we have covered the possibilities.

5 Let's see how --

6 Dr. Nagy and then Dr. Tepfer.

7 DR. NAGY: Actually, I'm not going to simplify,

8 but make it more complicated. We have one more, actually,

9 scenario, which I would like to add that I don't think we

10 are only discussing the possibility of recombination, but

11 also that the newly emerging recombinants are going to be

12 more a fit than the original y type virus here.

13 For this respect I would like to add indeed I

14 would agree with Dr. Allison that probably the possibility

15 of recombination is going to be increased in transgenic

16 plants.

17 However, the newly made recombinants might need

18 to fight against the same type of resistance, which is now

19 making the original, the y type virus less fit into that

20 transgenic plants.

21 This is an added factor that I think is very

1 difficult to predict whether the new recombinants would be
2 -- I mean the plant would not be resistant against the new
3 recombinant.
4 DR. ROBERTS: Thank you. Dr. Tepfer.
5 DR. TEPFER: I wanted to sort of in support to
6 Richard Allison's proposal to provide a bit of data, part
7 of which is not published yet, we're in the process of
8 doing this.
9 As I mentioned previously we are in the process
10 of studying recombination in transgenic and non transgenic
11 plants using reverse transcription PCR.
12 RTPCR is a very nice protocol for doing this.
13 It is extremely sensitive.
14 And in quite a lot of experiments in plants
15 infected with two cucumo viruses, non transgenic plants,
16 we routinely find are able to detect recombinants on the
17 order of 5 to 10 percent of the plants.
18 Now when we do exactly the same type of
19 experiment with transgenic plants expressing a coat
20 protein gene, we infect with a related cucumo virus. Here
21 we see a much higher frequency of detection recombinants.

1 It is on the order of 30 to 50 percent.

2 This is I think in support of what Richard was

3 saying previously.

4 DR. ROBERTS: Yes, Dr. Nagy.

5 DR. NAGY: I would like to go back. The new

6 recombinants have increased fitness? DR.

7 TEPFER: Actually I don't think that's the question of

8 this particular point that's being discussed here. The

9 question is not the fitness, but -- if you read the

10 question that's posed, it is not the fitness. We're just

11 looking at whether the transgenic plants are going to be a

12 greater or lesser site for recombination to occur. Is

13 that correct.

14 DR. KRAMER: That's correct.

15 DR. TEPFER: I don't think that's part of the

16 question at this precise moment. DR. ROBERTS:

17 Yes, Dr. Bujarski.

18 DR. BUJARSKI: I would like to add one thing that

19 has not been mentioned. Isn't it possible that the

20 expression of viral MRNA can actually trigger RNAI

21 pathway? And in this aspect then in a way the

1 recombination likelihood might actually decrease.

2 DR. ROBERTS: Dr. Allison.

3 DR. ALLISON: We have experiments that are

4 intended to address that. Our hypothesis is that, in

5 fact, in the situations where RNA is -- RNAI is invoked,

6 then we anticipate and hypothesize that there will be much

7 less recombination in which case, although this is not

8 relevant to this particular question, there may be a

9 superior technique to coat protein mediate a resistance.

10 DR. ROBERTS: Are there other panel members with

11 experience in this that want to weigh in?

12 The panel is divided.

13 DR. KRAMER: Since we have no agreement, can I

14 for clarification ask if Dr. Tepfer's comments were the

15 only ones actually supported by data? He mentioned some

16 unpublished studies.

17 Is anybody else referring to anything besides

18 theory in answering this question?

19 DR. ROBERTS: Dr. Melcher.

20 DR. MELCHER: I think Dr. Allison presented some

21 data too.

1 DR. ROBERTS: Dr. Allison, would you clarify
2 that?
3 DR. ALLISON: In our experiments we have been
4 able to recover numerous recombinants, many of which are
5 blind alleys (ph) in that the recombinations have led to
6 pieces that are not truly going to be a virus because the
7 open reading frame is not maintained.
8 But these were in transgenic plants that were
9 expressing a part of the coat protein gene. But those
10 experiments were not done in direct comparison with non
11 transgenic plants.
12 DR. ROBERTS: Dr. Falk.
13 DR. FALK: And I also alluded to some data we
14 published in PNS a few years ago where we did show that
15 unrelated viruses do, in fact, recombine in cells and do
16 make functional MRNAs. But we also did not then have the
17 other control being transgenic plant in those experiments.
18
19 DR. KRAMER: Thank you.
20 DR. ROBERTS: Dr. Hammond.
21 DR. HAMMOND: I have no cause to query the data

1 that's been presented. However, one of the conclusions
2 from the 1995 USDA APHIS and AIBS workshop is that the
3 question that should be posed is at what level of use does
4 the risk associated with recombination in transgenic
5 plants outweigh the agricultural and environmental
6 benefits of resistance.
7 I think that that question cannot yet be
8 answered, but that there is definitely significant benefit
9 from deploying resistance.
10 DR. ROBERTS: Dr. Allison.
11 DR. ALLISON: I definitely agree that the
12 benefits outweigh the risks. The worst thing we can get
13 is a new plant virus. And new plant viruses emerge by
14 natural, more natural mechanisms all the time.
15 DR. ROBERTS: Again, these are sort of big
16 picture comments. They are important, but I would like to
17 -- we can certainly tackle those at the end. I would like
18 to sort of get through the more technical questions that
19 are being posed first, though. Are there any
20 other comments on question 12? Dr. Sherwood.
21 DR. SHERWOOD: I just wondered if EPA is

1 interested in changing that question to what conclusions
2 can be drawn as to whether the likelihood of viable
3 recombination and/or heterologous encapsidation occurs.
4 Insert the word viable since that's really what
5 would be of interest. If it is non viable, it really
6 doesn't matter if recombination events occur. It only
7 occurs if you are going to get an infectious virus out of
8 it.
9 DR. KRAMER: I guess the thought would be that
10 production of a viable recombinant would be an extremely
11 rare event, which would be even more difficult to study
12 directly.
13 If we can show just an increase in overall
14 recombination rates, we would then assume that you would
15 get an increase in overall production of viable
16 recombinants.
17 If that's an incorrect assumption, then please
18 comment on that.
19 DR. ROBERTS: Dr. Tepfer will.
20 DR. TEPFER: In the experiments that we have
21 done at least with the non transgenic plants with 2 wild

1 type viruses, these are not mutants, these are perfectly
2 viable viruses, we haven't -- I think we have done about 2
3 dozen of the recombinants. We have looked at the
4 biological properties afterwards.
5 About half of them -- I don't have numbers in
6 mind. But about half of them are non viable. But there
7 are quite a few of them that are viable. Some of them
8 have other sort of more minor defects.
9 They affect their ability to move within the
10 plant, things like that, but the truly dead viruses are
11 not by any means a majority.
12 DR. ROBERTS: What is the time frame for
13 publishing those results, Dr. Tepfer?
14 DR. TEPFER: It's in press -- we're in the
15 process of writing that up. There are 2 articles that
16 will be appearing in the next couple months.
17 DR. ROBERTS: Thank you. Dr. Zaitlin.
18 DR. ZAITLIN: As you can see, there is
19 disagreement here, but the question doesn't give us the
20 opportunity to say that the possibility is unchanged. It
21 only gives us increased or decreased.

1 DR. KRAMER: That would certainly be an option.
2
3 DR. ROBERTS: Yes, I think if someone wants to
4 articulate that -- I believe the beginning Dr. Nagy's
5 comments indicated that's a possibility as well or I'm
6 sorry Dr. Bujarski's comments.
7 Dr. Tepfer.
8 DR. TEPFER: I want to say that from my personal
9 point of view, this is not the important question either.
10 I think that if you consider that there is going to be a
11 vast array of recombinants created in transgenic and non
12 transgenic plants, the question is fitness and biological
13 properties.
14 Just the fact that we may be seeing a higher
15 rate of appearance recombinants in transgenics is not
16 necessarily a factor of risk.
17 We do see it. That's what we have seen, but I
18 don't believe that that's a problem.
19 DR. ROBERTS: Dr. Allison.
20 DR. ALLISON: In terms of viable recombinants,
21 of the recombination events that we have recorded, by far

1 the majority are non viable recombinants. It is only
2 through means of selecting for viable recombinants that we
3 have been able to recover them successfully.
4 In our experience competing those that were
5 viable with the wild type virus, the wild type virus was
6 always a superior virus and outcompeted in a period of 2
7 to 3 passages the recombinant virus, which was then
8 excluded from the plant.
9 DR. ROBERTS: Dr. Nagy.
10 DR. NAGY: A question for Richard Allison. Did
11 you test the viability in transgenic plants, resistance
12 against the Y type virus?
13 DR. ALLISON: No. It was not done in resistant
14 plants. Is that what you mean? DR. NAGY: Yes.
15 DR. ROBERTS: Dr. Bujarski, do you want to add
16 something?
17 DR. BUJARSKI: I would like maybe try to
18 recapitulate somehow what we are saying. That no matter
19 if the rate of recombinants is low or higher, later on
20 there is some more important biological bottlenecks,
21 bottlenecks that can simply somehow define the final

1 outcome of the viral RNA population.

2 In this aspect, indeed, I would agree that

3 really it doesn't matter if there is a decrease or

4 increase likelihood of recombination, but what is

5 important is this more downstream final outcome.

6 DR. ROBERTS: Dr. Kramer, I think that probably

7 our response will include the point made by the panel,

8 that the fitness of the recombinants is an important

9 factor ultimately in determining risk.

10 Dr. Allison.

11 DR. ALLISON: I have a way of interjecting

12 things at inappropriate times in this meeting. However, I

13 think I would like to add one more thing to the record at

14 this point, because it ties together something that Dr.

15 Zaitlin has brought up, subliminal virus infections and

16 the possible use of those in recombination events.

17 So viruses infecting the transgenic plant are

18 well adapted to the plant species. Therefore, the useful

19 incorporation of genetic information through recombination

20 may be a rare event and a recombinant would be rapidly

21 excluded by the wild type virus.

1 In contrast, subliminal virus infections, those
2 viruses that replicate in the initially infected cell, but
3 lack the necessary mechanism for systemic infections, may
4 find all or part of the viral transgene useful.
5 While the coat protein structural role is
6 consistent among viruses, various additional functions,
7 including systemic movement, have been identified in
8 different viruses.
9 Laboratory experiments investigating the
10 availability of the transgenic transcript for
11 recombination with a replicating virus have been
12 criticized as providing an unreasonably high degree of
13 selection pressure.
14 However, subliminal viruses undergo a similar
15 selection pressure as the genome that obtains the key to
16 open the door to the cell next door will find an expanded
17 reproductive environment.
18 DR. ROBERTS: Dr. Melcher.
19 DR. MELCHER: I'm trying to recall the
20 literature on coat protein involvement in movement through
21 the plant. And I may not recall it correctly. But my

1 impression is that the coat protein is required for
2 movement beyond the initial clump of cells, that the
3 initial movement is really just what the movement protein
4 function is.
5 So in that case, the subliminal infection would
6 not result -- being aided by a coat protein that's
7 transgenic would not result in further movement. But I
8 may be wrong there because my memory is not so good.
9 DR. ROBERTS: Dr. Falk.
10 DR. FALK: I was going to make a similar comment
11 to Dr. Melcher's and say that, yes, it is unlikely, then,
12 in my interpretation of what Professor Allison said, that
13 the virus would ever get out of that initially infected
14 cell and if it doesn't it is not going to move on to
15 another plant.
16 DR. ROBERTS: Dr. Tepfer.
17 DR. TEPFER: I think it depends on what viral
18 group you are talking about. There are some where the
19 coat protein is absolutely essential for both local and
20 long distance movement, in which case at least
21 theoretically this kind of thing could occur.

1 But it is not the case of all plant viruses, obviously.

2 DR. ROBERTS: Everybody is nodding. That might

3 mean it is a good time for a break.

4 We'll take advantage of this moment. It is

5 10:25. Let's take a 15 minute break. Come back. We'll

6 decide whether or not we're finished with question 12. If

7 we aren't, we'll finish it. If we are, we'll move to the

8 next one.

9 (Thereupon, a brief recess was taken.)

10 DR. ROBERTS: Let me be sure that we have

11 actually finished off 12 before we go to 13. Let me ask

12 the panel members if they have anything they would like to

13 add on 12.

14 I don't see anything. Let me ask Dr. Kramer if

15 there is any clarification.

16 DR. KRAMER: Could I ask directly once again an

17 issue that came up at the end. That is, would the panel

18 say it is an incorrect assumption that an overall increase

19 in the frequency of either heterologous encapsidation or

20 recombination would also increase the frequency of a

21 significant event of heterologous encapsidation or viable

1 recombinant?

2 MEMBER: Could you repeat that? I'm sorry.

3 DR. ROBERTS: Yes, you may have to repeat the

4 question.

5 DR. KRAMER: I guess I sensed from the panel

6 before that there was some discomfort with the premise of

7 this question. That is that there would be any overall

8 significance in an increase in the rate of these events.

9 And the assumption behind this question is that

10 an overall increase in recombination per se would lead to

11 an increase in the production of a viable recombinant. Is

12 that an incorrect assumption?

13 DR. ROBERTS: Dr. Allison.

14 DR. ALLISON: I believe that just looking at the

15 odds, the more recombinants you make, the more likely you

16 are to come up with a viable recombinant. You interpret

17 that correctly.

18 DR. ROBERTS: Dr. Tepfer and then Dr. Falk.

19 DR. TEPFER: I think my point of view on this is

20 that if you have the same types of recombinants that are

21 present under the sort of circumstances, simply increasing

1 the frequency doesn't change the impact.

2 The real question is the nature of recombinants

3 that are created and not their frequency. If it is the

4 same ones, then it is not going to have a different

5 impact.

6 DR. ROBERTS: Dr. Falk, then Dr. Bujarski.

7 DR. FALK: I agree with what Mark just said

8 about the nature of the recombinant. Richard used the

9 term viable. But I think it is really the nature or the

10 potential or the significance of that recombinant.

11 And I would argue that lumping heterologous

12 encapsidation and recombination and sort of -- and

13 considering them similarly here, which it sounds like from

14 the question, that I think they are different.

15 I would say for heterologous encapsidation it is

16 no.

17 DR. ROBERTS: Dr. Bujarski and then Dr. Melcher.

18

19 DR. BUJARSKI: As regarding recombination, I

20 would like to agree with Mark and Bryce Falk.

21 DR. ROBERTS: Dr. Melcher.

1 DR. MELCHER: I detected disagreement, I think,
2 between Mark and Bryce. No, I'm sorry. Dr. Allison
3 spoke first. Right?
4 DR. ALLISON: Yes, I did.
5 DR. MELCHER: I agree with the assumption and I
6 don't see any way that the mechanisms that generate
7 recombinants have a way of detecting whether the
8 recombinants are going to be viable or not viable.
9 I think that if there is a small proportion of
10 viable recombinants that could be generated, increasing
11 the general frequency of recombination will increase their
12 general frequency also.
13 DR. KRAMER: So there is disagreement on this
14 issue as well?
15 DR. MELCHER: Apparently.
16 DR. ROBERTS: Dr. Nagy, then Dr. Tepfer, then
17 Dr. Hammond.
18 DR. NAGY: I would like to make sure this is a
19 theoretical assumption. We don't really have exact data
20 about the significance of the recombinants generated in
21 transgenic plants.

1 DR. ROBERTS: Dr. Tepfer.

2 DR. TEPFER: I guess what I was trying to say is
3 the -- of course if you have -- you are going to generate
4 more recombinants if you have a higher frequency of
5 recombination. I don't think that that's anything that
6 anyone would like to question. It is just a question of
7 whether this is significant.

8 I guess there is a difference between whether
9 you think the abundance of the recombinants is limiting or
10 not in terms of their emergence.

11 I think that what I'm trying to argue is that it
12 is quite likely that the abundance of the recombinants is
13 not the eliminating (ph) factor, but their ability to be
14 viable and to outcompete other types of molecules in the
15 plant and in the ecosystem.

16 In which case, if you increase their absolute
17 numbers, you haven't really changed anything if you are
18 talking about the same populations of molecules that are
19 being produced.

20 DR. HAMMOND: I think we have -- I don't think
21 any of us are questioning that an increased frequency of

1 recombination will lead to an increased frequency of
2 viable molecules. The question as Mark Tepfer has just
3 said is whether there is any significance.
4 And I believe that there is no a priori reason
5 to believe that recombination between a virus and a
6 transgene will lead to any more significant a recombinant
7 than a recombination between 2 viruses and a mixed
8 infection.
9 DR. ROBERTS: Dr. Allison.
10 DR. ALLISON: I would just agree with that.
11 DR. ROBERTS: Anyone else want to weigh in? Dr.
12 Kramer?
13 DR. KRAMER: I think we can move on to the next
14 question.
15 DR. ROBERTS: I was going to say I think our
16 response is as clear as it is going to be at this point.
17 Let's move on to question 13.
18 DR. KRAMER: How effective is deleting the 3
19 prime untranslated region of the PVCP gene as a method for
20 reducing the frequency of recombination in the region of
21 the PVCP gene.

1 Is this method universally applicable to all
2 potential PVCP PIP constructs. Would any other method
3 work as well or better. What methods are sufficiently
4 effective and reproducible such that actual measurement of
5 rates to verify rate reduction would be unnecessary?
6 DR. ROBERTS: Is there any background on this
7 question you want to give us that might help us?
8 DR. KRAMER: This is really looking at what the
9 practicality might be in the usefulness of having a factor
10 that's similar to what is listed in I think question 16
11 such that a product could be shown to have reduced risk
12 essentially because it had employed such a method.
13 DR. ROBERTS: Dr. Bujarski, could you lead off
14 our discussion on this one?
15 DR. BUJARSKI: Yes. In terms of recombination
16 rate, the ability of viral RNA to undergo replication
17 enhances the chances for recombination not only because
18 replication propagates and increases the level of
19 recombination substrates, but also because of the
20 mechanism of recombination involved.
21 Template switching by the replicase enzyme

1 during replication, RNA replication is a commonly accepted
2 mechanism for viral RNA recombination. Therefore, the
3 removal of 3 prime UTR is expected to reduce a combination
4 rate due to the bar (ph presence.
5 However, to be effective, to remove 3 prime
6 UTR must carry to the signals of RNA replication that are
7 recognizable to the viral replicase. Thus, in some cases,
8 the replication signals other than the 3 prime UTRs must
9 be removed from the transgene MRNA to reduce its replicate
10 ability.
11 Other methods of alleviating the risk of
12 recombination would involve the reduction in sequence
13 homology, for instance, by changing the third base in the
14 cordon triplet between PVCP PIP and the viral RNA or
15 reduce the sequence or modify the sequence composition GC
16 versus AU content.
17 Also, it must be said that the transgene MRNA by
18 itself might induce RNA silencing events that could reduce
19 the levels of recombining RNA substrates.
20 It is, however, questionable whether all these
21 methods are sufficiently effective to warrant the rate

1 reduction beyond the safe level. And this remains to be
2 established experimentally.
3 Several general aspects must be considered here.
4 First, it is likely that each particular viral system
5 responds differently to the bav (ph preventive approaches
6 because each value has its own set of tools securing
7 molecular interactions during recombination.
8 Second, although not sufficiently proven,
9 recombination may depend upon the host genome environment.
10 In other words, the same values might recombine with a
11 different rate because of host -- different host factors
12 involved.
13 Third, there might be different mechanisms other
14 than copy choice (ph) supporting viral RNA recombination.
15 For instance, breakage and 3 ligation (ph) and then the
16 removal of replication signals might not completely
17 prevent to avoid crossovers.
18 In reality, however, the Naston (ph recombinants
19 might be overpowered by selection pressure with best fit
20 virus that outcompete the recombinants especially in field
21 situations where viruses replicate under so-called low

1 selection pressure.

2 Overall, there is nothing universal approach in

3 eliminating the risk of viral RNA recombination. And

4 although we might reduce its probability, the measurement

5 of recombination rates might be necessary.

6 DR. ROBERTS: Dr. Melcher.

7 DR. MELCHER: I agree with Jozef's original

8 comment on the 3 prime untranslated region.

9 But I would like to go into it a little bit more

10 because I think it is just a matter of words and so forth,

11 because Dr. Kramer yesterday did present in her slide that

12 besides removing the 3 prime untranslated region one would

13 exclude replicase recognition sites or other hot spots.

14 So the method of removing the three prime

15 untranslated region is not universally applicable to all

16 potential PVCP PIP constructs. Deleting the 3 prime

17 untranslated region will only be important for those

18 viruses in which this region contains the promoter for

19 minus strand synthesis, which is not all plant viruses.

20 A more important statement would be that all

21 viral promoters minus strand plus strand and subgenomic

1 ought to be avoided in such constructs.

2 I have 2 other ways that have already been

3 mentioned, but would like to make sure that they occur

4 here, that one could reduce -- what are we trying to do,

5 we're trying to reduce the frequency of recombination.

6 One is limiting expression of transgenes to

7 those tissues in which the virus from which the transgene

8 comes. That's a desirable method for reducing the

9 frequency.

10 And the other is making sure that the level of

11 transcription is the minimum required for the protective

12 effect, so that the frequency of recombination is reduced.

13

14 As far as whether this is effective and

15 reproducible enough to preclude measurement of rates, I

16 really do not have any knowledge and I would defer to my

17 colleagues that do have that knowledge.

18 DR. ROBERTS: Thank you. Dr. Allison.

19 DR. ALLISON: If I can again read into the

20 record, I just want to state that I agree with Dr. Melcher

21 about the non universal placement of replication signals

1 in the 3 prime UTR, untranslated region. And that's not
2 addressed in the statement here.
3 The 3 prime untranslated region has been
4 maintained in genetic constructs partly because it is
5 believed to provide stability to the RNA transcript within
6 the cytoplasm due to their typical secondary structure and
7 partly because they are associated with the original
8 clone.
9 As numerous CDNA clones used to establish coat
10 protein mediated resistance used primers that hybridized
11 to the 3 prime terminus of the virus. This is especially
12 true of poly idinolated (ph) viral RNA such as poty
13 viruses.
14 Due to the biochemical rules of DNA and RNA
15 replication, the replication processes initiated at the 3
16 prime end of the virus utilizing a sequence/structure
17 maintained in this 3 prime UTR. As it was stated this is
18 not 100 percent, but it is frequently found there.
19 Thus by including the 3 prime UTR in the
20 construct, a related virus may recognize the viral
21 transcript as a template and initiate replication on the

1 transgene transcripts.

2 Two things may happen. The replication complex

3 may use this initiation point to begin replication and

4 switch back to the viral RNA as a template during

5 recombination.

6 Or the replication complex may make a

7 complimentary copy of the viral transgene which may also

8 be available for RNA recombination.

9 Since all viral RNA viruses must use the 3 prime

10 UTRs as replication initiation sites, it makes good sense

11 to withhold these sites from the viral transgene as it

12 would appear that they enhance recombination activity of

13 the transgenic transcript.

14 In laboratory experiments where transgene

15 constructs have been involved in recombination events,

16 removal or destruction of the 3 prime UTR, untranslated

17 region, have reduced recombination to below experimental

18 detection limits.

19 If transcript stability becomes an issue, other

20 nonviral RNA stabilizing sequences could be added.

21 Now, in addition to removal or destruction of

1 the 3 prime UTR, several additional methods have been
2 suggested for reduction of recombination events involving
3 the transgene.
4 These include using the smallest resistance
5 generating viral fragment possible to generate resistance.
6 Disruption as Jozef has said disrupting a potential AU
7 rich recombination hot spots. Providing point mutations
8 and or deletions in the transgene that would enable --
9 that would disable a functional aspect of the coat protein
10 when identified.
11 In addition, Dr. Melcher has indicated using the
12 smallest amount of transcript as possible.
13 These methods do not have to be used
14 independently. They can be combined to help ensure that
15 either recombination does not occur or that the
16 incorporated segment is useless to the recombinant.
17 DR. ROBERTS: Dr. Hammond.
18 DR. HAMMOND: This question is based around
19 reducing the frequency of recombination. But as we have
20 already discussed previously, there is a difference
21 between the frequency of recombination per se and the

1 frequency of viable recombinants.

2 Removing the 3 prime end certainly reduces a lot

3 of the possibility of recombination as does the deletion

4 or avoidance of incorporation of other replicase

5 recognition sequences such as subgenomic RNA promoters.

6 Similarly, minimizing the transgene expression

7 levels by deliberate induction of RNA silencing, but then

8 this little point of using the coat protein gene itself,

9 as it is not necessary to have the coat protein gene

10 expressing coat protein to induce RNA silencing, there are

11 many other ways by which one can make any potential

12 recombinant less viable by introducing mutations.

13 But I think those are dealt with more

14 appropriately and in one of the succeeding questions.

15 I'll leave it at that. DR. ROBERTS: Thank you.

16 Any other comments? Dr. Kramer.

17 DR. KRAMER: Can I reiterate what I heard then?

18 You've listed a number of different strategies that could

19 be employed to reduce recombination. It's sounds like

20 there is not a single strategy that would be universally

21 applicable to all constructs. That, rather, it would have

1 to be looked at on a case-by-care basis.

2 It sounds like -- actually, I think there was

3 only one person that directly addressed the last part of

4 the question as to whether any of these methods are

5 sufficiently effective and reproducible such that you

6 would not need to actually measure rates to verify that

7 they had worked.

8 Is there agreement -- I think what we heard

9 from one person was that these methods were not -- you are

10 not willing to commit to say that these methods were

11 sufficiently effective and reproducible.

12 Is there any other discussion on that?

13 DR. ROBERTS: Any disagreement with that

14 statement? Dr. Allison.

15 DR. ALLISON: I agree with the statement. That

16 at this point none of these methods are going to be 100

17 percent efficient. While the easiest recombinants to

18 recover are those that are viable and have resulted from

19 homologous recombination events, there are numerous, at

20 least in our laboratory we have recorded numerous

21 heterologous recombination events leading to nonviable

1 viruses.

2 In each one of those cases it would appear that

3 these types of approaches would not have inhibited those

4 recombination events.

5 DR. KRAMER: But would the overall rate be

6 decreased if one of these methods were employed?

7 DR. ALLISON: Yes.

8 DR. KRAMER: Without measuring it? You can be

9 confident of that?

10 DR. ALLISON: I feel confident.

11 DR. ROBERTS: People can weigh in verbally on

12 that. Dr. Tepfer.

13 DR. TEPFER: I wanted to agree with Richard

14 Allison on that. I think that when you remove the 3 prime

15 non coating region, the only recombinants that can occur

16 that would be viable are double recombinants.

17 If the frequency of crossing over at any given

18 site is x, then you are increasingly enormously the -- you

19 have to multiply the two frequencies in order to be able

20 to recover the double recombinant.

21 Just mathematically, physically, I think we can

1 all feel rather confident that doing this, forcing double
2 recombinants to be the only viable ones is indeed a way of
3 decreasing the frequency.
4 I would be surprised if that were in itself
5 controversial. Whether that is sufficient or not, of
6 course, is going to depend.
7 DR. ROBERTS: Dr. Melcher.
8 DR. MELCHER: I guess when I said I would defer
9 to my colleagues, I thought that Dr. Allison would make it
10 clear that his conviction is based on experiments from his
11 laboratory, which I think is correct.
12 DR. ALLISON: That's correct.
13 DR. ROBERTS: Any other follow ups?
14 DR. KRAMER: No. Thank you.
15 DR. ROBERTS: Let's move on to the next
16 question.
17 DR. KRAMER: Are any methods for inhibiting
18 heterologous encapsidation or transmission by insect
19 vectors universally applicable to all PVCP PIPs? Which
20 methods are sufficiently effective and reproducible such
21 that actual measurement of rates to verify rate reduction

1 would be unnecessary?

2 Just as a clarification where this question is

3 coming from, we're looking at a factor -- to judge

4 qualification of a product we would want something -- we

5 want to know how specific we can be in laying out that

6 factor. Is there something that is going to be known to

7 work in all cases and also looking at the practicality of

8 it.

9 If rates needed to be measured, that's important

10 to know.

11 DR. ROBERTS: Dr. Falk.

12 DR. FALK: I would say in answer to what you

13 just said, can anything be applied in all cases, I would

14 think that at this time, no. But I think that the more we

15 learn about factors that are important in these two

16 events, yes, things could be done. But I think it would

17 vary on a case-by-case basis.

18 And I think it would only be important to do

19 that if these were determined to impose a real risk.

20 I think the first question here is asking two

21 different things. And I think it is important to realize

1 that heterologous encapsidation and transmission by insect
2 vectors is not synonymous. That I think, yes, you do need
3 to have heterologous encapsidation to get transmission.
4 But because you have heterologous encapsidation
5 does not necessarily mean that you will have insect
6 transmission. So it is important to remember that.
7 If we were talking about affecting heterologous
8 encapsidation, obviously, it would be important to just
9 delete or mutate amino acids -- the cordons in coating
10 (ph) for the amino acids that are important in virion
11 assembly.
12 In many cases, we don't know this. But we do
13 in many cases. Certainly all filamentous rod shape
14 viruses have 3 amino acids conserved spread out among the
15 protein itself.
16 I'm not positive that those are important in
17 virion assembly. But since they are conserved among all
18 filamentous plant viruses, and this would be serine,
19 arginine and aspartic acid that something like that could
20 be considered if heterologous encapsidation was important.
21

1 Certainly, in regards to insect transmission, we
2 do know for some viruses we do know important features
3 that are important in some cucumo virus transmission, poty
4 virus transmission and luteo virus transmission.
5 This was given in the background information.
6 It has been brought up in discussion yesterday. Those
7 domains or amino acids also could be considered if
8 heterologous encapsidation and subsequent transmission
9 were deemed to be a risk.
10 DR. ROBERTS: Dr. Tepfer.
11 DR. TEPFER: Just in following up on Bryce
12 Falk's comments, with which I agree quite entirely, I
13 think there is only one case that's been described where
14 it has been possible to interfere with the virus assembly
15 process which is to say with heterologous encapsidation
16 and to conserve the resistance phenotype.
17 This is from Edgar Mize's lab (ph). So I think
18 that would be a rather difficult test to try to
19 generalize. I think there is also evidence from tobacco
20 mosaic virus in Roger Beach's lab(ph) that the assembly
21 process itself is important in the resistance that's

1 conferred.

2 So that might be a counter example where it may

3 not be possible to interfere with potential heterologous

4 encapsidation without losing the resistance.

5 I'm sort of maybe more positive than Bryce is

6 about interfering with the vector transmission itself.

7 Admittedly, there are these three cases that are

8 rather well known groups of viruses where we know how to

9 interfere with the vector interaction in a way that does

10 not prevent the resistance phenotype.

11 I think what is important is sort of the

12 principle involved that where necessary this kind of

13 strategy can be developed so that there may be other virus

14 groups in which it may have not been studied yet which may

15 be desirable to prevent heterologous vector transmission

16 to occur.

17 And then this can be explored using the same

18 sorts of principles as has been developed with these three

19 virus groups that are very clearly understood.

20 So I think that in a sense from my thinking you

21 are getting close to at least what could be a considered a

1 potential universal principle in the case of preventing
2 vector transmission. Where as I would say that preventing
3 the encapsidation itself from occurring is not likely to
4 be universally possible.
5 DR. ROBERTS: Dr. Melcher.
6 DR. MELCHER: I guess I should first address the
7 question of interfering with assembly that was brought up.
8 The residues that Dr. Falk mentioned, the serine, the
9 arginine, the aspartic acid, at least two of those are
10 directly involved in the assembly of particle.
11 As Dr. Tepfer mentioned that's probably
12 important for the protection effect. So the two go hand
13 in hand.
14 On the other hand, there are other residues in
15 these coat proteins that are not as universally conserved,
16 but play roles in forming the structure, and it might be
17 possible to engineer a coat protein gene so that it has
18 one of three residues altered so that it would not co-
19 assemble with the invading virus.
20 The hazard of doing that is that it might make
21 it possible to co-assemble with still another virus. So I

1 think that's a very speculative risky sort of thing.
2 However, there are other things that one can
3 think about. There are two kinds of viruses, the
4 isometric ones and the elongated ones. Each of them have
5 components on their surface that are mostly the ones that
6 are involved in interaction with other organisms. For
7 example, the vector.
8 So restricting the portion of the PVCP transgene
9 to that encoding the structurally essential core may be a
10 somewhat universal strategy for limiting adverse effect of
11 trans encapsidation, but not for limiting the trans
12 encapsidation itself.
13 I'm saying the vector transmission part may be
14 reduced by concentrating on the core particles. For
15 isometric viruses, that would mean using only the s
16 domains, omitting the p domains that interact with those
17 other organisms.
18 For helical viruses, maintaining the four helix
19 bundle core and the internal nucleic acid binding loops
20 while dispensing with the surface displayed n terminal and
21 c terminal ends would reduce the chances of nonwanted

1 vector transmission, but still likely retain the
2 effectiveness as a plant protectant.
3 Trans encapsidation could be prevented or
4 inhibited in another way if the plant contains resistance
5 genes for all the viruses that are capable of infecting
6 that plant.
7 Such a plant would not allow infection by an
8 invading virus, thus there would not be a genome of an
9 infecting virus to be trans encapsidated. The difficulty
10 of this approach of course is manyfold, I suppose.
11 But two that I can think of; one is identifying
12 all the viruses capable of infecting the plant, it may be
13 not possible, not only its major pathogens, but other
14 viruses too. And there is of course the technical
15 challenge of creating such plants. Another
16 universal strategy and one that I think is well recognized
17 is to limit the amount of transgene protein produced. The
18 lowest concentration consistent with protection should be
19 used to reduce the frequencies of trans encapsidation just
20 as for recombination.
21 DR. ROBERTS: Dr. Hammond.

1 DR. HAMMOND: I believe that there is no
2 universal approach. Different virus groups assemble
3 differently and are transmitted differently. And I think
4 that we know enough about most virus groups to be able to
5 suggest approaches that work for specific virus groups
6 such as removing the DAG triplet from most poty viruses,
7 removing the read through domain from luteo viruses or
8 ablating the RNA binding sites in some instances so that
9 particles cannot be assembled.
10 However, there are other virus particles that
11 are stabilized rather than by protein RNA interactions,
12 solely by protein protein interactions. And in that case,
13 you have no RNA binding site to ablate.
14 So that requires a different approach and would
15 need to make modifications to the surface motifs that are
16 involved in vector transmission.
17 Closest thing to a universal approach is to get
18 away from using coat protein and go to RNA silencing,
19 which is kind of a different approach, and then it is not
20 relevant to coat protein, which we're discussing here.
21 With reference to assembly mutants, there is a

1 potential problem with modifying subunit subunit
2 interactions that would prevent assembly. Because in some
3 instances, those may induce a hypersensitive response that
4 would be deleterious to the host plant. I think
5 that's been shown with Jim Colver's lab (ph) that some of
6 the TMV coat protein subunit subunit interaction sites
7 when mutated induced the hypersensitive response as well
8 as inhibiting particle assembly.
9 To do that does not necessarily solve the
10 problem.
11 DR. ROBERTS: Other comments. Dr. Falk.
12 DR. FALK: I think the last thing did solve the
13 problem because you are probably not going to be able to
14 regenerate that plant.
15 DR. ROBERTS: Dr. Kramer.
16 DR. KRAMER: I'm going to follow up with given
17 that there is not a single method that would be
18 universally applicable, given that we might have methods
19 that would be known to work in a particular virus group,
20 could you answer affirmatively for the second part of the
21 question that for certain situations, certain virus

1 groups, certain modifications within those groups you may
2 not need to actually verify rate reduction because those
3 methods are known to occur?
4 DR. ROBERTS: Panel response. Dr. Hammond.
5 DR. HAMMOND: I would believe that to be true.
6 DR. ROBERTS: Dr. Tepfer is nodding for the
7 record. Dr. Falk.
8 DR. FALK: I'm not aware, though, that those are
9 done in transgenic plants. Are you?
10 DR. HAMMOND: Which?
11 DR. FALK: The methods that are sufficiently
12 effective and reproducible such that actual measurements
13 to verify rate reduction would be unnecessary. I think we
14 have done these things with virus mutants and things and
15 now we're suggesting that they should work in transgenic
16 plans, but I don't know that they have.
17 DR. ROBERTS: Dr. Tepfer.
18 DR. TEPFER: Isn't there work published on
19 deleting the DAG motif in poty viral --
20 DR. FALK: Yes.
21 DR. TEPFER: I think there is. I think also

1 that some of the transgenic CMV CP plants are using a
2 nonaphid transmissible strain, and I think that that was
3 verified that --
4 DR. FALK: I know you are right.
5 DR. TEPFER: For the poty, I'm sure. In any
6 case, the CMV I know was used with a nonaphid
7 transmissible strain. Whether that was actually verified
8 after the fact, I'm not absolutely certain.
9 DR. ROBERTS: Dr. Hammond.
10 DR. HAMMOND: As far as I'm aware, removal of
11 the read through domain of luteo viruses has been
12 demonstrated to ablate insect transmission. Removal of
13 the immuno (ph) terminus of poty viruses ablates aphid
14 transmission.
15 Mutation of the RNA binding site in poty viruses
16 prevents encapsidation and still allows effective
17 resistance. That's work from Edgar Mize (ph).
18 There are certainly aphid nontransmissible
19 isolates of cucumber mosaic virus that have been
20 identified and many other instances.
21 There is good evidence for some virus groups. I

1 would not say that there is good evidence for all virus
2 groups, but for a significant number of virus groups.
3 DR. ROBERTS: Dr. Melcher.
4 DR. MELCHER: I guess to be specific, I would
5 say that if there is good evidence that the virus without
6 these groups -- whatever the modification is, are not
7 insect transmissible, then there should not be any need to
8 require further documentation for that particular gene in
9 a transgenic plant.
10 DR. ROBERTS: Is there agreement with that among
11 the panel members? Let the record show lots of heads
12 nodding.
13 Any other follow ups, Dr. Kramer.
14 DR. KRAMER: No, thank you.
15 DR. ROBERTS: Let's go ahead and take the next
16 question, then.
17 DR. KRAMER: How technically feasible would it
18 be to measure rates of recombination, heterologous
19 encapsidation and vector transmission in PVCP PIP
20 transgenic plants in order to show that rates are reduced?
21

1 DR. ROBERTS: Dr. Melcher.

2 DR. MELCHER: I guess I quibble with words

3 sometimes. So bear with me. Recombination rate is

4 defined as the number of recombination events occurring

5 per unit of time. The virologically meaningful unit of

6 time is a replication cycle.

7 Unfortunately, we do not have accurate numbers

8 for how many replication cycles occur between the initial

9 infection of a plant and the harvest of the virus.

10 The number depends on how many cells are on the

11 pathway that the infection takes, whether a new

12 replication cycle must occur in every cell, in the pathway

13 and whether multiple cycles occur in a single cell.

14 These factors are likely different for different

15 viruses. Fortunately, there is another quantity that is

16 easier to measure and directly related to recombination

17 rate, and that's the frequency of recombinants.

18 The rate of recombination is equal to the

19 frequency of recombinants divided by the number of

20 replication cycles or some other measure of time.

21 Since the question really implies an interest in

1 whether recombination rates are reduced, it can be
2 answered confidently if the frequency of recombinants is
3 reduced. I assume the comparison is between an
4 unmodified PVCP PIP transgene and a PVCP PIP transgene
5 modified to address some of the concerns that have been
6 dealt with elsewhere in the discussion.
7 So with that proviso, then, going on, it is
8 technically feasible to measure the frequency of
9 recombinants in a population of viruses.
10 One popular approach is to create multiple
11 molecular clones of a region of the viral genome in which
12 the combination may have happened and determine their
13 nucleotide sequences scoring whether or not they are
14 recombinants.
15 A large number of clones is of course required
16 to obtain statistically valid results, but with current
17 costs of nucleotide sequencing, such an undertaking is not
18 prohibitive.
19 A second approach is allele specific real time
20 prolimiraese (ph) chain reaction.
21 In this approach, two pairs of primers are

1 designed, one pair in which each primer will only prime
2 from the genome of the infecting virus and the other pair
3 in which each primer will only prime from the transgene.
4 Three or four PCR reactions are carried out.
5 Those with each pairs I just described. They serve as
6 controls to measure the amounts of nonrecombinant
7 sequences and those in which the pairs exchange partners
8 allowing amplification of the reciprocal recombinants.
9 When the PCRs are performed in real time mode,
10 reliable estimates of the amounts of each template present
11 are obtained. The frequency of recombinants is the
12 quotient of the sum of the exchange values over the sum of
13 all values.
14 A third approach is possible if the sequences
15 differ in multiple restriction and nucleus recognition
16 sites. Restriction will generate unique recombinant DNA
17 fragments whose quantity can be determined by densatomety
18 of gel laphritic (ph)
19 separation of the digestion products, and comparisons with
20 quantities of the corresponding non recombinant fragments
21 will result in a value that is the frequency of

1 recombinants, which is relevant.

2 Those are the methods I know about as far as

3 recombination goes. The question includes I think also

4 trans encapsidation and vector transmission.

5 So trans encapsidation relative to this, the

6 rate concept has even less relevance than for

7 recombination. For trans encapsidation to be a problem,

8 the rate needs to be very close to that persistent

9 encapsidation otherwise there will not be any trans

10 encapsidated variance.

11 But, again, as with recombination, the frequency

12 of trans encapsidated genomes can be a useful measure for

13 the potential of trans encapsidation.

14 The frequency of these trans encapsidated

15 genomes can be measured reliably by at least one of two

16 methods, and probably my colleagues will add some more.

17 Immuno capture PCR or RTPCR in real time format should

18 reveal how much of the viral nucleic acid target of the

19 reactions is encapsidated in particles that contain the

20 PVCP that's the target of the antibody that's used.

21 An antibody against the transgene PVCP is used

1 to separate virions that bear sub units from the
2 transgenes from those that do not. The nucleic acid in
3 them is released and quantified by a quantitative PCR or
4 quantitative RTPCR, RT being reverse transcriptase, as
5 appropriate to the virus.
6 Concurrently, the total virion population is
7 also subjected to release in quantitative PCR or RTPCR,
8 and that value serves as the denominator for the quotient,
9 but gives the frequency of trans encapsidated genomes.
10 The second technique for trans encapsidation is
11 amino electron microscopy in the plant bearing PVCP
12 transgene. The only genome encapsidated can be assumed to
13 be that of the invading virus.
14 The question then becomes what proportion of the
15 virions formed have a capsid that is composed in part of
16 sub units provided by the transgene.
17 Staining of virions with tagged, for example, by
18 peritin (ph) antibodies against the transgene PVCP should
19 allow distinction of stained virions from unstained ones.
20 A difficulty with this approach is only a small
21 percentage of capsid sub units usually react in such

1 studies.

2 When only a small percentage of the sub units

3 derived from the transgene, many virion particles may

4 escape scoring as being trans encapsidated.

5 However, since the aim of the assay is to test

6 for reduction of the frequency of trans encapsidated

7 genomes, the underestimation resulting from this problem

8 is minimized as long as there is an appreciable evidence

9 of trans encapsidation in the case of the non modified

10 PVCP transgene.

11 That's the second part. That's the trans

12 encapsidation. Then there is the vector transmission.

13 Vector transmission plays a role in two

14 scenarios. When PVCP transgenes are used as plant induced

15 protectants, plant incorporated protectants, it is often

16 the case that the protection afforded the plant is not

17 absolute.

18 So low levels of the virus may result or the

19 plant may even eventually escape from the protection.

20 The second scenario occurs when infection is by

21 a non targeted virus and trans encapsidation has occurred.

1 So the assays for vector transmission are diverse because
2 the diversity of organisms that vector plant viruses are
3 diverse.
4 They are arthropods. They are fungi. They are
5 nematodes. They are mammals. Mechanical transmission
6 through contact.
7 If the virus vector is well understood, there
8 usually are assays available for measuring the efficiency
9 of vector transmission. For example, in the case of
10 arthropods, the experiments involved caging arthropods
11 with disease plants for a specified period of time, the
12 acquisition access period, and then transferring them to
13 healthy test plants for a second period of time, that's
14 the inoculation access period of time.
15 For viruses whose transmission mode is
16 circulative and or propagative, an additional period of
17 feeding on healthy plants intervenes between those two to
18 allow the virus to circulate and replicate in the
19 arthropod before it's delivered.
20 Scored are percentages of test plants that
21 become infected. These tests are pretty standard in

1 vector entomology. So in summary, for all three aspects
2 of the question, it is technically feasible to test
3 whether modification of the PVCP gene reduces
4 recombination, trans encapsidation and vector
5 transmission.
6 That's my opinion.
7 DR. ROBERTS: Thank you. Dr. Falk.
8 DR. FALK: I agree mostly with Ulrich and I think such
9 analyses are technically feasible. However, I must again
10 say that I think the important question is the biological
11 relevance if such interactions are even occurring.
12 And if they are, if they are of no relevance, I
13 don't see why you would want to go ahead and lower them.
14 And if they are important, these interactions, I think it
15 would be important then to determine not just can you
16 lower them, but how low do you need to lower these.
17 Ulrich talked about measuring rates of trans
18 encapsidation and I agree largely with the approaches that
19 he suggested. I do think that it is not simple. And even
20 techniques like amino capture RTPCR, it is important to
21 have care and appropriate controls to make sure that we're

1 detecting biologically active heterologous encapsidated
2 RNAs.
3 And such approaches are a little bit difficult.
4 I think measuring vector transmissibility as
5 Ulrich said can be done, but then again the significance
6 of transmissibility is important in terms of whether it is
7 transmissible or whether it is not transmissible because
8 the results here only really reflect the experimental
9 conditions.
10 But yes, you could compare them relative to a
11 control, but I'm not sure about the biological
12 significance.
13 I defer to my two colleagues in terms of talking
14 about the recombination.
15 DR. ROBERTS: Dr. Nagy.
16 DR. NAGY: Actually, I agree with both Ulrich
17 and Bryce that it is technically feasible to measure both
18 the recombination frequencies and heterologous
19 encapsidation and vector transmission.
20 The next thing I would like to add is that I
21 think it is feasible and relatively easy to do is to

1 integrate plans with the two known viable transcripts to
2 see the recombination frequency with those.
3 That's I think a very good way to measure.
4 DR. ROBERTS: Let me get to Dr. Bujarski and
5 then we'll take Dr. Tepfer. DR. BUJARSKI:
6 I do not have much to add besides some other possible
7 techniques including for instance quantitative western
8 blott when a specific epitope is for instance recovered
9 due to recombination when you use a specific monoclonal
10 antibody. Thank you.
11 DR. ROBERTS: Dr. Tepfer.
12 DR. TEPFER: I just want to add some sort of
13 bench level reality to this. We have been working a lot
14 on detecting recombinants in non transgenic plants. And
15 the techniques that we have finally developed are
16 extremely sensitive.
17 And even so that we're very close to detection
18 limits. So the recombinant molecules in these plants are
19 extremely rare. And what we have been able to
20 do with cucumo virus so far at least has not been able to
21 be applied to other viral groups for a variety of reasons

1 which could be due to an even lesser abundance of the
2 molecules in question, differences in sensitivity of the
3 techniques used, but it is a very difficult kind of
4 experiment to carry out. We have had to work very hard on
5 this.
6 I'm a little bit concerned about trying to go
7 from sort of hypothetical ways of doing this and to the
8 actual realities of carrying it out. So I think it is
9 much, much harder than it looks.
10 So I would be very uncomfortable with mandating
11 something that was not feasible. So I think that doing
12 that type of experiment would only be feasible for perhaps
13 a very small number of viral groups where it has been
14 demonstrated it can be done.
15 In the area of vector transmission, I have more
16 of a question for my colleagues. I know that one can
17 measure this rather nicely with aphids and perhaps other
18 insect vectors. What about fungi and nematodes?
19 Can you do also do feeding experiments? It
20 seems rather more difficult by it. It is not my area of
21 expertise. I'm just asking the question whether this can

1 be done with non insect vector systems.

2 DR. ROBERTS: Let's see if there is any response

3 from the panel then we will go to Dr. Hammond for a

4 comment. Does anyone have experience with other vector

5 systems? Dr. Melcher.

6 DR. MELCHER: No, I don't have experience. If

7 someone has experience, they should go ahead. I have some

8 knowledge.

9 DR. SHERWOOD: I have some experience with

10 polymyxin and wheat soil born mosaic virus. It is very

11 difficult to get a virus free and maintain a colony of

12 that fungus.

13 DR. ROBERTS: Dr. Cooper.

14 DR. COOPER: With the nematodes, certainly,

15 tricadorits (ph) it is certainly possible and the other

16 nematodes too. Technically exacting, but possible

17 DR. ROBERTS: Dr. Hammond. You had another

18 comment.

19 DR. HAMMOND: I essentially agree with the

20 methods that have been proposed for dealing with

21 recombination and with vector transmission. Vector

1 transmission is difficult to do and hard to quantify, but
2 it can be done.
3 I have a correction or suggestion compared to
4 the detection of heterologous encapsidation in that amino
5 specific electron microscopy is very difficult to use as a
6 measure of the degree of encapsidation. There is an
7 enormous variability within the viral population from
8 transgenic plants in the proportion of transgene coat
9 protein that is incorporated.
10 We have attempted to do this and have published
11 on it using a monoclonal antibody that is specific for the
12 transgene coat protein. We are readily able to detect the
13 transgene coat protein in particles of viruses able to
14 infect these transgenic plants, but it is extremely
15 difficult to quantify.
16 Some particles have no transgene coat protein
17 incorporated. Some particles appear to have a few
18 molecules of the trans coat protein and some particles are
19 almost entirely encapsidated in the transgene coat
20 protein.
21 You cannot quantify on that basis because the

1 number of particles you would need to look at and count
2 and try to quantify is just too large to get meaningful
3 results. However, if you have a transgene coat
4 protein specific monoclonal antibody or a suitably
5 specific polyclonal antibody, then it is possible to use
6 various forms of ELISA or western blotting to determine
7 the proportion of the transgene coat protein that is
8 present in a virus preparation.
9 I would suggest it is much easier to do that in
10 a purified preparation than to do it in extract because of
11 the relative difficulty of quantifying and determining
12 relative amounts out of the same extract if you are using
13 crude sap rather than a purified preparation.
14 But you can then use RTPCR, amino (ph) capture
15 RTPCR using antibody specific for the transgene coat
16 protein or you can use a polyclonal and RTPCR or the
17 Western and direct ELISA methods. But ISCM is a very
18 difficult method to use for quantification.
19 DR. ROBERTS: Other comments from panel members?
20
21 Dr. Tepfer.

1 DR. TEPFER: I said it previously I just want to
2 reiterate the extreme difficulty of a lot of these things.
3
4 I'm sort of in the same way that we were talking
5 previously about the difficulties of reducing the impact
6 of gene flow in terms of how many populations and how many
7 states you needed to study and so on in the wild
8 populations.
9 Here I think we're in a sort of similar
10 situation. What you are trying to do is extraordinarily
11 difficult. Particularly in the case of heterologous
12 encapsidation in most situations, probably not worth the
13 effort.
14 So that needs to be taken into account.
15 DR. ROBERTS: Dr. Melcher.
16 DR. MELCHER: I would like to ask Dr. Tepfer
17 relative to the recombination what method he did use that
18 was successful and which methods he tried that were not
19 successful.
20 DR. TEPFER: In our hands what we have done is
21 reverse transcription PCR. It is very easy on paper. But

1 since the molecules we're detecting are extremely rare, it
2 turned out very difficult to do. This is obviously non
3 quantitative.
4 This is sort of a plus minus sort of situation.
5 So what we can see is what are the proportional of plants
6 in which we see recombinants, but we can't quantify them.
7 Colleagues at the Tobacco Institute in
8 Bejerak (ph) have used real time PCR using some of the
9 same primer pairs and they have been able to do that to
10 detect recombinants with cucumo viruses. So that has
11 worked.
12 The things I was referring to that did not work
13 were basically in fact using the same kind of reverse
14 transcription PCR type protocol to other virus groups.
15 It is colleagues within a EC funded project.
16 Edgar Mize working with poty viruses in particular tried
17 very, very hard to get the same strategy that we use to
18 work on potys and he never could get it to work.
19 So it is just a warning that even what looks easy on
20 paper can be difficult if not impossible.
21 DR. ROBERTS: Dr. Bujarski.

1 DR. BUJARSKI: I would like to add following
2 what Mark says that PCR, these RTPCR techniques require
3 numerous controls.
4 For example, we have to make sure that RTPCR by
5 itself does not generate recombinants.
6 DR. ROBERTS: Any other comments or suggestions
7 in response to this question?
8 Dr. Kramer. Any follow ups.
9 DR. KRAMER: I think the answer is fine. Thank
10 you.
11 We have one more question on this general topic
12 area. Let me suggest that we go ahead and take this
13 question number 16 and then break for lunch.
14 DR. KRAMER: Please comment on how necessary
15 and/or sufficient each of these conditions is to minimize
16 the potential for novel viral interactions.
17 Please address specifically what combination
18 would be most effective or what conditions could be
19 modified, added or deleted to ensure that potential
20 consequences of novel viral interactions in PVCP PIP
21 transgenic plants are minimized.

1 Number one, the genetic material of the PVCP PIP
2 is translated and/or transcribed in the same cells,
3 tissues and developmental stages naturally infected by
4 every virus from which any segment of a coat protein gene
5 used in the PVCP PIP was derived.
6 Number 2. The genetic material of the PVCP PIP
7 contains coat protein genes or segments of coat protein
8 genes from viruses established throughout the regions
9 where the crop is planted in the United States and that
10 naturally infect the crop into which the genes have been
11 inserted.
12 Number 3. The PVCP PIP has been modified by a
13 method scientifically documented to minimize
14 recombination. For example, deletion of the 3 prime
15 untranslated region of the coat protein gene.
16 Number 4. The PVCP PIP has been modified by a
17 method scientifically documented to minimize heterologous
18 encapsidation or vector transmission or there is minimum
19 potential for heterologous encapsidation because no
20 protein from the introduced PVCP PIP is produced in the
21 transgenic plant or this virus does not participate in

1 heterologous encapsidation in nature.

2 DR. ROBERTS: Dr. Kramer, is there any

3 background you want to give on this question?

4 DR. KRAMER: I think the background is the same

5 for the question about gene flow that we were considering

6 before. That is that we're really looking at whether we

7 can come up with a set of factors that the agency could

8 use to determine products that are extremely safe.

9 And that is that in order to -- when we're

10 asking whether these conditions are necessary, in order to

11 conclude that they are not necessary, one would have to

12 say that under no circumstances with any virus in any

13 plant planted in any region in the United States would

14 there be a concern for the condition that the factor is

15 attempting to address.

16 DR. ROBERTS: Thank you. Dr. Tepfer.

17 DR. TEPFER: I must confess that I find this a

18 particularly difficult question. What I'm going to do is

19 go through the 4 sort of suggested, the questions, the sub

20 questions, the points that are raised briefly. And then

21 sort of make a few comments at the end.

1 The first one has to do with having the gene
2 expressed in the same tissues as the virus is usually
3 present in normally infected plants.
4 Admittedly, this is perhaps helpful to prevent
5 the virus in interacting with distantly related viruses
6 that would have different cell tropiziums. But this is a
7 rather marginal sort of circumstance.
8 There are of course plant viruses which are
9 phloem restricted. But most plant viruses tend to go
10 pretty much through a wide range of cell tissues.
11 So this doesn't strike me as an extraordinarily
12 useful thing to insist upon. The second thing,
13 the second point has to do with only using coat protein
14 genes or segments thereof, which I thought was an
15 interesting thing to have added in from viruses that are
16 prevalent in the area where the genes -- where plants
17 would be deployed. Here again, this doesn't
18 seem to me to really be very discriminatory. It seems to
19 me that this is going to be the case in nearly all
20 circumstances.
21 I think that the types of plant viruses that we

1 see tend to be rather broadly distributed. For instance,
2 with cucumber mosaic virus you tend to see the same types
3 of strains almost worldwide with a few exceptions -- with
4 group 1 b, which is more prevalent in Asia than elsewhere,
5 but that's changing very rapidly.
6 Here, again, this strikes me as a rather poorly
7 discriminant sort of a suggestion to make.
8 We have talked already in the third point
9 previously. The third point has to do with deleting the 3
10 prime untranslated region. This I think we all agree will
11 decrease the frequency of recombination in transgenic
12 plants.
13 Whether that remains adequate or not is
14 something also that we discussed again previously. It has
15 to do with to my sense again value judgments as to what is
16 acceptable in terms of frequency recombination and whether
17 recombination does lead to molecules that we would not
18 like to see being created.
19 The fourth point has to do with preventing
20 heterologous encapsidation or vector transmission.
21 And I thought one thing in here again we have

1 talked about this can be done. The question is whether
2 this is necessary. Whether there really are cases where
3 we need to do something about this or whether we can
4 simply say, well, heterologous encapsidation is not a big
5 problem except perhaps for one or two cases that would
6 stand out from this.
7 And then one thing that struck me in the
8 formulation of this question is that it then goes on to
9 suggest what about transgenes from which no protein is
10 synthesized.
11 I would simply like to suggest that this last
12 possible idea is probably the only really good suggestion
13 among them.
14 I think if you are really concerned about
15 heterologous encapsidation, if you really want to
16 absolutely minimize the degree of recombination, probably
17 the best thing to do is to simply abandon coat protein
18 protection to start with and to focus on RNA mediated
19 group protection that is related to post transcriptional
20 gene silencing also known as RNAI.
21 It seems to me that -- my take on this question

1 is really, well, you have all these sorts of things that
2 will sort of attenuate possibly a little bit or maybe a
3 little bit more some of these things, questions that are
4 being raised. You are talking about sort of small
5 adjustments.
6 But if you are really concerned, why not simply
7 head for bigger adjustments and say, well, if it is really
8 a concern, we should do RNAI and not protein mediated
9 protection at all.
10 That's my provocative conclusion to this. I
11 don't think most of these are extremely useful, but I
12 don't think most of them really address questions that are
13 particularly all that critical.
14 DR. ROBERTS: Dr. Sherwood.
15 DR. SHERWOOD: I would have to agree
16 considerably with Dr. Tepfer's well-stated answer and he
17 dissected the question I think quite well.
18 Again, number 1, not particularly useful. I
19 think it would be difficult to put the proof on the person
20 that was proposing that they are able to demonstrate that
21 considerably.

1 Secondly, in regards to distribution in the
2 United States, that only putting the transgenic plant in
3 an area where the virus is naturally found prevents, say,
4 for example, if you see an epidemic in South Carolina one
5 year and have a good idea that it might be in Georgia the
6 next, if it prevents the next growing season not being
7 able to deploy the control measure without first having
8 gone through a disease year.
9 We have kind of beat number 3 to death, I think,
10 in terms of the untranslated region or the adequacy and
11 frequency of that.
12 Certainly, I think in number 4 would agree that
13 -- whether or not the trans encapsidation is really an
14 issue that is of any significant long term ecological or
15 environmental impact.
16 Again, coming to this meeting I would have to
17 agree with him that, and it has been said earlier
18 yesterday, that whether or not this technology that we
19 were talking about in terms of expression of coat protein
20 is kind of something that's the initial model T on how
21 we're going to bring about transgenic resistance and

1 whether it is really even going to be relevant to how we
2 go about doing transgenic virus disease control in the
3 future with things that are now being understood about the
4 RNI mechanism.
5 DR. ROBERTS: Dr. Hammond.
6 DR. HAMMOND: I agree essentially with what Mark
7 and John have said before me. I do not believe that there
8 is significant risk. However, I believe that there are
9 ways to reduce the perceived risks.
10 I have already stated that it is better to use
11 endemic isolates than exotic isolates as a source of
12 transgenes.
13 I have also expressed that I think it is better
14 to use constructs that have been engineered to minimize
15 accumulation to reduce perceived risks rather than real
16 risks. And that RNAI is probably the way to go.
17 Having said that, the second part of the
18 question here about viruses that are established
19 throughout the regions where the crop is planted, I would
20 like to point out that there is considerable variability
21 of the isolates present within the crop and that sometimes

1 this is very highly variable and that certainly for some
2 viruses the variation present within a region is
3 equivalent to the variation between regions.
4 So there may be variation in some instances
5 between the isolates that are present in different
6 geographic areas of the world. However, with some viruses
7 it has been demonstrated that the variability within one
8 region is equal to the variability that has been observed
9 between geographic regions. And one example of
10 that that I can point out to is some recent work that was
11 done on hosta virus x where variability within the United
12 States is equivalent to the variability within isolates in
13 Korea. And that there is essentially no difference in the
14 variability between the two regions.
15 So that isolates variations that occur in Korea
16 are very similar to variance that occur in America.
17 Examples of the variability within isolates have
18 been presented for papaya ring spot virus and turnip
19 mosaic virus and for other poty viruses that have been
20 discussed extensively in the book, The Poty Verity put
21 together by Schuplar, Ward and Brandt (ph).

1 To oblate perceived risks, there are many things
2 you can do. You can modify the coat protein to prevent
3 RNA binding, to interfere with subunit, subunit
4 interactions, to eliminate the APHID transmission regions
5 that read (ph) through domains.
6 To use defective copies of genes that are known
7 to interact with other viruses, avoidance of replicase
8 recognition sequences, use of untranslatable or anti (ph)
9 sense sequences, defective interfering RNAs, permitting
10 genes that are effective through different mechanisms to
11 increase the level of resistance.
12 So there are many things that can be done. But
13 I believe that these would all be to reduce perceived risk
14 rather than actual risk.
15 DR. ROBERTS: Dr. Tepfer.
16 DR. TEPFER: I must confess I'm extremely
17 uncomfortable with the idea of requesting that people do
18 things to deal with perceived risk. I think that this is
19 something that we have suffered enormously from in Europe.
20 I think if biotech is in the pitiful state that
21 it is in Europe presently, one of the great problems has

1 been trying to address perceived risk. It is an endless
2 process. You cannot get to the end of it.
3 And I think that some of the things that John
4 has been talking about could perhaps better be stated as
5 trying to deal with what is a very difficult question. We
6 may need to get at the end of this is what are the levels
7 of acceptable risk.
8 And I think that couched in those terms I would
9 be more comfortable with some of these suggestions that he
10 was making, but I think that talking about perceived risk
11 is just opening an absolutely horrendous Pandora's box.
12 Let's not do that, I would suggest strongly.
13 DR. ROBERTS: Dr. Melcher.
14 DR. MELCHER: I guess I'm confused about Dr.
15 Tepfer's attitude from the discussion we had earlier on
16 recombination. The attitude with respect to number 2, the
17 avoidance of putting the plants out in regions that
18 viruses don't naturally exist.
19 I ask you to think about maybe 5 or 10 years ago
20 in the United States where we did not have plumb pox
21 virus. We were awfully concerned that plumb pox virus

1 would come into the United States.

2 If at that time we had put out orchards which

3 had a VCP PIP for plumb pox virus and perhaps there would

4 have been a recombinant and then we would have the

5 equivalent of plumb pox virus if they would have

6 recombinant with some other poty virus that was in that

7 vicinity.

8 I would have thought he would have had that kind

9 of a scenario in mind. But maybe he doesn't think that

10 that recombination could have happened. I'm not quite

11 sure.

12 DR. TEPFER: Thank you very much for bringing

13 that up. I think you are in fact absolutely right. I was

14 thinking of more like strain differences and things like

15 that. But it is true that if you are talking about the

16 rare situations where a virus is absent from a whole

17 region or continent, then, of course, the question is one

18 that needs to be addressed.

19 And obviously, I thank you very much for

20 bringing this up. It was not an exaggeration, but an

21 overgeneralization on my part. Thank you.

1 DR. ROBERTS: Dr. Nagy.

2 DR. NAGY: I have comments regarding the first
3 point. Actually, I think this is a relevant comment
4 because if you take the examples of barley yellow dwarf
5 virus, which is the coat proteins in -- natural infection
6 is being expressed only in the phloem.

7 If you have transgenic plants which express
8 coat protein everywhere, all tissues, I think that
9 incredibly increases the transfer combination. Not with
10 barley yellow dwarf, but other viruses which infect those
11 tissues.

12 I think this is unnecessary things to do. I
13 would actually recommend this first point to reduce the
14 chance for recombination between different viruses.

15 DR. ROBERTS: Dr. Tepfer.

16 DR. TEPFER: Here, again, I did say -- I was
17 talking about the most general cases. I did mention there
18 were exceptions, in particular, the phloem limited
19 viruses. I was suggesting this is not a universal
20 problem, but certainly in the cases you are talking about.
21 And I think I did mention that.

1 This is something that should be taken into
2 account.
3 DR. ROBERTS: Dr. Hammond.
4 DR. HAMMOND: Two points of clarification. In
5 the particular case of plumb pox virus, as far as I'm
6 aware there is actually no other poty virus that is known
7 to infect pronus (ph), other than plumb pox virus.
8 So the risk of recombination with another poty
9 virus infecting pronus is absent.
10 With respect to barley yellow dwarf, barley
11 yellow dwarf virus can actually replicate in mesafilled
12 (ph) protoplasts of cereals. I'm not sure about the
13 question with barley yellow dwarf itself, but with other
14 luteo viruses in mixed infections they can escape from the
15 phloem and multiply in other tissues.
16 This has been shown with potato leaf roll virus
17 in Scotland. I'm trying to think -- was it PVY?
18 DR. COOPER: Yes.
19 DR. HAMMOND: So in mixed infections, luteo
20 viruses can escape from the phloem and replicate in other
21 tissues. Again, the possibility of something happening in

1 transgenic plants as a result of expression of the
2 transgene in other tissues is not so very different from
3 what can occur in mixed infections.
4 DR. ROBERTS: Dr. Melcher.
5 DR. MELCHER: Responding to the plumb pox virus.
6 This was meant to be a hypothetical example and not
7 necessarily that specific.
8 But imagine the case where the virus does not
9 exist in the United States at all, but we have a desire to
10 protect our crop against that virus. We put in a
11 transgene. There may be some possibility for
12 recombination. That was the point.
13 DR. ROBERTS: Dr. Hammond.
14 DR. HAMMOND: If I may come back to that again.
15 If we had had pronus resistant to plumb pox virus, there
16 would have been many fewer orchards that were bulldozed
17 and destroyed to prevent the spread of plumb pox virus.
18 The cost of the introduction of plumb pox virus
19 is in the millions of dollars. And even beyond the
20 attempts to eradicate the virus locally, those orchards
21 cannot be replanted for another 7 years after the last

1 case of plumb pox infection is found in that area.
2 These costs for the growers in those areas are
3 enormous and the benefits of having had resistance to
4 plumb pox virus would have been tremendous.
5 DR. ROBERTS: Dr. Kramer.
6 DR. KRAMER: I have a couple follow-up
7 questions.
8 DR. KRAMER: I hear that there is some
9 disagreement over conditions 1 and 2, whether there would
10 be any use for them at all. If we just let that, correct
11 me if I'm wrong, but if we just let that disagreement
12 stand, could I ask whether conditions 1 or 2 -- could you
13 give me a sense of how onerous they would be?
14 How many products might not fit under these
15 conditions, or is it just the case that the people who
16 would think they are unnecessary just find them not
17 useful?
18 DR. ROBERTS: Dr. Sherwood.
19 DR. SHERWOOD: I guess in terms of condition 2,
20 I would view that as someone having essentially write a
21 section 18 for the use of coat protein mediated

1 protection.

2 Be an emergency label per se being able to use

3 that crop in a state. I think it would be a case of -- it

4 would be too little too late.

5 Essentially, you would have to go to, like I

6 said, go through a growing season and show that the

7 disease was now present in your state or area or whatever

8 that we're going to define that before you would be able

9 to use the technology.

10 DR. ROBERTS: Dr. Hammond.

11 DR. HAMMOND: I think that both really are

12 unnecessary. And with respect to question 1, going back

13 to the luteo viruses, the study by Thomas, et al., with

14 the plants that were transgenic for both potato leaf roll

15 virus and potato virus Y and field tested over, was it,

16 six seasons, and there was no evidence of any untoward,

17 any different viral activity, any difference of disease

18 symptoms present over that time.

19 That is the best evidence that we have at this

20 time and there is no evidence for any change in virus or

21 disease as a result of that even though potato leaf roll

1 is normally restricted to the phloem and the transgene was
2 expressed in essentially all tissues in that case.
3 DR. ROBERTS: Dr. Tepfer and then Dr. Allison.
4 DR. TEPFER: To answer, try to address more
5 directly the question of the cost of implementing
6 something like this, I think generally speaking, again,
7 there are exceptions for these, these are not costly
8 questions.
9 For instance, using preferably coat protein
10 genes from local or not too distantly geographically
11 isolates is a sort of thing that is relatively easy to do.
12 It is not a complex problem.
13 It is the sort of thing that one is likely to
14 anyhow since one tends to have better protection using
15 homologous viral sequences than using distantly related
16 ones. Although, there, again, there are exceptions to
17 that.
18 To the tendency is to try to use local strains,
19 local coat proteins. So it is sort of already more or
20 less general practice. That's all I wanted to say.
21 DR. ROBERTS: Dr. Allison.

1 DR. ALLISON: In reference to number 1, while
2 theoretically it might be practical, that, in reality,
3 most of these promoters that are tissue specific are very
4 leaky or leaky to some degree. There is really no
5 concrete insurance that you are not going to get
6 expression in these other cells.
7 DR. KRAMER: How many viruses are there that
8 would not be expressed in all cells anyway?
9 DR. ALLISON: I don't know if I can tackle that
10 one. In terms of a number, I can't give you a hard core
11 number. But there are certainly cell types that --
12 DR. KRAMER: I guess I could say are most
13 viruses infecting all cells in the first place? Is that
14 true?
15 DR. ALLISON: I would tend to go on that side of
16 the dividing line. Yes. There are, as we talked about
17 phloem limited viruses, there are parts of the plant --
18 there are viruses that don't appear to be in the seeds
19 because they are not seed transmitted. So they may not be
20 in that replicating tissue.
21 Probably my colleagues can add to that.

1 DR. ROBERTS: Dr. Melcher.

2 DR. MELCHER: Most viruses I think are poty

3 viruses and poty viruses are all over. They are the most

4 abundant kind of plant virus, a poty virus. Is that not

5 correct?

6 DR. ALLISON: More than 200 different poty

7 viruses.

8 DR. ROBERTS: I guess I didn't -- the last part

9 of it you turned away and talked to your colleagues. Most

10 of them are poty viruses and?

11 DR. MELCHER: Therefore -- poty viruses are

12 infecting all cells. So, therefore, most of the viruses

13 are infecting all cells.

14 DR. ROBERTS: Thank you. I just wanted to make

15 sure we got that heard and on the record.

16 Dr. Tepfer.

17 DR. TEPFER: Even going beyond poty viruses, of

18 course, most other virus groups are also rather general.

19 It is more I think the exceptions which are tissue

20 specific.

21 DR. FALK: A couple of very minor points here.

1 To say that most viruses are poty viruses I would rephrase
2 that a little bit and say that the virus family with the
3 most members would be the poty verity (ph). So yes.
4 It is not all cells. Because within the plant
5 there are many, many, many cells. They have the potential
6 to infect all living cell types, I would say, but they are
7 not infecting all cells for sure.
8 DR. ROBERTS: Dr. Kramer.
9 DR. KRAMER: Then I have another question.
10 There is still some disagreement, but we'll just let that
11 stand over whether there is any value in having conditions
12 1 and 2. But there does seem to be agreement that if
13 conditions 1 or 2 were met there would be no need for
14 conditions 3 or 4.
15 Correct me if that's wrong, but the real
16 question is if conditions 1 and 2 were not met for those
17 that would find some value in them, would satisfying
18 conditions 3 and 4 be sufficient, 3 alone, 4 alone?
19 Could you maybe expand on how these might be
20 used in the cases when 1 and 2 are not met?
21 DR. ROBERTS: Dr. Tepfer.

1 DR. TEPFER: I don't agree with your starting
2 premise. I don't think that meeting conditions 1 or 2 in
3 any way is a guaranty.
4 These are ways of minimizing a little bit
5 certain potential risks. I don't think it is going to
6 have a major effect. To my way of thinking, these are
7 things which may or may not be done and the difference is
8 not going to be great.
9 It was more in that sense that I was saying that
10 these are perhaps not particularly important except for a
11 few exceptional cases.
12 So I don't think that to my thinking they would
13 in any way exempt consideration of the other factors that
14 are being raised.
15 DR. KRAMER: But I understood -- I didn't hear
16 any support for the idea that conditions 3 and 4 would be
17 necessary.
18 DR. HAMMOND: I would agree that that I don't
19 think that conditions 3 and 4 are necessary. They are
20 things that would reduce perceived risk. Potential risk.
21

1 But I do not believe that they -- I do not
2 believe that the risks are significant that these
3 conditions need to be imposed.
4 DR. ROBERTS: Other viewpoints. Dr. Tepfer.
5 DR. TEPFER: I want to go back to -- among the 4
6 points that we have raised, the one that seems to be
7 potentially the more interesting to my thinking at least
8 has to do with point 3 as a means of reducing the
9 frequency recombination because I think that the potential
10 impact recombination is relatively great.
11 On the other hand, I think that again it is in a
12 way a weak measure. So that, in fact, my -- the reason
13 why I don't think that it is important to impose this
14 entirely is I don't think that it is, in fact, entirely
15 effective.
16 So instead of saying that it is -- we don't need
17 to impose any of these 4 conditions because this is merely
18 a question of perception, I would say we don't need to
19 impose them because I don't think they would be all that
20 effective to make it worthwhile. DR. ROBERTS:
21 Dr. Sherwood.

1 DR. SHERWOOD: I would have to agree with that.
2 Because I don't know -- particularly, 1 and 2, going back
3 to those, how you would ever establish the bar to measure
4 those.
5 As we have heard, there is a lot of variation
6 how viruses are distributed in plants and how mixed
7 infections impact that distribution. And certainly there
8 is a wax and waning of distribution of viruses in the
9 United States as epidemics come and go.
10 I don't know how you would over time -- what
11 would you use in order to establish the bar for meeting
12 number 2.
13 DR. KRAMER: I think I heard a suggestion from
14 Dr. Hammond earlier. What if number 2 were rephrased to
15 say that the virus -- the coat protein is from an endemic
16 isolate of the virus?
17 DR. SHERWOOD: I don't know if that is directed
18 to me.
19 DR. KRAMER: To anybody.
20 DR. SHERWOOD: You are going to have to define
21 endemic. The American Phytopathological Society in

1 conjunction with APHIS has been trying to develop a list
2 of endemic viruses for nearly 10 years.
3 We cannot come to agreement with APHIS on the
4 definition of endemic.
5 DR. HAMMOND: I would have to say that the use
6 of endemic isolates as I understand endemic isolates is by
7 far the most common and makes the most sense.
8 You do not have to go and get a permit to use an
9 -- to have in your possession an endemic isolate if it
10 comes from your own state.
11 It is in most cases the position of choice.
12 However, as I stated before, in many viruses there is a
13 great degree of variability and there is no significant
14 difference between the variability that is present in
15 local isolates and isolates that are present across the
16 world.
17 In some instances there is, but there are also
18 many isolates that have not been characterized, so the
19 degree of variability is probably greater than we're
20 currently aware.
21 DR. ROBERTS: Other viewpoints?

1 DR. FALK: What are you thinking now?

2 DR. KRAMER: Well, I heard that there was some

3 support for the idea that conditions 1 and 2 --

4 DR. FALK: I'm just talking about the last point

5 here.

6 DR. KRAMER: Whether we would be able to

7 identify endemic?

8 DR. FALK: Yes. Does endemic mean United

9 States?

10 DR. KRAMER: Yes.

11 Elizabeth was saying there is potential that we

12 could define endemic based on the region where the crop

13 might be planted, so that a virus isolate from the Virgin

14 Islands would not necessarily be acceptable for planting

15 in another area of the United States.

16 DR. FALK: The only reason I brought that up,

17 certainly the triple gene resistance squash is grown all

18 over the United States. And that's a -- I assume those

19 must be New York isolates of the virus that were used.

20 That's very effective everywhere.

21 United States is good, I think.

1 DR. ROBERTS: Dr. Kramer -- let's get Dr.
2 Sherwood. We had interrupted you. You were about to say
3 something about points 1 and 2.
4 DR. SHERWOOD: If United States is going to be
5 used as definition of endemic, I would certainly endorse
6 that. But if we use a state by state definition of
7 endemic that APHIS is using, then I see this very
8 difficult for any company to be able to bring a product to
9 market.
10 DR. ROBERTS: Dr. Kramer, you were about to sort
11 of give your impressions back on 1 and 2 and sort of get
12 some closure on where we stand on those.
13 DR. KRAMER: Right.
14 As I understand it, there is some support for
15 the idea that there would be some value in having 1 and 2
16 to minimize risk with the caveat that we would amend 2 to
17 define just that the coat protein was from an isolate
18 endemic to the United States.
19 There is not agreement on that, I understand,
20 but there are some people who feel that those would be
21 valuable. And that there is no agreement -- no one

1 supports the idea of conditions 3 and 4 under any
2 circumstances including when conditions 1 and 2 are not
3 met.
4 DR. ROBERTS: Let's see. Does everyone agree
5 with that assessment?
6 Does anyone disagree with that assessment? Dr.
7 Tepfer, Dr. Sherwood.
8 DR. TEPFER: I just -- I don't think that it
9 should be useful to impose condition 1. It seems that it
10 is only going to be a restraint for a small number of
11 virus groups. I'm still not convinced that that's
12 absolutely useful to do so.
13 DR. ROBERTS: Dr. Sherwood.
14 DR. SHERWOOD: That would be my question on how
15 would the agency evaluate that as a criteria. Is it
16 possible to evaluate that as a criteria considering the
17 discussion about the impact of mixed infections and other
18 things that we have talked about.
19 DR. KRAMER: I guess I would throw that question
20 back on you. Are you saying, then, that you don't think
21 that's possible to evaluate?

1 DR. SHERWOOD: No, I don't.

2 DR. ROBERTS: I have heard a lot of

3 reservations about the value of 1 and 2. Let me see. Are

4 there panel members that feel that 1 and 2 have value?

5 Dr. Falk?

6 DR. FALK: I was going to say I agree with what

7 Mark, the way he rephrased the statement. I don't see any

8 reason for one.

9 DR. ROBERTS: Dr. Allison.

10 DR. ALLISON: I believe it is worthless.

11 DR. KRAMER: Could I ask Dr. Tepfer if he is

12 suggesting that there might be certain cases that could be

13 identified now in which number 1 would be important?

14 DR. TEPFER: The only cases we talked about were

15 the phloem restricted viruses where perhaps in a risk --

16 minimizing risk to some extent, potential risk, perceived

17 risk below a level of -- the reason I object particularly

18 to basically all 4 of these is that it is I think very

19 dangerous to think that applying any of these would confer

20 any real protection against potential risk.

21 I think that that's why I think that it is

1 dangerous to mandate things that are not effective.
2 Considering the flexibility even of at least
3 some of the phloem restricted viruses such as the luteos,
4 if you have an infection by other type of viruses, this
5 can lead to the luteo being released from the phloem into
6 the other cell types.
7 Again, you are just going through more -- in a
8 sense you're creating possible sort of hoops that people
9 will need to be hopping through giving a false sense of
10 assurance that this does something. It also distracts
11 people from trying to think about what might in fact be
12 the real critical issues if there are any.
13 Even if you could sort of draw a very
14 hypothetical sort of proposal to say, well, yes, for
15 luteos let's try to do it with just phloem promoters,
16 which are more or less phloem specific, I think you are
17 still sort of fooling yourself into thinking this would
18 really be effective in preventing something.
19 DR. ROBERTS: Dr. Kramer, we can continue to poll the
20 panel. I wasn't sure I heard a lot of support for
21 conditions 1 or 2. I'm not sure how divided the panel

1 really is on that.

2 Dr. Melcher.

3 DR. MELCHER: I think the reasons for conditions

4 1 and 2 are that they will minimize recombination,

5 minimize trans encapsidation and minimize vector

6 transmission. So they are really covered by items 3 and

7 4. Given the hesitation, perhaps they are not necessary,

8 then.

9 If the company can convince that the PVCP PIP

10 has been modified to minimize recombination, maybe that

11 would be grounds for an exemption, regardless of how they

12 have done it. They might have done it by using 1 and 2

13 and convince you that that's the reason that they have --

14 that the risk is minimal. DR. ROBERTS: Dr.

15 Tepfer.

16 DR. TEPFER: I guess what we're seeing is two

17 different reasons for objecting to these 4 proposals. One

18 is that some people are objecting because they think that

19 -- because they don't think that the potential risks are

20 important and that reducing them is significant.

21 And another way of object is to say that they

1 aren't effective. I think we're seeing two different ways
2 of objecting to these 4 types of mitigating proposals.
3 DR. KRAMER: I guess you are one of the people
4 who says they are not effective. Maybe I'm actually
5 jumping back to a question from earlier today. Would you
6 suggest that the risks are significant enough that some
7 type of strategy should be employed, if not these, then
8 something else?
9 DR. TEPFER: That's a difficult one. I think
10 that -- we don't have any -- you are really getting to the
11 question what is acceptable risk. And that's an
12 extraordinarily difficult question to deal with.
13 I'm not in a regulatory sort of mindset myself.
14 I sort of avoid asking myself that question. I
15 understand for you that's the critical one.
16 DR. KRAMER: I guess what I feel that we haven't
17 gotten was when I was pushing so hard back on I think
18 question 9 was what the level of risk is.
19 And then the question of acceptable can come
20 later. But I don't really feel like we got a handle on.
21 I understand the panel seems to be saying that

1 in the vast majority of cases there is no real appreciable
2 risk at all, but people keep pointing out specific
3 examples in which there might be some appreciable risk.
4 I want to understand in those conditions, in
5 those rare cases what is the level of risk that we're
6 talking about.
7 DR. TEPFER: But it is something that we can't
8 quantify. What units are you talking about in risk?
9 There are no values that we can apply to any of
10 these potential risks that we have been talking to. You
11 can't quantify it in dollars. You can't quantify it in --
12 I think that you are asking us to do something
13 that is pretty difficult to do, if not impossible.
14 DR. ROBERTS: Dr. Melcher.
15 DR. MELCHER: Maybe the risk people should say
16 this. But risk is the product of frequency times hazard.
17 So hazard is probably measurable in dollars. Frequency
18 is something that we -- perhaps they are asking us to come
19 up with. I think we really don't have a good number for
20 frequency, but it certainly -- if we did, it could be
21 quantified.

1 Is that not correct?

2 DR. KRAMER: I think we're also asking for your

3 opinion on what the hazard is. In these rare cases what

4 really is the concern.

5 DR. ROBERTS: This is certainly -- I know this

6 is the bottom line question. And maybe what we ought to

7 do is return to this as the sort of one of the last

8 questions and we can try again. I mean, I know that's

9 what you are trying to get from the panel. But many of

10 the questions are worded fairly specifically.

11 And the panel is sort of focused on answering

12 those. And we can pose that broader question to the panel

13 and through some dialogue probably give you as good as we

14 can.

15 Let me propose that we do that kind of at the

16 end. I thought one of the things you were going to hit on

17 this one was if --let's assume for the moment that -- if

18 you are of the position that there is not an appreciable

19 risk, then these really don't matter.

20 If assuming for a moment that there is a risk

21 that you want to avoid, do these conditions help identify

1 those situations. And if these conditions don't, what
2 conditions -- and there seems to be some opinion these
3 conditions really aren't very useful, are there other
4 conditions that would be more useful for that purpose.
5 DR. KRAMER: Yes.
6 DR. ROBERTS: Let me pose that to the panel.
7 Dr. Hammond and then Dr. Tepfer. DR. HAMMOND:
8 I would like to quote a couple things from the OECD
9 consensus document on general information concerning
10 biosafety of crop plants. Specifically viral resistant
11 through coat protein gene mediated protection.
12 With or without the use of transgenic plants,
13 new plant virus diseases will develop that will require
14 attention.
15 And a quote from Dan Roshum (ph) of Agriculture
16 and Agri Food Canada, it is likely that current means of
17 detecting and controlling new diseases in this country
18 would be adequate to control any new virus resulting from
19 recombination between a transgene and another virus.
20 New viruses will arise whether from mixed
21 infections, from natural evolution and selection from as a

1 result of the quasi species nature of plant viruses or
2 from recombination between viruses and transgenes.
3 I don't see any difference in the quality of the
4 viruses that are likely to arise by recombination from
5 mixed infections or from a virus infecting a transgenic
6 plant. If there is no difference in the quality
7 of the virus that would result from the 2 cases, why are
8 we overly concerned with the possibility, and it is a
9 hypothetical of a different virus arising from
10 recombination within a transgenic plant.
11 DR. ROBERTS: Dr. Tepfer.
12 DR. TEPFER: I guess I'm going to start by
13 responding to some of that. I think that indeed now
14 viruses will appear. And that's one of the facts of life.
15 I think that one of the sort of the question we will need
16 to ask maybe at the end of this is to go into more detail
17 about is this a complete nonissue in which case we have
18 been wasting 3 days.
19 In any case, we're quite used to dealing with
20 plant viruses. There is no reason to be worried about new
21 viruses that will appear because we simply know how to

1 deal with it.

2 This has been proposed for many years, that we

3 should simply stop worrying about all this, because, after

4 all, plant pathologists and agronomists have been dealing

5 with viruses for decades and they know what they are

6 doing.

7 I think that's an interesting point of view. I

8 think we should put that off to the end. I think that's

9 one of the big picture questions we need to deal with.

10 Concerning whether new viruses will occur in

11 transgenics that are different from non transgenic plants,

12 I think that everyone believes there will be no

13 difference. I would just like to wait until we have a

14 little bit of data. That's what we're trying to do.

15 There is no reason to suspect otherwise.

16 Everyone's working hypothesis, everyone's I would say in

17 many cases conviction is that there will be no

18 differences. Nonetheless, I think it would be very

19 reassuring if we had some solid data showing that indeed

20 the same viruses are occurring by recombination in the

21 transgenics as in the non transgenics. But I think that's

1 where we're headed.

2 DR. ROBERTS: Back to my question, then. If you

3 accept that the possibility exists that there may be some

4 new viruses but don't see value in these particular set of

5 conditions, are there other conditions that would be more

6 useful. Dr. Tepfer.

7 DR. TEPFER: That's why I suggested at the end

8 of my first remarks on this question was that if that was

9 where we were headed, then we should simply abandon coat

10 protein expressing plants.

11 Because in the face -- if you decide that the

12 risks are significant, the 4 possible mitigation

13 techniques are relatively weak, then you should look for

14 something that is going to be quite different.

15 I think, for instance, RNAI type, PTGS

16 resistance is a good alternative. Either we decide that

17 they are important or they are not important and then in

18 accordance with that afterwards either contending (ph)

19 with the coat protein or we don't.

20 DR. ROBERTS: Let me ask for other viewpoints.

21 DR. FALK: Certainly there are some examples now

1 of effective coat protein. So I don't think -- in papaya
2 and in squash. We don't want to eliminate those. But I
3 think the future I think will be moving the direction that
4 you are suggesting.
5 I think we don't want to eliminate what we have
6 because it is working and it is good. It is showing
7 definite benefits.
8 DR. ROBERTS: Dr. Kramer, I think the panel sees
9 limited value in these conditions. But does not come up
10 with alternative conditions.
11 DR. KRAMER: Okay.
12 DR. ROBERTS: Dr. Sherwood.
13 DR. SHERWOOD: I don't have conditions, but I
14 would have to conclude after sitting here for 2 days that
15 the risk of using coat protein mediated resistance is no
16 greater than the risk of emerging viruses. And
17 one example in Georgia in the late 80s is tomato spotted
18 wilt mated from the West Coast with franklinia
19 accidentalis (ph). We were suffering losses of 40 million
20 dollars in our peanut crop and significant losses in
21 tobacco crop and also significant losses in our vegetable

1 crops due to that disease.

2 We know that transgenic resistance works very

3 well in tobacco. It also works very well in peanut. But

4 that technology is not going to be used because of lack of

5 consumer acceptance and other issues.

6 I don't perceive this as having any greater risk

7 than the normal risk that we face during the continued

8 either new strains or new viruses coming into our

9 production areas or viruses occurring there overcoming

10 resistance that has been deployed.

11 DR. ROBERTS: Dr. Kramer.

12 DR. KRAMER: Unless I can think of another way

13 to rephrase this question over lunch, I think I'm done for

14 now.

15 DR. ROBERTS: I will give you that opportunity.

16 Let's break for lunch.

17 If you come up with another way to rephrase it,

18 we will entertain it first thing right after lunch.

19 Otherwise, we'll move on to the next question. Let's

20 break for an hour. Come back at 1:30.

21 (Thereupon, a luncheon recess was taken.)

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