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FIFRA SCIENTIFIC ADVISORY PANEL (SAP)  
OPEN MEETING  
OCTOBER 13 - 15, 2004  
ISSUES ASSOCIATED WITH DEPLOYMENT OF A TYPE OF  
PLANT-INCORPORATED PROTECTANT (PIP), SPECIFICALLY  
THOSE BASED ON PLANT VIRAL COAT PROTEINS  
(PVCP-PIPS)

WEDNESDAY, OCTOBER 13, 2004

VOLUME II OF IV

(Afternoon session)

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

Reported by: Monica Knight Weiss, Stenographer

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A F T E R N O O N   S E S S I O N  
MR. ROBERTS: We decided that we were going  
to finish up question 3 after lunch and get a change  
to maybe think about the list a little more, and I see  
that the question has now been I wouldn't say amended  
but let's just say it's been completed by showing the  
footnote that it was inadvertently dropped of on the  
copy distributed indicating how the United States is

9 defined, and there's also a tentative list we'll call  
10 it of species based on discussion prior to lunch.

11 So let's then -- and I don't know what Dr.  
12 Stewart, do you want to sort of lead off the  
13 after-lunch discussion on this or maybe Dr. Tepfer  
14 might be a logical person. No, he's pointing to Dr.  
15 Stewart, Dr. Stewart can you lead off the discussion  
16 to finish off question 3.

17 DR. STEWART: Well I guess during lunch we  
18 discussed sugar cane as possibly having or that was  
19 before lunch, you know we discussed sugar cane as  
20 being more of a tropical crop, anyway there's minimal  
21 acreage in the U.S. that has sugar cane. Most of our  
22 sugar comes from sugar cane in the tropics, so shatter

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1 cane is a wild relative that could potentially be  
2 there.

3 The one thing that I would like to say about  
4 this list of plants is that it should be open for  
5 revision during the next few weeks as we really dig  
6 into it, so we'll add some things here, perhaps take  
7 some things away, I mean because I don't -- no one  
8 here has an encyclopedic knowledge of all plants, all  
9 crops, all wild relatives, and I would especially say  
10 in the trust territory of pacific islands.

11 MR. ROBERTS: We're deciding what we can do  
12 about that under fagu (ph).

13 MR. LEWIS: Thank you, Dr. Stewart. This is  
14 Paul Lewis again, designated Federal official for the  
15 SAP and again the intent of the meeting we have here  
16 is to have an open discussion of dialogue on all of  
17 the issues (inaudible) once the meeting is over the  
18 panel is writing its report basically summarizing the  
19 points that occurred here, so if we have any new ideas  
20 or suggestions after this meeting in terms of  
21 reflecting it as a panel consensus it's after the  
22 fact, so if you want to use the time today and

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1 tomorrow or the rest of the meeting time to look at  
2 this list and digest it and revise it you're welcome  
3 to do that, but once the meeting is over we want to  
4 come to a conclusion on the panel's position. Thank  
5 you.

6 MR. ROBERTS: Dr. Tepfer.

7 DR. TEPFER: I just want to suggest that we  
8 all continue to think about this seriously because if  
9 some of these minor crop plants could be exempted  
10 because they are no wild relatives this could make a  
11 huge difference in terms of what can be done from a  
12 biotech point of view because the burden of going  
13 through the regulatory hoops is extremely heavy  
14 particularly for minor crops, so I would suggest that  
15 all of us continue to think about this over the next  
16 day or two and we can try to toward the end of the  
17 three days try to make a more complete list of -- not  
18 that it would take very long, just to try to get as  
19 much on as we can.

20 DR. GENDEL: Actually having dealt with  
21 similar kinds of issues in other contexts I would like  
22 to suggest that maybe what the people who know this

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1 subject should think about is perhaps not making the  
2 list complete but developing a criteria or mechanism  
3 by which the Agency can look in the future to make  
4 these decisions because I don't think anybody's ever  
5 going to have a complete list that answers all these  
6 questions, so what I think the Agency probably needs  
7 are a set of guidelines to how to go about making  
8 these decisions in the future.

9 MR. ROBERTS: For the record that was Dr.  
10 Gendel. Dr. Cooper.

11 DR. COOPER: Could I make a suggestion that  
12 circulars go out to the plant breeders of these crops  
13 who might well have unpublished experiences which will  
14 be relevant to this and they could be collected  
15 together. At the present moment these are scattered  
16 and very often unpublished experiences which would be  
17 relevant to know answers to these questions and we  
18 don't have very many if any plant breeding represented  
19 here.

20 MR. ROBERTS: Other comments or suggestions,  
21 Dr. Hammond.

22 DR. HAMMOND: This list has a very small

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1 number of ornamentals on but there are a very large  
2 number of ornamentals for which various problems are  
3 significant for which genetic engineering is under  
4 investigation and could reasonably be exempted by  
5 virtue of lack of wild relatives certainly I think in,  
6 certainly within the continental U.S., and I think  
7 probably many of them not in the territory of the  
8 south pacific, so there are a large number of  
9 ornamentals that could usefully be appended to that  
10 list.

11 I could add a few now.

12 MR. ROBERTS: Okay. Go for it.

13 DR. HAMMOND: Ornothogolum (ph) is a crop  
14 that is currently under consideration in our own lab,  
15 is gladiolus on the list, we have engineered  
16 gladiolas. There are wild relatives of geranium --

17 DR. STEWART: Actually the geranium is not a  
18 geranium, the genus is not geranium, the wild  
19 geraniums are geraniums.

20 DR. HAMMOND: But the new guinea impatientis

21 --

22 DR. STEWART: They have wild relatives.

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1 MR. ROBERTS: For the record the discussion  
2 was between Dr. Hammond and Dr. Stewart. In the  
3 interest of time, and I'll get to Dr. Sherwood in just  
4 a second, maybe we can, individuals around the panel  
5 can sort of brainstorm this evening over dinner or  
6 lunch or something or during the course of the meeting  
7 and we can give the Agency our best impressions at

8 this meeting of what the species are and then perhaps  
9 can make some recommendations about how to construct a  
10 more formalized list, obviously people are sort of  
11 reacting off their knowledge, but would that be  
12 satisfactory to the Agency and we can work on that  
13 during the course of this meeting. Dr. Hammond.

14 DR. HAMMOND: There is certainly a relatively  
15 recent reference and review of which ornamentals have  
16 been engineered or people are trying to engineer and  
17 from that it would certainly be possibly look at which  
18 of them have known wild relatives and which don't.

19 MR. ROBERTS: Perhaps we can get a copy of  
20 that reference while offline or something here and  
21 that might assist us in constructing our list.

22 Dr. Sherwood did you want to add something.

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1 DR. SHERWOOD: Yes. In trying to address  
2 this question in regards to what was suggested rather  
3 than make a list the question poses with the  
4 stipulation of that which they can produce viable  
5 hybrids in nature and maybe that should be the  
6 criteria that in addition to the crop plants listed  
7 those plants including ornamentals and others should  
8 be considered exempt if they are known not to produce  
9 viable hybrids in nature, that might be something  
10 added as an addendum to expand the list.

11 DR. STEWART: This is Neil Stewart, for many  
12 of these plants, especially once we get into the minor  
13 crops I'm not sure the data available.

14 DR. TEPFER: I just want to point out that as  
15 we get into more and more minor plant species some of  
16 them could also be invasive in themselves, so we need  
17 to not go toward exempting species that are  
18 potentially invasive.

19 MR. ROBERTS: Dr. Kramer, it seems that we  
20 need a little more time to work on this one, so we  
21 will do that and before we close out we will revisit  
22 this question and give you our updated view on the

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1 species.

2 DR. KRAMER: Should we go onto question 4  
3 then?

4 MR. ROBERTS: Let's go ahead and go onto  
5 question 4.

6

7 DR. KRAMER: What laboratory techniques used  
8 to achieve genetic exchange between species, for  
9 example, embryo rescue, use of intermediate bridging  
10 crosses, protoplast fusion, are not indicative of  
11 possible genetic exchange between these species in the  
12 field? Conversely, what techniques, if any, used in  
13 laboratory or greenhouse experiments provide the most  
14 reliable indication of ability to hybridize in the  
15 field?

16 MR. ROBERTS: Dr. Stewart, you're up again.

17 DR. STEWART: Lab-intensive methods to  
18 combine germ plasm such as embryo rescue and

19 protoplast fusion are not predictive of gene flow in  
20 the field. Hand crosses in growth chambers in  
21 greenhouses are marginally useful. Hand crosses can  
22 show if species are sexually compatible and if a

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1 transgene at a particular locus is transmittable and  
2 at what frequencies it provides a best-case scenario  
3 for transmission.

4 In the field there are factors that could  
5 prevent hybridization and integration including  
6 non-overlapping flowering, pollen competition, non  
7 selection linkage to equilibrium (ph), genetic  
8 exclusion, and competition within plant communities.  
9 So the short answer is that the lab techniques are not  
10 very useful in predicting gene flow in the field.

11 MR. ROBERTS: Okay. Dr. Cooper.

12 DR. COOPER: I would argue that they give you  
13 a worst-case scenario which is probably what would be  
14 very useful in a risk assessment, at least the hand  
15 crossing would even though the (inaudible) means a  
16 delivery of the pollen in that species would be an  
17 animal like a bee. Clearly the use of bees in glass  
18 houses in many hives and other things is a perfectly  
19 convenient technology, it takes a little time, it  
20 doesn't produce necessarily very high rates of  
21 transfer but they may be more realistic, but the rates  
22 I would argue of transfer is possibly not so relevant

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1 as the absolute occurrence of it.

2 So clearly wind and insect pollination is  
3 going to require different approaches towards the  
4 suitable delivery of the technology, but I would  
5 disagree with what seminus (ph) said, seminus said  
6 hand crossing is of little concern, I personally think  
7 it's a reasonably convenient way of measuring what can  
8 happen in the field albeit perhaps not at the rate  
9 level, but the fact that it can occur.

10 MR. ROBERTS: Dr. Hammond.

11 DR. HAMMOND: In general I agree with that,  
12 certainly wind pollination and bee pollination can be  
13 managed under contained conditions with some care and  
14 modifications, there are a number of other techniques  
15 that can be used to demonstrate crossability that have  
16 very little relevance in the field and I would add to  
17 those chromosome injection, application of pollen to  
18 cut styles or pre germination of pollen, cases where  
19 emasculation is necessary to achieve pollination  
20 because some species are normally self-fertilized and  
21 exclude foreign pollen and especially there are some  
22 species that are cryptogomist, that fertilize within

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1 their closed flower before the flower is even open,  
2 and of course there are cases where pollen storage is  
3 used to, collected from spring flowering species and  
4 used to pollenate fall-flowering species or vice-versa  
5 in that, that certainly has been used in our group  
6 with some tree species that where some flower in the

7 fall and others in the spring, so pollen storage for  
8 long periods is definitely not relevant to what  
9 happens in the field.

10 I think that's about all I had to add.

11 MR. ROBERTS: Dr. Melcher and then to Dr.  
12 Tepfer.

13 DR. MELCHER: I would agree with Dr. Cooper  
14 that the laboratory-type crossings are probably very  
15 useful because some of the impediments that Dr.  
16 Stewart mentioned are not really solid impediments,  
17 for example, the times of flowering, flowering times  
18 in plants are often determined by just a few genes and  
19 a mutation in one of those genes could very easily  
20 reverse the situation and thus make the cross  
21 pollination possible.

22 MR. ROBERTS: Dr. Tepfer.

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1 DR. TEPFER: If we're going toward the idea  
2 that pollination tests in the greenhouse -- our  
3 reviews, I just want to mention it's also a bit  
4 dependent on the genotype of the plants that you're  
5 working with so with certain brassicas you can get  
6 good crossing with one genotype and not another  
7 genotype even within the same species, so you have to  
8 be a little bit careful about that.

9 MR. ROBERTS: Over comments on this question,  
10 yes, Dr. Cooper.

11 DR. COOPER: Could I make a comment in  
12 relation to what John Hammond said, the concept of  
13 mentor (ph) pollen is sometimes brought up, it may be  
14 related to what you were talking about. So you use  
15 pollen to go to a different species perhaps even put  
16 it at the same time as your test species on the  
17 stigmatic surface and this does facilitate  
18 fertilization, this could be a realistic assessment in  
19 nature because it certainly could happen, but storing  
20 the pollen before you did that for long periods of  
21 time is obviously less relevant, but one of the ways  
22 in which is applied is of course to irradiate the

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1 pollen that you're using to help the process, that is  
2 clearly just removing a problem from your technology  
3 rather than anything else it doesn't actually say it  
4 doesn't happen in nature.

5 MR. ROBERTS: Other comments? Dr. Kramer is  
6 our response reasonably clear or would you like some  
7 clarification?

8 DR. KRAMER: I think that's fine, thank you.

9 MR. ROBERTS: If there are no other comments  
10 on 4 let's move onto number 5.

11 DR. KRAMER: Given that current  
12 bioconfinement techniques are not 100% effective, what  
13 would the environmental implications be of extremely  
14 low transfer rates of virus-resistance genes over  
15 time?

16 MR. ROBERTS: Dr. Cooper, could you lead our  
17 discussion on this one?

18 DR. COOPER: Yes, I would say potentially  
19 yes, a slow-burn impact that might escape  
20 recognition (ph) early enough to allow eradication  
21 of a problem, that's certainly a possibility. There  
22 are of course many uncertainties because of the

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1 variability of crop to wild gene-type flow, but the  
2 biggest gap concerns the actual magnitude of the  
3 resulting harm, not the fact of the movement.

4 If the transgenic gene was linked to  
5 agronomic traits including large seed size and other  
6 factors like that it's quite likely linkage drag would  
7 hold the process up, but there would be no stable  
8 introgression in that circumstance probably, however  
9 that benign outcome cannot necessarily be assumed to  
10 be overriding, for a variety of reasons the experience  
11 has been that hybrids survive poorly, but very rare  
12 and complex genetic exchanges do get perpetuated (ph)  
13 the brassica genome provides examples of a very  
14 complex series of apparent relationship changes that  
15 have taken place over a long period of time perhaps,  
16 but nevertheless the unlikely events do happen.

17 There is no reliable baseline to fall back on  
18 and as a need I would suggest for more specific  
19 hybrids between crops and wild relatives to be created  
20 and their fitnesses tested in the field, and that is  
21 obviously an area which was touched upon earlier this  
22 morning. We have little direct evidence of fitness

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1 over a whole lifetime in anything, it seems to me that  
2 our experiences with viruses and wild brassicas in the  
3 UK has revealed such complex in directions involving  
4 different vital genotypes and genetic diversity in the  
5 plants that prediction of the outcomes can certainly  
6 not be generic. In the UK the necessary planned  
7 releases would not necessarily be authorized and it is  
8 now appropriate to investigate the traditional -- in  
9 our particular case a specific virus that happened to  
10 be turnip mosaic virus resistance -- those that  
11 naturally occur in the species like brassica rapa and  
12 brassica nigra (ph) and to use those as surrogates of  
13 the transgenic which would not be so difficult to get  
14 permission to do, we need the information concerning  
15 the trait and its outcome and that would be one way of  
16 getting it.

17 Providing hybridization and stable  
18 introgression are possible genes from crops may  
19 increase infrequency when the gene confers greater  
20 lifetime fitness, that is the theoretical assumption  
21 behind this. This doesn't mean that hybrid plants  
22 would necessarily become more persistent or invasive,

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1 simple they could be an affect on biodiversity  
2 including some description of genetic integrity of  
3 local ecotypes, the hybrid-derived wild species may  
4 become more genetically uniform and never do, but  
5 these biodiversity changes may be important in certain

6 circumstances.

7           There may be fixation of the new genotype in  
8 a scattered subpopulation and if this was known that a  
9 wild species was sufficiently compatible for gene flow  
10 to occur the assumption should be made that the  
11 probability of hybridization overtime will be one, and  
12 if harm is anticipated it's clearly one of those  
13 things one wouldn't wish to authorize. Thank you.

14           MR. ROBERTS: Thank you, Dr. Cooper. Dr.  
15 Bujarski.

16           DR. BUJARSKI: I basically agree with Ian  
17 Cooper and since this is not within the specialty of  
18 my research I have no other comments to add.

19           MR. ROBERTS: Dr. Allison.

20           DR. ALLISON: Richard Allison. The rate at  
21 which a gene may escape has little to do with its  
22 establishment, rather it's integration into the

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1 population is dependent on its selective advantage in  
2 its recipient. The virus pressure in this PVY test  
3 the selective advantage, low transfer rates will  
4 influence the time required for integration, but low  
5 or high transfer rates should be treated similarly as  
6 they have little difference over time.

7           While low transfer rates decrease the  
8 likelihood of gene transfer within a given season we  
9 must evaluate the long term, if you know it's going to  
10 happen it is just a matter of time. We can look  
11 forward to better bioconfinement methods in the  
12 future, however at this point we should assume that  
13 given a sexually compatible recipient we should plan  
14 for gene escape.

15           MR. ROBERTS: Other comments. Dr. Falk.

16           DR. FALK: In terms of genoscape and its  
17 relevance to viruses I think I'm going to say what I  
18 said earlier is that we don't really know that plant  
19 viruses have a role in weed or natural plant survival  
20 or invasiveness. I think there are examples where  
21 virus resistance does not contribute to invasiveness.  
22 For example in California a serious virus disease,

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1 beet curly top (ph), and it is transmitted by the beet  
2 leaf hopper and both of these are introduced species  
3 that were introduced in the late 1800s and both have  
4 very wide host ranges.

5           The natural California vegetation which  
6 included perennial grasses and sage brush are  
7 resistant or immune to beet curly top, however many  
8 dicot species are susceptible. Through time these  
9 native species actually have been affected severely by  
10 cattle overgrazing and by other farming practices and  
11 the dicots that are susceptible to these viruses have  
12 spread now that these destructive practices that have  
13 led to elimination of the perennial grasses in sage  
14 brush have been stopped, these plants that are  
15 resistant have not at all moved back in or showed any  
16 sort of advantage to re colonize their original areas.

17 I think another point in thinking about gene  
18 flow is that gene flow and to related species and  
19 virus susceptibility are not also synonymous, so not  
20 all plants that are going to be related will be  
21 susceptible to the same virus.

22 MR. ROBERTS: Dr. Hammond.

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1 DR. HAMMOND: To follow-up some discussion  
2 that we had earlier, disease resistance genes have  
3 been deployed in crops through tradition grading  
4 methods for years and to date there has not been  
5 significant research done to look at the consequences  
6 of the introgression of these genes from the crops  
7 into wild species, but that does not appear to have  
8 been any obvious increase in weediness as a result of  
9 that potential integration. The methods exist now  
10 from genomics to go looking for those genes and to  
11 determine whether they have introgressed in crops and  
12 to determine whether those genes do have any influence  
13 in the persistence or weediness of the wild species  
14 and that should be done, but it seems to me that there  
15 is very little difference between transgenes that  
16 confer virus resistance and naturally occurring genes  
17 that confer disease resistance.

18 The tools are there, we have the option to go  
19 and look at it and I see no reason why virus  
20 resistance from transgenes should be of more concern  
21 than any other natural gene that has had the  
22 opportunity to introgress from crops to weeds.

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1 MR. ROBERTS: Other viewpoints, Dr. Tepfer.

2 DR. TEPFER: I'm just a bit concerned about  
3 generalization. I think we need to keep this in a  
4 case-by-case sort of perspective. I think that what  
5 this sort of emerging consensus that there seems to be  
6 no evidence for the ecological release having occurred  
7 in the past that we know of is one thing, but there  
8 are also cases where virus resistance could perhaps  
9 provide a sufficient booth to a wilder weedy species,  
10 I mean the obvious sort of hypothetical case here is  
11 the wild oat which is a terrific weed already, it is  
12 susceptible to BYOV, Bailey Yellow Dwarf Virus, I just  
13 would think we need to be a little bit careful and  
14 think about individual cases rather than trying to  
15 generalize.

16 MR. ROBERTS: Dr. Sherwood and then Dr. Falk.

17 DR. SHERWOOD: I think another thing to be  
18 considered is the plasticity of the viral genome and  
19 that although there would be no reason to think that  
20 virus resistance genes either by transgenes ones that  
21 were developed through convention breeding would be  
22 anymore stable in weeds than they would in the crop

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1 plant, and it's fairly evident that virus resistance  
2 is overcome on a fairly regular basis in crop plants,  
3 and so why would that not occur in weed species as  
4 well.

5 DR. FALK: I agree exactly with what John  
6 Sherwood just stated, I also think that the example of  
7 bailey yellow dwarf virus in wild oats. I don't think  
8 that the data do show in fact that bailey yellow dwarf  
9 viruses do have any effect on the natural incidents  
10 and colonization of wild oats.

11 MR. ROBERTS: Dr. Melcher.

12 DR. MELCHER: Relative to the wild oats and  
13 bailey yellow dwarf virus it occurs to me that maybe I  
14 should read to you part of the letter that was  
15 submitted, written by Roger Bechie (ph) where he says  
16 from the aspect of control of an epidemic disease  
17 control in weedy species is a positive thing since  
18 weed species are the source of most epidemics of plant  
19 viral disease worldwide, indeed one method to control  
20 such diseases is to remove alternative hosts, so he's  
21 saying that if the wild oats were to acquire  
22 resistance to the virus, the virus level would

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1 decrease in the reservoir in a reservoir for growth of  
2 the crop plants. It's probably not relevant to the  
3 question, but I thought it was worth reading.

4 MR. ROBERTS: Thank you. Other comments? It  
5 seems we have some differences of opinion on this  
6 which is fine, but I just wondered if there is anymore  
7 dialogue that we need to sort of clarify panel's  
8 position on this. I don't see any, so let me ask Dr.  
9 Kramer.

10 DR. KRAMER: I guess I would just asked if  
11 you could possibly clarify when you talk about a  
12 case-by-case evaluation is there any additional  
13 guidance you might provide about the criteria we would  
14 use in such an evaluation or is that something that  
15 might require more thought?

16 MR. ROBERTS: I assume your question is for  
17 Dr. Tepfer?

18 DR. KRAMER: Yes.

19 DR. TEPFER: I mean I want to come back to  
20 what Falk just said about the wild oats, of course  
21 this hasn't been demonstrated but I think this is a  
22 case I would be a little bit more concerned about than

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1 certain others because of the already weedy nature of  
2 the potential recipient species, so I would suggest  
3 that potential recipients that are already potentially  
4 weedy should be of particular concern. I think there  
5 are also cases where this is much less likely to occur  
6 in which the recipient is in an extremely limited sort  
7 of a ecosystem not very invasive, and that I would  
8 suggest would be reason to be a little bit less  
9 worried about this, I think it might be one criterium  
10 that should be considered.

11 MR. ROBERTS: Dr. Sherwood.

12 DR. SHERWOOD: I think this morning we had a  
13 very good presentation by AFIS (ph) about the criteria  
14 that could be considered in addressing this question  
15 and I believe those are reached by consensus of the

16 scientific community as important in making these  
17 case-by-case evaluations.

18 MR. ROBERTS: Dr. Stewart.

19 DR. STEWART: I would just add on the  
20 case-by-case basis, a lot of times there are several  
21 different categories of plants that you might be  
22 worried about and not worried about, so the ones with

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1 no wild relatives well of course that's when you don't  
2 worry about gene flow or increasing competitiveness or  
3 weediness of any wild relatives, on the other end are  
4 the wild oats the Johnson grass which is closely  
5 related to the sorghum are probably -- the Agency or  
6 any company will probably never go there as far as  
7 transgenics simply because the gene flow issue is  
8 going to be a done deal and nobody really wants to  
9 have liability over what would almost certainly be a  
10 transgenic weed that would persist in the environment  
11 for a long time, so I think that's one thing that Dr.  
12 Tepfer is kind of getting at on a case-by-case basis.

13 MR. ROBERTS: Yes, Dr. Nagy.

14 DR. NAGY: One additional comment I would  
15 like to make is that it's possible that if transgenic  
16 weeds can be widespread that it can change the  
17 selection pressure for a combination, so it is  
18 possible that in -- phonetically (ph) possible  
19 although I don't have any published information on  
20 that, that in those weed species no new viruses would  
21 have much better chance to emerge than currently  
22 (inaudible) or others, so this is an important issue I

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1 think for the record, now the selection (inaudible)  
2 can be changed in that situation.

3 MR. ROBERTS: Dr. Kramer, are there any other  
4 follow-ups on this question?

5 DR. KRAMER: No, thank you.

6 MR. ROBERTS: Let's go ahead then and take  
7 question number 6.

8 DR. KRAMER: Please comment on the prevalence  
9 of tolerance and/or resistance to viruses in wild  
10 relatives of crops..

11 MR. ROBERTS: A little bit open question, but  
12 we'll let Dr. Falk lead off our discussion on this.

13 DR. FALK: Tolerance resistance immunity to  
14 indigenous pathogens and viruses is present in wild  
15 population of many plants and this was an area of  
16 significance that particularly in early days virus  
17 biology and plant breeders of course have searched for  
18 germ plasm sources to use in breeding programs for  
19 resistance. One example is a book published in 1993,  
20 Resistance to Virus Diseases of Vegetables by Kyle --  
21 Kyle is the editor, in chapter-by-chapter going  
22 through that book they list sources of virus

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1 resistance found for cucurbets, for lettuce, for  
2 peppers, for tomatoes, for peas, for beans, and they  
3 document the sources as being various land races and

4 plant introduction lines or wild species basically,  
5 lettuce, resistance in lettuce like tucositiva (ph) to  
6 lettuce mozaic virus comes from related species like  
7 tucavirosa (ph) , like ticasuligna (ph) , like  
8 tucasariola (ph), and in some cases these are single  
9 gene types of resistance.

10 In the last chapter of that book Sorenson  
11 states that most of the sources of resistance that we  
12 utilize in breeding programs come from foreign germ  
13 plasm and we are increasingly relying on wild  
14 relatives of cultivated species for virus resistance  
15 genes. In barley there's a very good example of  
16 single gene resistance, barely yellow dwarf virus from  
17 Ethiopian wild barley -- let me see where I'm going  
18 because I thought we were doing this tomorrow -- I  
19 think also instead of just documenting resistance we  
20 can say that there must be resistance tolerance in  
21 weedy species when we introduce new crops to areas and  
22 viruses suddenly appear in those crops and there are

0029

1 many, many examples in the literature of that one is  
2 when cucow (ph) was planted in regions of Africa there  
3 was no indication ahead of time that cucow swollen  
4 shoot was an endemic virus in that area but now that  
5 an introduced crop species was planted there it was  
6 unable to be grown commercially and was basically  
7 eliminated, so the virus obvious was indigenous in  
8 native plants and was not causing detectable effects  
9 in the wild population.

10 Another think to think about I think in terms  
11 of resistance in wild populations and this is from a  
12 review article written by Jim Duffus (ph) in 1971  
13 where he talks about the role of weeds in plant virus  
14 incidents and epidemiology and he says that viruses  
15 can become pathogens of wild plants when susceptible  
16 crops are grown in their vicinity, that we grow  
17 susceptible crop plants that do become infected and  
18 now you have a uniform widespread source of inoculum  
19 that is a source of inoculum for the weeds and not  
20 vice-versa and we often think of the alternative.  
21 That's all I have.

22 MR. ROBERTS: Thank you, Dr. Falk. Dr.

0030

1 Zaitlin.

2 DR. ZAITLIN: I would like to point out that  
3 most plants and we're including the wild relatives  
4 we're talking about disease is the exception,  
5 resistance is the norm. I mean that pertains to all  
6 kinds of pathogens of plants and animals. If we  
7 became diseased in response to every pathogen we came  
8 in contact with we would be in a bad way.

9 Now on thing and I will talk about this a  
10 little later in my response to question 8, we I looked  
11 at some of these types of resistances they are not  
12 conventional resistance gene induced as Dr. Falk  
13 talked about, so they're so-called subliminal  
14 infections, that the virus is able actually to infect

15 the initial cell into which it is placed but it can't  
16 move from there, so there's a restriction and a  
17 capacity of this virus to move out of the infection  
18 court.

19 MR. ROBERTS: Dr. Sherwood.

20 DR. SHERWOOD: I would just like to build on  
21 that a little bit. When we think of these terms of  
22 virus resistance and virus tolerance they are I think

0031

1 defined differently by different groups of people and  
2 the direction of this discussion that I'm hearing is  
3 that resistant is an absolute, either the virus is not  
4 there or the plant's susceptible, and that's generally  
5 not the case, particularly in transgenic plants and  
6 most of the studies that have been done in the field  
7 there's been a delay of symptom development which is  
8 what you're really looking for from a plant production  
9 side is an absence of disease phenotype even though  
10 the virus may be there, and so probably the same thing  
11 is going to happen in natural populations if there are  
12 quote unquote "resistance" genes there that there's  
13 going to perhaps be less virus replication or delay in  
14 symptom development and not in absence completely of  
15 virus there's probably going to be very little impact  
16 that occurs.

17 In some of the work that I did when I was at  
18 Oklahoma State on virus resistance to weight soil born  
19 you know there's delay of movement out of the roots of  
20 the plant and in a resistant cultivar that's modulated  
21 by temperature and so building on what Dr. Zaitlin  
22 said you had these subliminal or limited infections

0032

1 that occur in a tolerant or resistant plant, but they  
2 are not resistant in the absolute sense that we thing  
3 that virus is not replicating there.

4 MR. ROBERTS: Dr. Zaitlin, follow up?

5 DR. ZAITLIN: Yeah, I think it goes back to  
6 the definition as John talked about as to what plant  
7 virologists consider resistance and what say plant  
8 breeders consider resistance. I remember some years  
9 ago we have a well-known plant breeder, Cornell Henry  
10 Munger, the resistance of various vegetables, he said  
11 come into the greenhouse I want to show you my  
12 resistance squash, well they sure look sick to me, but  
13 then he showed me the plants, their parents, and they  
14 were less sick than their parents, that was the  
15 criteria he used.

16 MR. ROBERTS: Yes, Dr. Melcher.

17 DR. MELCHER: Speaking of definitions I have  
18 a feeling listening to the comments that perhaps my  
19 definition of tolerance not quite the same as the  
20 others, the way I think of tolerance is that the plant  
21 is loaded with virus but it has absolutely no  
22 symptoms, and this is definitely something that is

0033

1 known for a number of plant and virus combinations and  
2 I'm not sure whether that's what the other people were

3 thinking, that's my thought.

4 DR. SHERWOOD: I would certainly agree with  
5 Ulrich when it comes to weeds, that you will have  
6 weeds that are just loaded with virus but have no  
7 phenotypic symptomatic expression of being diseased.

8 MR. ROBERTS: Dr. Hammond and then Dr.  
9 Cooper.

10 DR. HAMMOND: I think just to go back to that  
11 we've already talked about the fact that many times  
12 wild weed species have multiple infections with no or  
13 insignificant apparent symptoms, so they are obviously  
14 tolerant to a significant degree and there is little  
15 affect of many viruses on many wild species.  
16 Plantagolancilatra (ph) is the weed with which I'm  
17 most acquainted, that is naturally infected by at  
18 least 26 different viruses and the three phytoplasmas  
19 and experimentally known to be infected by at least 13  
20 other viruses and at least one other phytoplasma and  
21 most with minimal symptoms, a great deal of tolerance  
22 in wild species.

0034

1 MR. ROBERTS: Dr. Cooper.

2 DR. COOPER: I just put in a new work which  
3 is immunities, specific virus immunity occurs in wild  
4 plants in plastoanegra (ph) seedlings were collected  
5 from a span of a few kilometers and manually  
6 challenged with turnip yellow mozaic virus which is a  
7 readily transmittal virus of that sort. The  
8 proportions of immune seedlings and that were 21 out  
9 of 31 so that you can actually put real numbers which  
10 we have done for manually inoculated plants and for a  
11 virus like turnip yellow mozaic that's not a probably  
12 wholly unreasonable thing, you've got an easy virus to  
13 detect, the means of detecting it's illogically  
14 disease is normal, except in that population where  
15 there was not disease from turnip yellow mozaic and no  
16 turnip yellow mozaic occurred.

17 Another thing I would say that no brassica  
18 negru systemically invaded but beat western yellow's  
19 (inaudible) virus and never showed symptoms in any of  
20 the plants that we looked at, so that we can put  
21 numbers and we have indeed gotten numbers for  
22 proportions of those categories which I'm defining

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1 immunity as absolute exclusion of virus and detectable  
2 amounts.

3 The other terms where we did make an attempt  
4 tivengernsinine (ph) a couple of papers in  
5 phytopathology a few years ago to define the terms and  
6 reconcile the usage by plant breeders and plant  
7 biologists of the terms in resistant, susceptible and  
8 immune, and they have been accepted by many, but  
9 perhaps not by all.

10 MR. ROBERTS: Other comments by panel members  
11 on this question? Dr. Kramer have we confused the  
12 things with regard to terminology?

13 DR. KRAMER: I think so. I would like to

14 draw everyone's attention to the appendix that we, the  
15 Agency provided for resistant and tolerant and just  
16 ask for maybe some direct comment on whether these  
17 definitions are acceptable or if what I'm  
18 understanding from Dr. Cooper perhaps immune is really  
19 the word we're looking for to go with the definition  
20 for resistant.

21 MR. ROBERTS: Could you read these for us  
22 because I think I see people started scrambling around  
0036

1 to try and find those, but let's take them one at a  
2 time and maybe if you could read them and get some  
3 feedback.

4 DR. KRAMER: Let me start with the definition  
5 of tolerant which I didn't actually hear any  
6 disagreement about and see if we can maybe agree on  
7 that first. That definition of tolerant means the  
8 plant is able to sustain the effects of a virus  
9 infection with negligible or mild symptom expression  
10 and negligible or mild effects on fitness or growth  
11 despite the presence of the virus within the host.

12 MR. ROBERTS: Sound good panel. They're  
13 nodding for the record.

14 DR. KRAMER: The definition we have for  
15 resistant is -- means the plant is not infected by or  
16 is a non host of the virus concerned. And I guess I  
17 would ask a two-part question here, one, what term  
18 would you use for that definition is really the main  
19 question we have, and then the second part would be if  
20 you would use another term for example immunity for  
21 that definition then could you also provide a  
22 definition for resistant.

0037

1 MR. ROBERTS: Dr. Melcher.

2 DR. MELCHER: Well I can start with the -- I  
3 guess there are different levels of resistance in my  
4 view. Immunity is when the virus enters the cell and  
5 nothing happens, it does not replicate in that initial  
6 cell. The next level of resistance is what Dr.  
7 Zaitlin talked about, the subliminal infection. It's  
8 able to enter a cell and replicate in that cell but it  
9 does not spread any further than that one cell. And  
10 then there's another level where the virus is limited  
11 to a small area which is typical of the hypersensitive  
12 response and that's what I think a lot of people think  
13 of as resistance, although probably resistance refers  
14 to all three levels of those levels in my opinion.

15 MR. ROBERTS: Dr. Zaitlin.

16 DR. ZAITLIN: This brings me back to my  
17 comment of this morning about the definition of  
18 PVCP-PIP and you were talking about it refers to virus  
19 infection, here we're talking about disease, we're  
20 having situations here where a plant actually is  
21 infected but there's no disease, I think you ought to  
22 go back and look at that definition you gave us.

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1 MR. ROBERTS: Let's go ahead and give Dr.

2 Kramer some feedback an resistance, make sure we give  
3 a clear response on that. Dr. Sherwood.

4 DR. SHERWOOD: What I want to do is ask the  
5 other panel members if they knew of cases of PVCP-PIPS  
6 in which there is an absolute non host resistance  
7 conferred, because all of the literature I'm familiar  
8 with is that there is a delay in replication of the  
9 virus, the amount of virus or a delay in symptoms  
10 that's produced or in case of gene silence and  
11 recovery.

12 MR. ROBERTS: Dr. Tepfer.

13 DR. ZAITLIN: I don't know whether that's  
14 been really investigated like the situation that Dr.  
15 Cooper gave earlier did they actually look to see if  
16 whether there was anything like a subliminal  
17 infection?

18 DR. COOPER: Can say that they were tested  
19 rigorously, serialogically and in other ways to detect  
20 a virus which is generally a very abundant and easily  
21 detectable agent.

22 DR. ZAITLIN: But what I'm talking is did it  
0039

1 fact replicate in the initially infected cell.

2 DR. COOPER: The initially infected cell  
3 might have been so rare as to have the consequences  
4 diluted by the proportion of cells surrounding it that  
5 were not infected, however the net result was that you  
6 could not detect by normal means the presence of the  
7 virus, but the absolute presence of the virus in a  
8 single cell somewhere in the plant was not rigorously  
9 sought.

10 DR. ZAITLIN: I remember years ago there was  
11 a study by a fellow who was at the Los Angeles  
12 Arboretum and he had a wealth of plants at his  
13 disposal so he tried to infect them with the back (ph)  
14 mozaic virus and then to see whether in fact he could  
15 recover virus from them. Now the tools that he had  
16 available to him are very different from those we have  
17 today, he essentially tried to extract it and test it  
18 biologically, but interestingly enough he found in  
19 many, many species and I can't enumerate but I  
20 remember it included things like ferns, he showed that  
21 they were in fact hosting those viruses but to a very,  
22 very limited degree.

0040

1 MR. ROBERTS: I think Dr. Tepfer was going to  
2 respond to Dr. Sherwood's question.

3 DR. TEPFER: I think I was. Just wanted to  
4 mention that there are cases in the literature of the  
5 PTGS mediated resistance in which there is no apparent  
6 infection to start with. Now I would presume that  
7 there was at least at the early stages something that  
8 would have to be at least a subliminal sort of  
9 infection, but there are cases where it's not a  
10 recovery-type behavior, but where you simply see no  
11 virus and no infection.

12 MR. ROBERTS: Dr. Hammond.

13 DR. HAMMOND: I can address that in one  
14 particular instance where we have plants that express  
15 antisensaronate (ph) to being yellow mozaic virus and  
16 we had some lines that went through the typical  
17 recovery infection and recovery state, but we had one  
18 line which we were never able to detect infection even  
19 in the inoculated leaves even using a hundred  
20 micrograms per mil of viral inoculum violizer (ph), we  
21 did not go to the level of PCR so we cannot guarantee  
22 that there was no subliminal infection but using the

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1 ilizo (ph) which is pretty sensitive using monofinal  
2 (ph) antibodies we were not able to detect virus in  
3 the inoculated leaves, so in that case that appeared  
4 -- I described that as immunity.

5 DR. SHERWOOD: So would it be safe to say  
6 then that the expression of a transgene it would be a  
7 rare event that would make a plant immune to a plant  
8 virus.

9 DR. COOPER: I think that would be true and  
10 the cases of plant expressing coat protein we never  
11 observed lines in which we could not detect infection.  
12 There were some lines in which a high proportion of  
13 plants escaped infection, but there were no coat  
14 protein expressing lines that I have worked with that  
15 were not infected at some level or other.

16 MR. ROBERTS: I don't mean to interpret but  
17 we are working towards answering Dr. Kramer's question  
18 about a definition about resistance.

19 DR. KRAMER: If I understand correctly I  
20 think we got consensus that the term for absolute  
21 exclusion of the virus would be immunity --

22 MR. ROBERTS: The panel is nodding. So

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1 you're right then to the second part, how would you  
2 define resistance.

3 MR. ROBERTS: Dr. Allison, you were not  
4 nodding.

5 DR. ALLISON: I just want to contribute  
6 something that may help to define subliminal  
7 infections from resistance, and that is it seems that  
8 in the subliminal infection the virus lacks the  
9 ability to move from the originally infected cell,  
10 that is it can't open the door, it can't get through  
11 the plasma desmina (ph), however it still maintains  
12 the ability to replicate so it has that sort of  
13 machinery available to it, and we've done experiments  
14 where we've taken animal viruses and put them into  
15 barley protoplast and watched the replicate, however  
16 there is no way that that animal virus, flock house  
17 virus in this case, was able to move within a barley  
18 plant, so if that helps the definition of subliminal  
19 infection.

20 MR. ROBERTS: Dr. Kramer, did you get your  
21 definition of resistance to?

22 DR. MELCHER: I think she was asking whether

0043

1 we had definitions for resistance and it seems if I'm  
2 interpreting Dr. Sherwood's comments there's two  
3 different uses of resistance, there is resistance to  
4 infection and there is resistance to disease and they  
5 may not be the same, is that right?

6 MR. SPEAKER: That's correct.

7 DR. COOPER: Well the susceptibility and  
8 sensitivity are two parts of a see-saw but go into the  
9 disease expression scenario, but the resistance is  
10 very often one that might additionally involve  
11 nonacceptance by a vector or even deterrence by a  
12 vector at some distance remote from the plant so that  
13 the vector component and resistance should also be  
14 considered that the plant could be resistant to the  
15 deliver of a virus into it by a vector which didn't  
16 like it, but it is relative amounts of infection in  
17 proportion to inoculum would seem to be something like  
18 the definition of resistance, but it's a variable  
19 always in my eyes anyway.

20 MR. ROBERTS: Well this seems to be an  
21 important sidebar for our discussion because we're not  
22 using terminology consistently we're going to have a

0044

1 lot of problems providing clear feedback, so please  
2 feel free to use as much time as you need for it, so  
3 not only for the benefit of the Agency understanding  
4 what's said but among the panel so we're all using  
5 consistent terminology to the extent possible. Dr.  
6 Tepfer.

7 DR. TEPFER: I think that some of the  
8 difficulty we're facing has to do with a tradition  
9 plant pathologist or division of susceptible  
10 resistance and the definition that is proposed in the  
11 appendix which specifically focuses on transgenic  
12 plants which is a bit of a different situation. We  
13 have lots of cases that don't seem to exist in  
14 transgenic plants and things that may exist in  
15 transgenics that don't exist elsewhere, so that's part  
16 of the source of the confusion I think.

17 DR. KRAMER: I think we have the information  
18 that we need, but could I also ask, I'm not sure if  
19 this is the correct place, but I wanted to go back to  
20 something Dr. Zaitlin said about the definition of a  
21 PVCP-PIP.

22 MR. ROBERTS: Sure.

0045

1 DR. KRAMER: I just wanted to read into the  
2 record again the definition that we're using because  
3 I'm not quite clear on what point you're trying to  
4 make. PVCP-PIP means a plan incorporated protectant  
5 created from the gene or a segment of the gene that  
6 coats where a coat protein of a virus that naturally  
7 infects crop plants. So within --

8 DR. ZAITLIN: I was taking -- a slide that  
9 talked about controlling virus infection, that's the  
10 words I saw on one of those slides.

11 DR. KRAMER: Looking at the slides here this

12 is the definition that we're using for a PVCP-PIP. I  
13 think probably what you're referring to may be the  
14 taking that and using that to how we define it as a  
15 pesticide, but the definition of a PVCP-PIP in and of  
16 itself is simply the plan incorporated protectant  
17 created from the gene or a segment of a gene that  
18 coats with the coat protein of a virus that naturally  
19 infects crop plants and that's -- if you disagree with  
20 that definition then please comment.

21 MR. ROBERTS: I don't hear a disagreement.  
22 Have we handled at least for now the

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1 terminology issues?

2 DR. KRAMER: I think so.

3 MR. ROBERTS: Then let me ask you, this came  
4 up as I had asked you about our response to this  
5 question 6 are there other follow-up questions are  
6 related to this, is our response reasonably clear on 6  
7 the one we're just finishing up?

8 DR. KRAMER: Yes.

9 MR. ROBERTS: Let's go ahead and do question  
10 7.

11 DR. KRAMER: Please specify techniques that  
12 do or do not provide measures of tolerance and/or  
13 resistance that are relevant to field conditions. And  
14 I guess in light of our prior discussion I would  
15 change this to say please specify techniques that do  
16 or do not provide measurements of tolerance and/or  
17 immunity that are relevant to field conditions.

18 MR. ROBERTS: Dr. Stewart.

19 DR. STEWART: Well since I'm not a virologist  
20 per se I'm probably not the best guy to lead off with,  
21 but I will go ahead and take a stab on the basis of  
22 other transgenic plants that provide some type of

0047

1 difference in fitness, but this will be short and I  
2 certainly hope my associate discussants will come to  
3 the fore here.

4 Greenhouse and growth chamber experiments are  
5 marginally predictive of selection pressure in the  
6 field as the result of environmental effects and local  
7 viral load that very spacially and temporally or I  
8 would say that one would expect a very spacially and  
9 temporally in the field, that said there seems to be  
10 merit in using greenhouse and growth chamber  
11 experiments in assessing viral tolerance if for a  
12 number of viral strains and plant genotypes is used.

13 It is preferable to use specific transgenic  
14 events into wide range of viral strains and challenge  
15 experiments. Viral load can be assessed by alisa (ph)  
16 or other molecular slash biochemical techniques,  
17 disease can be assessed by visual asas (ph) in many  
18 cases, crop yield however integrates among resistance,  
19 tolerance, and other variables as a rough index for  
20 fitness.

21 MR. ROBERTS: Dr. Hammond.

22 DR. HAMMOND: I looked at this

0048

1 mechanistically and thought about things that you  
2 could measure that would reflect tolerance or  
3 resistance and I would go to resistance in this case  
4 rather than immunity, immunity is immunity, there is  
5 not much you can do to it to measure it except say it  
6 exists.

7       There are degrees of resistance that may be  
8 useful or may not be, but tolerance can be measured as  
9 high viral titer without symptoms or with minimal  
10 symptoms and resistance can be measured as reduced  
11 titer compared to a susceptible plant. Measures of  
12 resistance can be utilized through production of total  
13 biomass, yield of specific components, height of the  
14 plant, leaf number, effects on shooting, flower  
15 number, seed number, seed size, lack of symptoms, and  
16 persistence of the plant in the environment.

17       And any of those components can be affected  
18 by the environment under which the plant and the virus  
19 are growing, so some things will have little effect at  
20 high temperature and have much more pronounced effect  
21 at low temperature or vice-versa depending on the  
22 virus and the plant concern, for example I have one

0049

1 virus that I have been working with recently and using  
2 a house plant and a cotyledon (ph) and under  
3 most conditions it produces a mosaic or mottle, but if  
4 we grow the plants in a growth chamber under low  
5 temperature conditions we get first localized necrosis  
6 and then systemic necrosis, so you can have something  
7 that normally does very little to the size of the  
8 plant but produces visible symptoms and under low  
9 temperature conditions produces necrosis starting out  
10 localized and ending up with terminal necrosis which  
11 essentially ends the productive life of the plant, so  
12 it is growth chamber experiments under a variety of  
13 conditions necessary for a full assessment and there  
14 are many measures by which you can determine the  
15 degree of resistance or tolerance.

16       MR. ROBERTS: Dr. Falk.

17       DR. FALK: I will further confuse this, so I  
18 think in terms of this question I would add provide  
19 measures of tolerance or resistance or susceptibility  
20 that are relevant to field conditions, and I think  
21 that screening -- it is very difficult when we are  
22 doing any sort of screens in greenhouse or growth

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1 chambers or whatever because typically if the virus  
2 we're using is mechanically transmissible that's what  
3 we do, it is much easier, this is really quite  
4 artificial, where typically in mechanical inoculations  
5 we have a much greater inoculum load than is  
6 encountered naturally than would be encountered in the  
7 field and susceptibility from mechanical inoculation  
8 must be interpreted in that light I believe.

9       I think that under natural conditions the  
10 great, great, great majority of plant viruses are

11 going to be spread by specific vectors that transmit  
12 them, and things like pubescence or the hairs on  
13 leaves will affect natural infection and  
14 susceptibility in nature whereas the plant itself may  
15 be fully capable of supporting virus replication if  
16 you mechanically inoculate it, but if it is  
17 transmitted by aphids in the non circulative,  
18 nonpersistent manner the plant can show effective  
19 resistance under natural conditions.

20 Similarly if the aphid or other insect has to  
21 feed in the flowum (ph) the plant can be perfectly  
22 susceptible to virus replication and transport, but if  
0051

1 the vector cannot find the flowum where the virus has  
2 to be delivered the plant will issue effective  
3 resistance, so I think inoculation protocols do not  
4 give good measures of tolerance or resistance and must  
5 be considered. Even contemporary inoculation  
6 procedures using Agrobacterium to deliver clone DNA's,  
7 the Agrobacterium has its own defined host range and  
8 we can make mistakes as we have where we can say the  
9 plant is resistant to the virus when in fact it was  
10 resistant to the Agrobacterium that was used to  
11 deliver the virus.

12 In regards to susceptibility, I think when we  
13 measure phenotypic or biological effects after  
14 experimental inoculations those also give a false  
15 interpretation of the significance and natural effects  
16 due to virus infection as both the co-discussants here  
17 have mentioned, environmental conditions and et  
18 cetera, time of infection, all of these effect the  
19 severity of the symptoms and how we might measure  
20 susceptibility resistance or tolerance.

21 MR. ROBERTS: I would like to ask other  
22 members of the panel to contribute to this, Dr.

0052

1 Tepfer.

2 DR. TEPFER: I want to ask for a bit of a  
3 clarification, could we consider perhaps a mechanical  
4 inoculation as sort of a worst-case technique for  
5 inoculation. Are there cases where you have better  
6 resistance with using a mechanical inoculation as  
7 compared to vector mediated infection because in our  
8 hands it usually seems to correlate fairly well with  
9 the few viruses that we've worked with, are there  
10 cases where it doesn't work.

11 MR. ROBERTS: Dr. Falk.

12 DR. FALK: I'm not sure about how answer  
13 that, in what I was talking about here it says  
14 relevant to field conditions and that was my point of  
15 bringing things up like leaf pubescence because we  
16 know those plants are perfectly susceptible and we  
17 could inoculate them mechanically and we could  
18 inoculate them if we forced aphids on there, but under  
19 natural conditions they do show effective field  
20 resistance or tolerance, so I'm sure there are  
21 examples both ways in terms of what you're saying, but

22 I'm just trying to say -- what I was trying to say and  
0053

1 what I represented earlier is that I think we have to  
2 take care in interpreting the significance from what  
3 we've done under these controlled conditions and how  
4 those really relate to what's happening under natural  
5 conditions.

6 DR. COOPER: I would just say that resistance  
7 is very difficult to measure except you can make a  
8 model of the field in a plant pot in a glasshouse and  
9 you can take soil containing nematods (ph) of fungi  
10 carrying the viruses and put your plant under test in  
11 it and that's another way to get an estimate of  
12 ability infect relative to another species of cultivar  
13 or whatever -- we routinely do that.

14 There was viruses that we study that way of  
15 course you transmit mechanically but very often  
16 inefficiently and therefore you don't chose to use  
17 that, it's really where the viruses have a particular  
18 close relationship with the vectors you can't neglect  
19 that reality in the assessment of resistance and  
20 that's the best you can do with some of the systems.

21 Others of these vectors, the mites and the  
22 other animals are sometimes very difficult to handle

0054

1 and they can't easily be mimicked.

2 MR. ROBERTS: Other comments from panel  
3 members, Dr. Nagy.

4 DR. NAGY: I would like to add one thing to  
5 Dr. Tepfer's comments is that I think it's lots of  
6 times the tolerance and how we measure it is also,  
7 there is a delay in a symptom induction in this kind  
8 of thing, so I would like to add that the time of  
9 measurements is a major factor in estimation.

10 DR. KRAMER: I'm just wondering, we heard  
11 from Dr. Falk's examples of techniques, specifically  
12 manual inoculation would perhaps not provide an  
13 accurate measure of tolerance or resistance relevant  
14 to fields condition but could you maybe suggest  
15 something that would, would field experiments for  
16 example be necessary?

17 DR. FALK: I think that mechanical  
18 inoculation is what we do because we can do it, I was  
19 only trying to suggest that it's not perfect and that  
20 we remember that. I think in terms of any then to  
21 interpret what we see from our experimental  
22 inoculations in the greenhouse to field conditions we

0055

1 will have to do field experiments of course, I'm not  
2 trying to argue against mechanical inoculation, we're  
3 all going to do that, right, but it's just not to  
4 assume that because things work that way that they're  
5 going to apply to the field.

6 MR. ROBERTS: Dr. Sherwood and then Dr.  
7 Melcher.

8 DR. SHERWOOD: Just to add to that in a plant  
9 improvement program I was involved in resistance was

10 just a component of that overall program and so when  
11 you began working with plant breeders they're looking  
12 at another agronomic features, not just resistance  
13 that have to go hand-in-hand and basically they rely  
14 on the disease nursees (ph) which are conducted out in  
15 the field so the two go hand-in-hand to bring  
16 something along as a variety and as Dr. Zaitlin said  
17 you know you're looking for perhaps one that's less  
18 sicker than the parents were.

19 MR. ROBERTS: Dr. Melcher.

20 DR. MELCHER: I'm not sure I am going to get  
21 the words right but I would like to ask Dr. Falk if on  
22 the laboratory inoculations is the problem that one is

0056

1 seeing false positives but not false negatives, in  
2 other words it over estimates the resistance of the  
3 tolerance but if there was a resistance or a tolerance  
4 that -- there's not going to be a resistance or  
5 tolerance that shows up in the field that does not  
6 also show up in the laboratory, I guess that's the  
7 crux of it.

8 DR. FALK: I'm not sure I understand what you  
9 said. I think what I was trying to say was I think  
10 there is effective natural resistance tolerance to  
11 infection that can be missed by experimental severe  
12 inoculation in the greenhouse.

13 DR. MELCHER: I'm confused, I withdraw the  
14 question.

15 MR. ROBERTS: Let me pose a follow-up  
16 questions, are there any techniques that are not  
17 relevant to field conditions such that you would say  
18 that it really has no value, I don't mean to put words  
19 in Dr. Kramer's mouth, but as I read this you know I  
20 think they're trying to get information from us in  
21 terms of which of these techniques have value and  
22 which don't and it sort of has a tendency to kind of

0057

1 dichotomize them and I realize that there are shades  
2 of gray and they have value, but to the extent that  
3 which we can identify techniques and that just really  
4 have no relevance and shouldn't reply on them from  
5 making decisions about what's going on in the field  
6 are there any that the panel can identify?

7 DR. FALK: I didn't mean to say that non of  
8 them are useless because they are all useful, but I  
9 just thought that all of the things that we do must be  
10 interpreted in the context of how we've done them is  
11 all I'm trying to say.

12 MR. ROBERTS: Dr. Hammond.

13 DR. HAMMOND: May I ask what the relevance of  
14 this question is to the charge of the panel because  
15 farmers aren't going to grow something that does not  
16 have relevant tolerance or resistance.

17 DR. KRAMER: We're really looking at whether  
18 it would be possible to identify wild or weedy  
19 relatives having already tolerance or resistance to  
20 the virus whose coat protein was inserted into the

21 transgenic plant.

22 MR. ROBERTS: Dr. Tepfer.

0058

1 DR. TEPFER: That puts a very different light  
2 on it because I think we know of several cases of  
3 plants that when you take them into the greenhouse,  
4 wild plants you can infect them using mechanical  
5 inoculation which are simply never infected in the  
6 field, so I think that doing the experiment we were  
7 all thinking in the other sense, you have possibly  
8 resistant crop plant, what happens when you take it to  
9 the field, but going in the other direction I think  
10 that we are in a complete, a rather severe black box  
11 in fact, I don't think we have very much knowledge  
12 about how easy it is and what are the predictable ways  
13 of doing it in the greenhouse, that type of experiment  
14 maybe other people...

15 DR. HAMMOND: I would like to follow-up on  
16 that because we find especially with some houseplant  
17 virus combinations that you get nothing most of the  
18 year but a few weeks in the fall and a few weeks in  
19 the spring you can do some useful work, and there are  
20 a lot of environmental variables, the growth stage of  
21 the plant, the quality of the day length, the  
22 physiological state of the plant whether it's in

0059

1 active growth, whether it's entering a reproductive  
2 phase that have enormous influence on how easily you  
3 can infect it, you can put plants in the dark for a  
4 day before you inoculate them and then succeed in  
5 infecting something that you cannot ordinarily succeed  
6 in infecting, and that is a very artificial means and  
7 is not relevant to what's going on with weeds in the  
8 field so you have to be much more careful in drawing  
9 inferences about weed plants which is why I asked what  
10 the relevance of this question was because I was  
11 looking at it from the crop end.

12 If you're looking at it from the weed end you  
13 do have to be very careful with the environmental  
14 conditions under which you look at it and look at the  
15 natural vectors rather than mechanical inoculation and  
16 look at it with low vector populations and high vector  
17 populations. In that case it is very relevant and if  
18 you do it mechanically and under ideal plant growing  
19 conditions you will see things that will be of no  
20 relevance at all in the field.

21 MR. ROBERTS: Dr. Kramer if it sounds all  
22 right with you perhaps in our response to this we will

0060

1 clarify at the beginning of the response that you  
2 clarified that we were really talking about this from  
3 the weed and that way it will be easier for our  
4 response to be understood, so let's just as a note to  
5 us when we're writing our response we just indicate  
6 that we clarified that and with that clarification our  
7 response is and then -- Dr. Stewart.

8 DR. STEWART: And so this really does pertain

9 to wild plants, not weeds per se.

10 DR. KRAMER: I would say both.

11 DR. STEWART: Because weeds are -- most weeds  
12 that weed scientists would call weeds are recently  
13 evolved entities are very different from wild plants,  
14 that's just a note of clarification. We can take a  
15 look at all of things I suppose.

16 MR. ROBERTS: Dr. Sherwood.

17 DR. SHERWOOD: I would just like to add that  
18 one just has to look back to the beginning days of  
19 plan virology where one would go out and collect  
20 native species and use those as a range of indicator  
21 host to look at what the reaction of various viruses,  
22 what their reaction of various viruses was and

0061

1 certainly they would not be ones that you would find  
2 in them in nature but could artificially inoculate  
3 them to either systemic infection to the virus  
4 purification or local lesions in order to isolate  
5 individual isolets, so all the work through the 20s  
6 and 30s was done with wild species or native species  
7 to differentiate plant viruses before you had the  
8 tools of today.

9 MR. ROBERTS: With the clarification then  
10 we've had, Dr. Hammond and Dr. Tepfer had certainly  
11 responded and I want to just ask the panel that with  
12 that clarification does anyone else want to respond  
13 differently than what they responded for? Okay. Then  
14 back to Dr. Kramer, with that clarification is the  
15 response from the panel reasonably clear or do you  
16 want to ask some follow-ups?

17 DR. KRAMER: I think it is reasonably clear  
18 that we don't know a whole lot about how to do this  
19 relevant to field conditions if I understand  
20 correctly.

21 MR. ROBERTS: Is there any disagreement among  
22 the panel on that statement? I don't see any.

0062

1 Let's go ahead then and take question number  
2 8.

3 DR. KRAMER: How do environmental or other  
4 factors for example temporal variations effect  
5 tolerance and/or resistance given the expected  
6 variability what measures of tolerance and/or  
7 resistance would be reliable. And again I would say I  
8 think we mean to use the term immunity as we've just  
9 discussed here rather than resistance.

10 MR. ROBERTS: Dr. Zaitlin.

11 DR. ZAITLIN: I just knocked my name tag over  
12 the edge of the desk.

13 First of all my response is a repeat of  
14 something I've already said, anyway it says that most  
15 plants are resistant to most viruses, disease is the  
16 exception, in many cases the disease resistant plants  
17 exhibit no symptoms but it is also apparent in the  
18 relatively few cases that have been investigated the  
19 virus can infect the resistant plant, the initial cell

20 of entry, but the virus cannot spread from that site  
21 so no disease results, thus much resistance is  
22 affected by an inhibition of cell-to-cell movement

0063

1 rather than a restriction on virus replication per se,  
2 environmental factors can affect this mode of  
3 resistance, but it has not been studied extensively.

4 Principally elevated temperatures can  
5 encourage virus movement and such movement may break  
6 conventional resistance, this is particularly evident  
7 when resistant results in a necrotic local lesion.  
8 This phenomenon has been well studied and can result  
9 in a systemic movement of the virus, furthermore when  
10 the ambient temperature is reduced subsequently the  
11 whole plant then can become necrotic.

12 Resistance to plant viruses generated by  
13 plant breeding involving incorporation of resistance  
14 intecrops from other cultivars or species is often not  
15 stable because viruses can replicate in such plants,  
16 and as we discussed before resistance is often scored  
17 as a reduction of symptoms and thus there is a  
18 selection of variant virus isolets (ph) that can  
19 overcome the resistance. Fewer than 10 percent of the  
20 54 host virus resistant gene combinations enumerated  
21 by phrase and gurwitz (ph) in review in 1987 remained  
22 effective over a long period.

0064

1 The effect of the environment on this process  
2 has not been investigated although it is probable that  
3 environmental conditions that enhance virus  
4 replication would increase the probability that  
5 resistant breaking virus isolets could be induced or  
6 selected for.

7 On the other hand, coat protein induced  
8 resistance has proven remarkably stable in the most  
9 prominent case papaya ring spot virus in Hawaii. The  
10 resistance is viral strain specific or isolet  
11 specific, but ring spot isolets from either regions of  
12 the world could overcome the resistance in laboratory  
13 tests, it does not happen in the field. And I have  
14 recently inquired at my friends at the University of  
15 Hawaii who confirmed that. They are concerned however  
16 that there may be some isolets being generated at  
17 papayas other than the big island where the papaya  
18 ring spot virus resistant plants are grown may be  
19 evolving.

20 They are trying to actually overcome this by  
21 pyramiding virus resistance sequences to these other  
22 isolets. The other commercial application of coat

0065

1 protein made of resistance out of viruses in squash is  
2 more complicated in that the plants have resistance to  
3 three viruses, thus the probability breakdown of  
4 resistance would be enhanced, but I know of no reports  
5 of that happening and perhaps Keith Reddenbaugh (ph)  
6 who is here from Siminus (ph) could confirm or refute  
7 that charge.

8           The conclusion is that resistance breaking is  
9 a function, is most probably a function of changes in  
10 the virus not the plant in both conventional  
11 resistance and coat protein (inaudible) resistance.  
12 And ask my colleagues if they really know of instances  
13 where the plant gene itself, resistant gene itself is  
14 modified to breakdown the resistance. I think  
15 comparing the two types of resistance, the natural and  
16 the coat protein mediated resistance, it seems to be  
17 the most stable and most reliable.

18           MR. ROBERTS: Dr. Melcher.

19           DR. MELCHER: Regarding the first example  
20 that Dr. Zaitlin mentioned this is I believe the case  
21 of tobacco mozaic virus interacting with the N gene of  
22 tobacco and in that case it is an indirection between

0066

1 the virus and the plant that is breaking down, so I  
2 don't think that we can really attribute it to either  
3 the virus or the plant, it's the two working together.

4           There is another case where temperature has  
5 in effect on the breakdown of resistance and that is  
6 in an effect that is well known for all organisms and  
7 that's heat shock, when there's a sudden increase in  
8 temperature the organism shuts down the synthesis of  
9 most of its proteins and turns on another set of  
10 proteins called the heat shock proteins, the ones that  
11 are turned down should include probably the proteins  
12 that are involved in resistance, so as far as  
13 temperature goes that's definitely a environmental  
14 factor that would affect tolerance and/or resistance  
15 and I still want to call it resistance and I will get  
16 to why in a second.

17           I don't know about other factors, perhaps I  
18 can rely on my colleagues, other factors might be  
19 plant water status, light intensity, light durations,  
20 solenity and so forth, they may have effects on a  
21 breakdown of resistance, but I am not sufficiently an  
22 expert to say anything about them.

0067

1           Tolerance is important for the survival of  
2 the tolerant species, but it allows the creation of  
3 virus reservoirs for transmission to other species  
4 that are neither tolerant nor resistant and I really  
5 don't know anything about environmental factors  
6 relative to tolerance but they are probably important  
7 there. What I may know something about is the  
8 measures used for measuring these things and they were  
9 discussed in response to the previous question to some  
10 extent but not completely, so maybe it's worth my  
11 going into that a little bit now.

12           The lowest level of resistance which is what  
13 I call immunity and I think some of my colleagues  
14 agreed with me on that, there is not replication. To  
15 test for that the test is either to take isolated  
16 cells from the plant and try to infect that and see  
17 that there is no replication in those isolated cells  
18 or to do some kind of a detection where you can look

19 at single cells and say the leaf, one way would be to  
20 use a virus that is tagged that will express for  
21 example gene fluorescent protein and then after the  
22 appropriate incubation time look at the leaf and see

0068

1 if you can find single cells that are fluorescent  
2 green, if you cannot find any then there was no  
3 infection of even a single cell.

4 The same assertive technique can be used for  
5 the next level, subliminal section, subliminal  
6 infection, if you find just a single cell without a  
7 cluster of cells being fluorescent then that is a  
8 reflection of the inability of this virus to move out  
9 of a single cell and would be a subliminal infection.  
10 The further levels I think then you begin to get into  
11 things that have been mentioned before, eliza (ph)  
12 various nuclaic (ph) acid detection techniques like  
13 hybridization and RTPCR.

14 I think we're supposed to say how these would  
15 be reliable as far as looking at environmental  
16 factors, I think the extrapolation is obvious that if  
17 you're interested in how the environmental factors  
18 affect resistance or tolerance with these methods you  
19 have to do the experiments under a variety of  
20 conditions, variety of temperatures, light  
21 intensities, and so forth, and I believe that's all I  
22 can offer, not very much I'm afraid.

0069

1 DR. KRAMER: I just wanted to make a point of  
2 clarification, I've not sure if it's necessary, but  
3 given the misunderstanding of the last question I  
4 thought I might go ahead and do that.

5 This question number 8 directly follows from  
6 question number 7 where we're really looking at  
7 whether it would be possible to identify tolerant  
8 resistant immune plants that were relatives of any BCP  
9 transgenic plant and therefore you might be less  
10 concerned about the transfer of any type of resistance  
11 to that population and so when we're looking at how  
12 well environmental factors may impact those measures  
13 are really considering whether it's possible at all to  
14 measure those given the types of variations that you  
15 would expect under natural conditions.

16 MR. ROBERTS: Dr. Cooper.

17 DR. COOPER: A lot of would argue the least  
18 you can do is you can collect seeds from wild  
19 populations, bring them in the glasshouse, you  
20 challenge them with a virus that's in measure which  
21 you are going to then follow-up as we described  
22 earlier. It is not an absolute measure and it doesn't

0070

1 easily relate to viruses obligated transmitted by  
2 pollen or in some sophisticated way, but at least it  
3 gives you one measure and then you look at the plants,  
4 you test the plants and then you come into the area of  
5 considerable debate as to whether you use this word to  
6 describe what you found as some other word.

7 MR. ROBERTS: Dr. Sherwood.  
8 DR. SHERWOOD: And I would add that tolerance  
9 is probably very easily measured out in the field  
10 because most of the weeds that are seen are  
11 nonsymptomatic for virus disease yet will test  
12 positive for viruses under conditions and so that plan  
13 I guess in the terms that we're using today will be  
14 tolerant to that virus infection since it was  
15 nonsymptomatic yet positive in some test for the  
16 virus.

17 DR. STEWART: I have a question to the  
18 virologist about some other environmental effects that  
19 I haven't heard about, whether these could be  
20 important or not such as increased UVA or UVB,  
21 increased ozone or soil contaminants, say heavy metals  
22 or whatever, could that affect tolerance or infection

0071  
1 or disease for that matter, and I'm thinking  
2 especially now if we bring wild plants or other plants  
3 into the greenhouse or growth chamber where these  
4 things are probably not going to be factors does that  
5 make any difference.

6 DR. ZAITLIN: I think light has been  
7 investigated in this case and is it has to be light of  
8 photosynthetic quality, it's a common practice if  
9 you're conducting an assay, you put the plants in the  
10 dark beforehand and then you can then infect them  
11 better, but you put them in the dark in the absence of  
12 CO2 I think it won't work well.

13 DR. TEPFER: I will just sort of answer  
14 rebounding from what Dr. Cooper said that you can  
15 still use I think the test by mechanical inoculation  
16 in the greenhouse as distinguishing at least between  
17 susceptibility and non susceptibility, so that if you  
18 admittedly all of these things like ozone or water  
19 stress or UV in a particular way the plants are  
20 generally more susceptible and if you can then still  
21 not infect them that probably means that that might be  
22 rather difficult to infect, but if they are infectible

0072  
1 in the greenhouse in these rather soft conditions then  
2 you have a harder question to answer.

3 DR. ZAITLIN: I would like to ask my  
4 colleagues if they know of any case where the actual  
5 plant gene itself has broken down. I know as Dr.  
6 Melcher pointed out I mean these resistances are an  
7 interaction between a virus and the plant gene whether  
8 it be a transgene or a natural gene, but I don't know  
9 of any cases numerated where shown that a mutation in  
10 the plant gene has in and of itself caused the  
11 resistance to break down, does anyone know?

12 MR. ROBERTS: For the record there was no  
13 positive response.

14 DR. STARK: Just a comment and maybe a point  
15 of clarification for me because I'm not a virologist  
16 as well. It would seem to me, I agree with what's  
17 been said over here by Mark, you want to start with

18 the worst-case scenario to see if you can infect a  
19 plan but then ultimately what would really be good  
20 would be to have some standardized techniques. We've  
21 only done this in toxicology quite a bit as well where  
22 different labs do things very differently, they expose

0073

1 organisms in a different manner, at different times of  
2 the day, at different life stages, things like this,  
3 and it can all have great influence in susceptibility  
4 on toxicants and I assume the same thing would hold  
5 true with disease, so it would be nice to have a  
6 series of standardized approaches when trying to  
7 investigate whether or not you're going to have  
8 problems like gene flow and other disease  
9 transmission.

10 MR. ROBERTS: I get the sense that the  
11 Department is asking us for advise about how you might  
12 construct those tests or what they should look for in  
13 those tests, especially with your clarification, Dr.  
14 Kramer, on 7 and 8 is ability to do tests other than  
15 field tests that would have some sort of predictive  
16 value and what can you do, what can be reasonably  
17 done, what sort of techniques can be used, and given  
18 the fact that environmental factors can vary what  
19 needs to be paid attention to, what would you need to  
20 vary or sort of work into you -- what would be the key  
21 things to work into your studies to be sure that you  
22 produced results that might have value in the field or

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1 predictive value for the field, is that where you're  
2 headed?

3 DR. KRAMER: Yes.

4 MR. ROBERTS: Okay. Dr. Tepfer.

5 DR. TEPFER: In just response to John Stark's  
6 precise point, I'm afraid that's a very sort of  
7 utopian idea, if you you've been working on a small  
8 number of plant species, each single plant species has  
9 a different sort of optimum situation for infection  
10 and indeed there are lots and lots of factors that  
11 intervene in terms of just growing the plants in the  
12 greenhouse, the age of the plants, the quality of the  
13 inoculum, the list is extremely long, so I don't think  
14 that for going from one plant species to the next or  
15 even to one virus to the next on a given plant species  
16 you can come to some sort of standardized sort of  
17 protocol, all you can do is say well things like viral  
18 plants will be often more susceptible when they're  
19 younger or well if the leaves are sort of softer it  
20 would seem to be easier to infect, and so can sort of  
21 make a catalogue of generally how to increase  
22 susceptibility, for there to go to standard conditions

0075

1 I don't see how we can do it given the diversity of  
2 the plants the viruses.

3 DR. STARK: Good point, even within the  
4 animal realm you run into the same kinds of issues,  
5 good point.

6 DR. STEWART: So speaking of things in the  
7 utopian realm, since viruses aren't fairly simply as  
8 far as things go, could there ever be any proteomic  
9 type approaches where you look at protein interactions  
10 to be able to predict susceptibility, if you could  
11 take the whole gumish from the plant and probe that on  
12 some type of viral platform, since we are running  
13 ahead of time.

14 MR. ROBERTS: We can have some of this side  
15 bar discussion, but my concern is I don't think we're  
16 really giving the information or the advise they're  
17 looking for on frankly 7 or 8, and I think we may need  
18 to work a little more to try and get back to that, but  
19 we can entertain your discussion, your question a  
20 little bit loosely. Dr. Melcher, were you going to  
21 respond to that?

22 DR. MELCHER: Well, yes, with proteomics as I  
0076  
1 understand it you need to know something about the  
2 proteins that that organism makes, and if we're  
3 talking about of these wild and weedy relatives I  
4 doubt that there would be very much information about  
5 the proteiiums of those to do any comparisons with.

6 DR. COOPER: I would also say that from  
7 experience with brassica proteomics and genomics they  
8 environmental conditions are very influential on the  
9 outcome of what you see, and therefore the environment  
10 is definitely a parameter that you have to build into  
11 your experiments at great expense.

12 MR. ROBERTS: Back to questions 7 and 8. DR.  
13 Sherwood.

14 DR. SHERWOOD: I will give a shot at that, I  
15 think the use of mechanical inoculation and or  
16 appropriate vector inoculation will help in  
17 determing whether a weed species is a host or not a  
18 host, but in terms of hooking at the specific  
19 parameters in regards to virus replication, how it is  
20 going to act in the field, that that is not going to  
21 be -- there's not going to be an approach to do that  
22 in the growth chamber or the greenhouse, that would

0077  
1 have to be done under field conditions.

2 MR. ROBERTS: In doing those studies looking  
3 at question 8, are there some key variables that you  
4 need to address in your study which I think is what  
5 question 8 -- with your understanding about  
6 environmental or other relevant factors, what kinds of  
7 things would you need to look at or incorporate into  
8 those studies? Dr. Sherwood.

9 DR. SHERWOOD: I think they have all been  
10 mentioned and generally as a virologist you are trying  
11 to put the host in the most susceptible condition, so  
12 inoculating young vigorous growing plants, preparing  
13 your inoculum so it has the highest degree of  
14 infectivity, darkening the plants before inoculating  
15 them, we used to put wet paper towels over the plants  
16 after they were inoculated so the leaves didn't dry

17 out, whether you're going to use carborundum (ph) or  
18 carbide, or whatever else you're going to use, all of  
19 those things are kind of in-house, I don't know,  
20 witches brew or each lab differs a little bit  
21 differently and how they go about inoculating plants.

22 DR. KRAMER: So can I just try to reiterate  
0078

1 --

2 MR. ROBERTS: Yes, please do, and if we are  
3 still not getting this right please let us know.

4 DR. KRAMER: From what I'm understanding is  
5 that it may be a fairly simple task to identify a  
6 plant that is the is tolerant or resistant, if you  
7 able to show that through manual inoculation or other  
8 such techniques that you aren't getting a virus  
9 infection that's a reasonable conclusion to make.

10 The converse isn't necessarily true and then  
11 if you are able through laboratory techniques to show  
12 that you are able to get infection can't necessarily  
13 apply that directly to a field scenario and in that  
14 that circumstance you're just faced with a much more  
15 daunting task to try to demonstrate that there would  
16 be true tolerance or immunity under natural conditions  
17 given the type of variation that we can expect under  
18 environmental conditions.

19 DR. COOPER: I will just make one final  
20 comment on this, Rothomstead (ph) a well know  
21 virological center at one time, the Scottish Crop  
22 Research Institute another one, had kinopoteium

0079

1 quinoris (ph) test plants. The kinopoteium in each of  
2 those two glasshouses reacted very differently to a  
3 whole range of viruses, they were as far as one could  
4 judge the same species but for unknown reasons they  
5 were very different and would not help your approach.

6 MR. ROBERTS: Despite that comment is -- I  
7 want to be sure it is, do we agree with Dr. Kramer's  
8 sort of summary back of what she heard us say on this  
9 point, yes, yes, all right, good. Dr. Hammond.

10 DR. HAMMOND: I essentially was going to back  
11 up what Dr. Cooper said and the people -- the  
12 (inaudible) in the Netherlands had collected  
13 ketopoteium seed from various sources and inoculated  
14 it with a number of viruses and found considerable  
15 variation in the susceptibility.

16 MR. ROBERTS: All right. Dr. Kramer, do you  
17 think we have done this?

18 DR. KRAMER: I think so.

19 MR. ROBERTS: As good as we're going to do.  
20 Let's do one more before we go to break.

21 DR. KRAMER: Question 9, what would be the  
22 ecological significance if a plant population acquired

0080

1 a small increase in viral tolerance and/or resistance  
2 above a naturally-occurring level. And perhaps I can  
3 just start off with a verification of this question  
4 and that will be we're really considering again if we

5 can identify that there is natural tolerance or  
6 immunity within a plant population that's a wild or  
7 weedy relative of a VCP transgenic plant is there any  
8 ecological significance of conferring upon that plant  
9 additional tolerance and/or immunity from the  
10 incorporation of the PCP transgene.

11 MR. ROBERTS: Dr. Stewart, you're a popular  
12 guy as a lead discussant.

13 DR. STEWART: Well, so this is going to touch  
14 a little bit on, this question and the next question,  
15 and I'm going to through in some stuff on this last  
16 clarification so if someone wanted to be provocative  
17 earlier this will be as provocative as I can get.

18 So gene flow is defined by the formation of  
19 hybrids and back cross hybrids is not a risk per se,  
20 gene flow is not a risk, the consequences of gene flow  
21 may be, theoretically a small boost in viral tolerance  
22 or resistance under constant and viral pressure could

0081

1 cause an increase in relative fitness, an increase in  
2 fitness would theoretically cause an increase in  
3 transgene frequency that would eventually be fixed in  
4 a population.

5 This scenario would not necessarily confer  
6 increased competitiveness in plant communities however  
7 and this scenario pertains to a directly transformed  
8 plant of an isogenic line that is a crop not a wild  
9 plant. There are many generations from a transgenic  
10 crop to introgress near isogenic transgenic wild  
11 plants. In F1 hybrids the host genome will contain  
12 proportional genomic constituents of the two parents.

13 In BC1 hybrids with back crossing onto the  
14 wild plant and selection for the transgene and  
15 assuming equal size parental genomes an average of 25  
16 percent crop genome would be in the BC1's along with  
17 the transgene, and BC 2's 12.5 percent, the BC 3's  
18 will have 6.25 percent crop genome and 93.75 percent  
19 wild genome on average. And this is as far as back  
20 crossing usually gets for testing of wild relatives,  
21 so while most BC3 plants will appear to be very  
22 similar to the wild host, they're expected to contain

0082

1 around 2,000 crop genes along with a single transgene  
2 or two or three transgenes affecting, altogether  
3 affecting the fitness landscape, that's on average in  
4 an average in advance transgenic background back cross  
5 plant such as BC3 the transgenic effect can be  
6 expected to be swamp by the hitchhiking crop genome  
7 effect, that's ecologically insignificant. With  
8 apologies to John Dunn no gene is an island.

9 So if you are not back crossing onto a  
10 tolerant wild relative you could look at putting in a  
11 little bit of gasoline onto a raging fire, therefore  
12 once again it would have an even less effect than if  
13 you were back crossing onto a susceptible wild  
14 relative host. If there's already tolerance you know  
15 what's a little more tolerance, and so if you add to

16 the natural barriers to introgression, physical  
17 containment, genous restriction technology, transgene  
18 mitigation technologies, and male sterility, there  
19 would be almost complete barriers to introgression.

20 MR. ROBERTS: Dr. Stark, do you have anything  
21 to add to this?

22 DR. STARK: No, I'm going to pass on that, I  
0083

1 think we covered it very well.

2 DR. COOPER: Probably not much effect, the  
3 breeding system might be crucial, out crosses would be  
4 less affected than inbreeding types and importantly I  
5 think in any increase in the magnitude of the virus  
6 would have a potential to create more viruses  
7 available for evolution to take place if you had a  
8 tolerance sort of situation in that circumstance, so  
9 if that simply addresses the question probably not  
10 much.

11 DR. SHERWOOD: I just take and using the  
12 words as we are, with immunity I don't see if the  
13 plant isn't immune already I don't see how we could  
14 increase immunity and so that then gets us to viral  
15 tolerance, and if we're using tolerance as we are what  
16 would occur perhaps is an increasing amount of virus  
17 in the plant, but let's take us out of ecological  
18 consideration in that the rate limiting step in all of  
19 this is going to be the movement of the virus by the  
20 appropriate vector and that's going to be the gateway  
21 as to whether or not it's going to have an ecological  
22 impact, and the question would then be increase if you

0084

1 increase the amount virus in a plant it's more  
2 tolerant is that necessarily going to lead to more  
3 transmission within the crop and within the weed and I  
4 don't think if we really know that in terms of whether  
5 a high virus content plant is a much better source of  
6 virus leading the epidemics than a plant that has you  
7 know maybe half as much virus, where does that break  
8 point occur.

9 DR. ALLISON: I believe that my comment is  
10 basically the same as yours, in terms of resistance if  
11 there's a lesser amount of virus within the wild  
12 population then there's a less inoculum for the crops  
13 species itself and this is what Dr. Beechie was  
14 referring to in his comments.

15 DR. MELCHER: I guess I can try to go one  
16 step further and consider population dynamics over a  
17 longer period of time, if the transgene gets into the  
18 wild species does provide some sort of advantage even  
19 though I agree with Dr. Stewart is not very likely,  
20 that means that there is a gradual reduction in the  
21 virus population that will be effecting that species  
22 in that particular region and with the reduction of

0085

1 the virus population there is a reduction in the  
2 selective pressure to keep the transgene, so even  
3 though the transgene may be established temporarily,

4 in the long run it will be reduced probably to low  
5 levels. I'm not a population geneticists, but that  
6 seems reasonable to me, maybe others can correct me.

7 DR. STEWART: There's several things swimming  
8 in my brain here as far as viruses and wild plants and  
9 crops and weeds, so virus evolution is faster than  
10 plant evolution, I guess that's a fair statement. So  
11 if we're talking about crops and I'm thinking about a  
12 monoculture here, monoculture of a crop where you have  
13 a big target for the virus, that's going to be a  
14 different situation than a wild plant community which  
15 would be fairly diffuse, that is the number of wild  
16 plants of a particular species that could be a wild  
17 relative would be of a much less dense than the crop,  
18 so I'm trying to figure out why wild plants really  
19 matter very much at all.

20 I can understand why weeds might matter  
21 because the weed density in crop fields can often be  
22 as high as the crop density, so weeds and wild plants

0086

1 are two different things, and I'm not sure where I'm  
2 going with this, but I'm hoping one of you can tell  
3 me.

4 MR. ROBERTS: Dr. Cooper.

5 DR. COOPER: Could I just make a comment on  
6 the context of biodiversity conventions that the U.S.  
7 may not subscribe to, but in other countries the  
8 diversity of the plant population whenever it grows  
9 could be relevant to a risk assessment and therefore  
10 wild plant numbers, diversity, performance, and such  
11 like could become an issue.

12 DR. STEWART: So on the risk assessment we're  
13 usually predisposed to think about creating increased  
14 weediness, increased invasiveness, if we're talking  
15 about decreasing the competitive ability or fitness of  
16 a wild plant species population whatever or creating  
17 hybrids that will place another species in jeopardy  
18 and I think that's a totally different thing.

19 Something that's often not appreciated, I  
20 don't know how many examples there are in real live  
21 where a single transgene might actually place a plant  
22 population or species in jeopardy, it's worth

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1 considering anyway, I think it is especially worth  
2 considering by the EPA, I will say that.

3 MR. ROBERTS: Any other points on this  
4 question? Dr. Kramer.

5 DR. KRAMER: I think that's fine, thank you.

6 MR. ROBERTS: Let's take a 15-minute break,  
7 let's try to reconvene at 3:50.

8 ( Break.)

9 MR. ROBERTS: As we begin our discussion I  
10 just want to give everyone notice that it's my intent  
11 to just take up one more today, number 10, because we  
12 will have a change in topic to viral interactions,  
13 we'll begin with that one first thing in the morning.  
14 I would like to go ahead and take 10 then I will offer

15 a brief opportunity for go backs, we don't do a lot of  
16 those, but if there's a comment that you in the  
17 discussion of the first ten questions that we've  
18 covered today that you forgot to make and we moved on  
19 since we're moving kind of quickly I'll give you the  
20 opportunity to go ahead and address that now and then  
21 I would like for the panel to meet in closed session  
22 just to discuss planning for the write-up for the

0088

1 minutes for today's first session.

2 So let's go ahead and take question 10.

3 DR. KRAMER: Please comment on how necessary  
4 and/or sufficient these conditioners are to minimize  
5 the potential for the PVCP-PIP to harm the environment  
6 through gene flow from the plant containing the  
7 PVCP-PIP to wild or weedy relatives. Would any other  
8 conditions work as well or better? If we go to the  
9 next slide then we actually have the conditions up  
10 there and I would like to read through those, number  
11 one, the plant into which the PVCP-PIP has been  
12 inserted has no wild or weedy relatives in the United  
13 States with which it can produce viable hybrids in  
14 nature, for example, corn, tomato, potato, or soybean.

15 Number two, genetic exchange between the  
16 plant into which the PVCP-PIP has been inserted and  
17 any existing or wild or weedy relatives is  
18 substantially reduced by modifying the plant with a  
19 scientifically documented method, for example, through  
20 male sterility. Or number three, it has been in  
21 periodically demonstrated that all existing wild or  
22 weedy relatives in the United States with which the

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1 plant can produce a viable hybrid are tolerant or  
2 resistant to the virus from which the coat protein is  
3 derived.

4 And it would just like to clarify this  
5 question to make sure people understand how the Agency  
6 is envisioning these three factors here, and that is  
7 the work group came together before getting the  
8 panel's advice and tried to come up with factors that  
9 the Agency could potentially use to evaluate when a  
10 product would be of such low risk, that it might not  
11 be necessary to undergo all regulatory requirements at  
12 the Agency. And these are the factors that we were  
13 able to come up with and now we're asking for the  
14 panel to comment on these particular factors.

15 MR. ROBERTS: Thank you, Dr. Kramer. Dr.  
16 Cooper, could you lead off for a discussion on this  
17 one.

18 DR. COOPER: Well I would say that we  
19 addressed question 1 to this morning to some degree  
20 and I have little to add. In question 2 it's  
21 important I think to recognize at least one mechanism,  
22 male sterility has the potential to impact on wild

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1 life because wild life eats seeds and even pollen, and  
2 therefore that aspect should be considered if not

3 given a lot of weight. And they have at least the  
4 potential to prove a risk of harm on wild life  
5 diversity I would suggest in the environment.

6 I generally subscribe to the if it can happen  
7 it will happen school and the evolutionary time scales  
8 therefore are rather more important than whatever have  
9 been implied in some of these statements earlier, but  
10 question 3 is certainly an area that I have greatest  
11 uncertainty with, perhaps even the least useful, it  
12 should be perhaps replaced with some reliance on the  
13 specific virus isolets that's being considered co  
14 evolving with the crop, viruses change and get  
15 selected for in local conditions.

16 Pathotype, the concept of the fact that a  
17 virus that infects one sort of plant may be a  
18 different virus in the genetic sense to the same virus  
19 that it doesn't infect the same plant, so pathotype is  
20 one of the terms used in circumstances like that where  
21 the host range is important.

22 Furthermore the virus from which the coat  
0091

1 protein was derived may be very different from the one  
2 that it's protecting against, so lettuce mozaic virus  
3 provided by my colleague here on my right was used  
4 genetically engineered brassica in hybridization  
5 experiments because there was a benefit against turnip  
6 mozaic virus, both related in the sense of being potty  
7 (ph) viruses but different viruses and I have no  
8 evidence that lettuce mozaic virus will be detectable  
9 under that name in brassica's, but I have to say I  
10 haven't personally looked for it.

11 There may be no effect for all the variety of  
12 reasons we talked about, linkage drag in particular  
13 this morning, but all I would say is that it is very  
14 difficult when you have the diversity of plants, the  
15 diversity of the viruses, and they should all be  
16 considered in your case-by-case risk assessment.

17 MR. ROBERTS: Dr. Hammond.

18 DR. HAMMOND: I have little to add to that.  
19 I think that in general if the coat protein is being  
20 deployed against the virus from which it came there is  
21 very little reason for concern and these conditions  
22 should therefore be suitable and appropriate.

0092  
1 DR. STEWART: I would agree with Dr. Hammond  
2 these seem to be fairly a conservative set of  
3 conditions to give something a free pass.

4 DR. TEPFER: Well since Neil is not being  
5 provocative I guess I should instead.

6 DR. STEWART: I was provocative on the last  
7 question.

8 DR. TEPFER: This is my turn. Just in  
9 regarding the second point in all seriousness I think  
10 a lot of the sort of confinement techniques trying to  
11 reduce the gene flow are not a hundred percent  
12 effective and therefore if we go along with what Ian  
13 was just saying if it can't happen it will, then we

14 need to really carefully consider other sorts of  
15 strategies. And I would simply like to suggest that  
16 it is time for us to overcome the tabu of talking  
17 clearly about the usefulness of gurt strategies as  
18 gene flow preventive mechanisms essentially.

19 I think it is extremely important, we could  
20 have a very, very, very valuable resistance genes put  
21 into plants that cross readily with terrible weeds, if  
22 we have a good girt strategy behind it that really

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1 will keep it from being transmitted we should be able  
2 to go ahead and do it. And I think that we're at a  
3 point where we need to come out and say it and so  
4 that's I'm doing so.

5 And another point I would like to say in this  
6 regard is that if there were a girt strategy that was  
7 made freely available so it's not a question of  
8 industrial strategies to try to take over the world of  
9 the seed markets and things like that which is a lot  
10 of the opponents in Europe are using is a way of  
11 knocking people over the head, trying to prevent girt  
12 strategies from being implemented is just the big  
13 companies trying to take over and so on, if there were  
14 gurts that were available to small companies to people  
15 in academic labs, particularly to work on some of the  
16 less important crop lands once for developing  
17 countries, this could really turn around an enormous  
18 perceptual issues about using gurts, what they're for  
19 because people completely forgot these are gene  
20 confinement strategies and not just ways of trying to  
21 monopolize germ plasm.

22 MR. ROBERTS: Dr. Cooper.

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1 DR. COOPER: I was intending to say something  
2 in this context in another part of the program, but  
3 recombination mediated by safety measures like girt,  
4 especially cre lux indegrays and G jix cleavage (ph),  
5 have the potential to release something that might be  
6 living in the genome of the plant and I think it would  
7 be prudent for an experimenter to have diligently  
8 investigated the genome of the host to look for occult  
9 virus genome segments that might be triggered as a  
10 result of this technology, so although the technology  
11 is perfectly appropriate and should be available I  
12 think it is prudent in this area where there is more  
13 and more information albeit not always public  
14 information, but at least the experimenter should have  
15 realistically have access to the genomic information  
16 pertaining the crop they're concerned with, and to  
17 investigate that as a prelude to or in parallel with  
18 doing the experiments would be useful in case genie  
19 gets let out of the box.

20 At the moment the only sorts of viruses known  
21 in these occult forms are I supposed DNA contained  
22 viruses that are rather a rare phenomena, but at the

0095

1 moment we have no clear measure of how prevalent they

2 are and it is a concern.

3 MR. ROBERTS: Other points?

4 DR. STEWART: Well I just might add so girt  
5 is for the record keepers gene used restriction  
6 technologies, g-u-r-t. There is always the tandem  
7 mitigation technologies where you combine transgene of  
8 interest that might confer fitness with another  
9 transgene either in a transgene fusion or at least in  
10 the tandem pair which would tend to decrease fitness  
11 or increase domestication, and those are reasonable  
12 things to look for in the future.

13 I think what the EPA would like though is to  
14 have certain conditions where if a company just  
15 brought them a product and I don't think we're going  
16 to see any gurts within the next five years coming up  
17 for commercialization, I hope I'm wrong, I hope it's  
18 sooner than that, but are these things reasonable, are  
19 these conditions reasonable, and I think they probably  
20 are reasonable.

21 And I would also add if something can happen  
22 it will but I'm not sure that, I'm still not convinced

0096

1 that introgression is going to happen even in  
2 something like the brassicas where there's lots of  
3 wild relatives and when we're talking about transgene  
4 introgression.

5 DR. COOPER: Well the truth of the matter is  
6 we don't yet have the atlas cooper (ph) making an  
7 interjection. The truth of the matter is we don't  
8 have the information which would give us assurances  
9 in that matter. On balance at the moment it does seem  
10 possible that stable introgression will occur in some  
11 (inaudible) species and at least in those we should be  
12 a little more careful perhaps.

13 MR. ROBERTS: Dr. Kramer.

14 DR. KRAMER: I just wanted to ask Dr. Cooper  
15 a question, would you then disagree with the other  
16 respondents in saying that you think that factors two  
17 and three would be inappropriate or I just wasn't  
18 clear if you were agreeing or not.

19 DR. COOPER: I think they are appropriate  
20 with care is about I would say, they are minor issues,  
21 I suspect they are minor issues associated with male  
22 sterility, there is a potential for an impact upon the

0097

1 wildlife which might not have been here or to  
2 considered, not all things eat pollen and not many  
3 things eat seed but clearly some do and they are  
4 potentially significant.

5 Especially I would have to say in England  
6 where the impact of transgenics on the bird population  
7 was an unintended and unexpected consequence of the  
8 current debates that are going on, so perhaps we're  
9 more sensitive in England to that sort of impact on  
10 wildlife.

11 As to question 3 it was really just to  
12 highlight the fact that not all viruses are the same I

13 suppose and that when you're looking for whatever  
14 you're looking for bear in mind that the virus that  
15 was used as a transgene may have no relationship to  
16 the one that you're protecting against, generally it's  
17 going to be similar, but at least it's a possibility  
18 that might not be.

19 MR. ROBERTS: Dr. Melcher, I think you have a  
20 comment.

21 DR. MELCHER: This would be changing the  
22 subject a little bit, this item 3 includes the

0098

1 phraseology are tolerant or resistant which I objected  
2 to earlier, I would like to at this point withdraw my  
3 objection, I had a discussion with Dr. Kramer on the  
4 break and I now understand what she means and what the  
5 Agency wants and I feel like I need to explain that to  
6 the rest of the panel because I think some of my panel  
7 members had similar opinions as I did.

8 I guess it is a matter of black and white,  
9 either the plant is tolerant and there is a lot of  
10 virus that replicates in the plant or I forget which  
11 black or white, but the other direction there is  
12 absolutely no virus in the plant, everything else in  
13 between is gray, and the grays are very difficult to  
14 handle in a regulatory sense because they are  
15 conditional and it is very difficult to establish all  
16 of the conditions that might be necessary to keep the  
17 gray from being important, is that I think a fair  
18 assessment of what we said?

19 DR. KRAMER: Yes.

20 MR. ROBERTS: Dr. Kramer, did you have  
21 something else you wanted to add?

22 DR. KRAMER: I did want to ask another

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1 question particularly in response to Dr. Hammond's  
2 comments, it seemed to me that you were really address  
3 the question of whether these criteria were  
4 sufficient, but I would also ask it from the other  
5 perspective do you think they're necessary at all,  
6 that is it necessary to have any criteria with which  
7 to judge a product on the basis of gene flow concerns.

8 DR. HAMMOND: Coat protein I'm not sure there  
9 is, I don't have any significant concerns about coat  
10 protein genes even if they do introgress.

11 DR. KRAMER: And could the other panel  
12 comment on whether they agree with that or not?

13 MR. ROBERTS: We can ask.

14 DR. STEWART: I think we have seen some  
15 written comments to that effect, and I would agree  
16 with them that I don't really see gene flow as being a  
17 big issue with coat proteins.

18 MR. ROBERTS: Any other panel members want to  
19 express an opinion one way or the other with that?  
20 Dr. Tepfer.

21 DR. TEPFER: For me we're at the situation  
22 where it's a matter of value judgments in a sense. If

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1 we can agree that a gene flow may occur, that this can  
2 lead to introgression, that this may confer -- this  
3 fitness advantage to the wilder weedy species  
4 conceivably all of this have never been demonstrated,  
5 this could lead to ecological release.

6 The question is what is the degree of  
7 uncertainty, what is the extent of imagined, because  
8 we're imagining now a damage that is acceptable,  
9 that's what we're talking about a this precise moment  
10 as I understand it, I don't particular care to engage  
11 in that kind of discussion, it seems like that's  
12 really getting quite a bit past what my scientific in  
13 this case allows me to pronounce on.

14 DR. ZAITLIN: I too think that we may be in  
15 some sense overreacting here to the transgene  
16 phenomena because we haven't really applied these  
17 standards to resistances which have been generated by  
18 more conventional means, so we're applying a different  
19 standard here. If we can demonstrate that there  
20 really were poor consequences of the natural  
21 resistance escaping maybe then we should be more  
22 concerned about this, but so far I haven't seen it.

0101

1 MR. ROBERTS: Anyone else?

2 DR. KRAMER: So I haven't heard anybody  
3 disagree with the statements of Drs. Hammond and  
4 Stewart that no criteria are necessary other than Dr.  
5 Tepfer who thought that really addressing this  
6 question at all is moving beyond the scientific issues  
7 as I understand it that are answerable with the data  
8 that we have.

9 MR. ROBERTS: Silence is ascent.

10 DR. MELCHER: I guess it is due to the lack  
11 of expertise in the gene flow field, so I am neutral.

12 MR. ROBERTS: I think that some members of  
13 the panel just may not feel comfortable expressing an  
14 opinion because it's not sufficiently within their  
15 area of expertise, really all we can do is ask those  
16 who feel comfortable enough to express an opinion to  
17 do so and I think that's where we are right now.

18 Any other follow-ups on 10? Follow-up  
19 comments from panel members or any questions from Dr.  
20 Kramer or the Agency related to number 10?

21 DR. STARK: Along the lines of Dr. Tepfer,  
22 this bothers me when we say I don't worry about

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1 introgression in gene flow with protein coats, we  
2 really don't know what might happen ultimately in the  
3 long term with these types of things granted the  
4 evidence of these are not a problem and the risk of a  
5 problem is very low, but I would be very hesitant to  
6 just say don't worry about it, you just don't know,  
7 there are too many unknowns.

8 MR. ROBERTS: All right. I think we have  
9 probably covered as much from 10 as we're going to  
10 get. Let me then ask the panel, we have covered ten  
11 questions today which is good progress, but we moved

12 through some of them very quickly and I want to give  
13 the panel an opportunity at the end of the session  
14 today for a go-back on 1 through 10, number 3 of  
15 course is still open and we're going to be working on  
16 that, but on the other ones is there any other  
17 comments that you know and now in thinking about them  
18 that you didn't make during our discussion that you  
19 did like to put into record or make now?

20 DR. STEWART: I would like to see  
21 clarification from the EPA, when they seek to regulate  
22 or cease to regulate something what is the time scale

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1 that is used, because we've heard things from  
2 ecological time scale to evolutionary time scale and  
3 I'm pretty sure we're going to get a some point the  
4 geological time scale and I'm sure the EPA's not  
5 worried about something that's ten thousand years out,  
6 but I mean what is the time frame that we should  
7 really be considering here, because we've had virus  
8 resistant plants with viral coat proteins out on the  
9 market for you know going on ten years now, eight to  
10 ten years and you know eventually it seems to me that  
11 we would see something if there's something to be  
12 seen, and if not at some point we need to make the  
13 determination that their equivalent to the  
14 conventional, so this is just -- since we have a  
15 little bit of extra time once again, what are we  
16 thinking about when we think about these regulations?

17 DR. KRAMER: I think I maybe look at Charlene  
18 Matten (ph) over there because this is an issue that  
19 obviously the Agency has had to deal with resistance  
20 management before, maybe Charlene you would have  
21 something to say here?

22 MS. MATTEN: This is Charlene Matten, I work

0104

1 in the biopesticides and pollution prevention division  
2 and I'm I guess more than just the Power Point mover  
3 today, but when we talked about insect resistance,  
4 management and that question was asked we truthfully  
5 did not define our time frame because our division  
6 director gave us a good example and I will share that  
7 with you, she said if we were looking at the murder  
8 rate would we say what a minimum murder rate would be,  
9 do we want three murders, five murders, ten murders,  
10 so she had said that it's best for us to say we want  
11 the least amount of murder as possible, and so in this  
12 case we want the longest time possible and for  
13 resistance management we said the longest time  
14 possible, but we also know that the models we were  
15 using were 15 year time frames and we also know the  
16 patent lifetimes are -- what are they 19 years, so we  
17 know that in terms of long term, it was at least 15  
18 but anything else, it was 15 to infinity, but we knew  
19 less than ten was not reasonable so that's the best  
20 answer I can give you and in all honesty it was not  
21 defined but it was anything below ten was not good,  
22 fifteen and above was good but what the outside was

0105

1 not defined.

2 MR. ROBERTS: Dr. Hammond, did you have  
3 something that you wanted to add on a previous  
4 question?

5 DR. HAMMOND: In response to that the average  
6 lifetime of an agricultural variety is less than ten  
7 years, I think it is six or seven years of wide use  
8 for most varieties, there are few that last longer  
9 than that.

10 DR. STEWART: But these transgenes really go  
11 beyond conventional variety since they get  
12 introgressed into various plant varieties, so I think  
13 it is worth while to take a long view, I'm just not  
14 sure what that long view really is.

15 MR. ROBERTS: Dr. McClintok do you want to  
16 respond?

17 DR. MCCLINTOK: I've worked in the pesticides  
18 program and also the toxic program which is OPPT and  
19 we have never openly discussed a time frame about  
20 products that we have regulated in terms of what we  
21 would look for. I would add though that as science  
22 changes and/or as the data comes in we would surely

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1 consider that information with the products that we do  
2 regulate, so in terms of a time frame I never had  
3 those discussions.

4 DR. ALLISON: If I understand this correctly  
5 that your decision is being made in order to allow for  
6 continued breeding with the transgene present, so in  
7 terms of a particular crop variety being available and  
8 useful for six years that's really not what we're  
9 talking about because we can use this, a company or  
10 individual breeders can continue to move this  
11 particular gene into other and better varieties as  
12 time goes on, is that correct?

13 So at what point, is there any point at which  
14 you revisit this as other techniques come along that  
15 may be superior. I always view this as kind of the  
16 model T of biotechnology, we're sitting around right  
17 now but certainly there's going to be sports car  
18 versions ten years from now or maybe less, at what  
19 point do you revisit these issues and say all right  
20 this technology is no longer appropriate because there  
21 is so much better technology available and companies  
22 should therefore use that is crossed out, is there any

0107

1 provision for that or thoughts along those lines?

2 MR. ROBERTS: Let me interject because my  
3 intent was to use this time to sort of get more  
4 comments on the record regarding the first ten  
5 questions. I mean I think it's a valid question  
6 you're asking, I'm not sure that it pertains directly  
7 to our answering these questions and it may be a  
8 question that's best addressed as a side bar to Agency  
9 folks during a break.

10 DR. ALLISON: It was actually intended to be

11 at the end of the questions.

12 MR. ROBERTS: Then let me ask you one more  
13 time, are there any go-back comments on 1 through 10?  
14 Dr. Kramer.

15 DR. KRAMER: I guess I will just comment  
16 partially on follow-up to the prior discussion that  
17 really especially when you are considering your answer  
18 to number 10 we would like to make sure that there is  
19 an inclusion there of your relative certainty of the  
20 estimate, remember that's one of the things that we  
21 want to try to understand for all of these questions,  
22 and part of the reason relates to this question of

0108

1 time frames and that is as the Agency is considering  
2 how to regulate these products there are certain  
3 things that the Agency can do that are not going to be  
4 -- that are -- our time frame is not necessarily the  
5 same as the products that are on the market right  
6 there, right now.

7 MR. ROBERTS: Okay, were there any follow-up  
8 clarification questions on 1 through 10, I will give  
9 you the opportunity at the end of each one, but sort  
10 of in retrospect looking back is there lingering  
11 questions, it may be best to take those now while the  
12 questions are still fresh on the minds of the  
13 discussants. If there aren't any that's fine.

14 DR. KRAMER: I don't have any.

15 MR. ROBERTS: Then let's go ahead and close  
16 today's session, I think we have made excellent  
17 progress in getting through the list of questions. We  
18 will -- let me ask Paul Lewis if he has any closing  
19 statements or announcements to make before we close  
20 the session. We're going to reconvene at 8:30  
21 tomorrow morning to begin with question number 11, and  
22 then I would ask immediately after the close of the

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1 session if the panel members would meet in closed  
2 session to discuss writing the minutes for today's  
3 session, but before we adjourn let me ask Paul if he's  
4 got anything he needs to say.

5 MR. LEWIS: Thank you, Mr. Roberts, and thank  
6 all the panel members for being engaged and  
7 contributing a great deal for the discussion we had in  
8 the course of today and I will be working with you  
9 this evening in terms of at least of trying to bring  
10 together some of your thoughts as a right to gather  
11 meeting minutes and looking forward to discussion  
12 tomorrow and for the public to again to be invited to  
13 hear our deliberations over the course of our meeting  
14 tomorrow morning and afternoon, thank you.

15 MR. ROBERTS: Then if there's no other  
16 business for today's session this session is closed,  
17 again we will reconvene tomorrow morning at 8:30 and  
18 again taking up the rest of the questions and again I  
19 would ask the panel members to meet immediately or now  
20 in the meeting room to talk about write-up of the  
21 minutes. Thank you.

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1 CERTIFICATE OF STENOTYPE REPORTER

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I, Monica Knight Weiss, Stenotype Reporter, do  
hereby certify that the foregoing proceedings were  
reported by me in stenotypy, transcribed under my  
direction and are a verbatim record of the proceedings  
had.

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MONICA KNIGHT WEISS