UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

OFFICE OF
PREVENTION, PESTICIDES, AND
TOXIC SUBSTANCES

June 29, 2009

MEMORANDUM

SUBJECT: Transmittal of Meeting Minutes of the FIFRA Scientific Advisory Panel Meeting Held March 31 - April 1, 2009 on Scientific Issues Associated with Designating a Prion as a 'Pest' under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and Related Efficacy Test Methods

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Director
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FROM: Myrta R. Christian
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THRU: Steven Knott
Executive Secretary
FIFRA Scientific Advisory Panel
Office of Science Coordination and Policy

Frank Sanders
Director
Office of Science Coordination and Policy

Attached, please find the meeting minutes of the FIFRA Scientific Advisory Panel open meeting held in Arlington, Virginia on March 31 - April 1, 2009. This report addresses a set of scientific issues being considered by the Environmental Protection Agency pertaining to Scientific Issues Associated with Designating a Prion as a 'Pest' under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and Related Efficacy Test Methods.

Attachment

cc:

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SAP Minutes No. 2009-05

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Scientific Issues Associated with Designating a Prion as a 'Pest' under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and Related Efficacy Test Methods

MARCH 31 - APRIL 1, 2009
FIFRA Scientific Advisory Panel Meeting,
Held at the
Environmental Protection Agency Conference Center
Arlington, Virginia

NOTICE

These meeting minutes have been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP). The meeting minutes represent the views and recommendations of the FIFRA SAP, not the United States Environmental Protection Agency (Agency). The content of the meeting minutes does not represent information approved or disseminated by the Agency. The meeting minutes have not been reviewed for approval by the Agency and, hence, the contents of these meeting minutes do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP is a Federal advisory committee operating in accordance with the Federal Advisory Committee Act and established under the provisions of FIFRA as amended by the Food Quality Protection Act (FQPA) of 1996. The FIFRA SAP provides advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP), and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an *ad hoc* basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Myrta R. Christian, SAP Designated Federal Official, via e-mail at christian.myrta@epa.gov.

In preparing the meeting minutes, the Panel carefully considered all information provided and presented by EPA, as well as information presented by public commenters. This document addresses the information provided and presented by these groups within the structure of the charge.

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SAP Minutes No. 2009-05

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

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MARCH 31 - APRIL 1, 2009
FIFRA Scientific Advisory Panel Meeting,
Held at the
Environmental Protection Agency Conference Center
Arlington, Virginia

Steven G. Heeringa

Steven G. Heeringa, Ph.D.
FIFRA SAP Chair
FIFRA Scientific Advisory Panel
Date: June 29, 2009

Myrta R. Christian

Myrta R. Christian, M.S
Designated Federal Official
FIFRA Scientific Advisory Panel
Date: June 29, 2009

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
March 31 – April 1, 2009

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INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) Scientific Advisory Panel (SAP) has completed its review of Scientific Issues Associated with Designating a Prion as a 'Pest' under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), and Related Efficacy Test Methods. Advance notice of the meeting was published in the *Federal Register* on December 17, 2008. The review was conducted in an open Panel meeting held in Arlington, Virginia, from March 31 - April 1, 2009. Dr. Steven G. Heeringa chaired the meeting. Myrta R. Christian served as the Designated Federal Official.

EPA is developing a Notice of Proposed Rulemaking (NPRM) that describes how EPA proposes to revise its regulations to include "prion" in the definition of "pests" that fall under the jurisdiction of FIFRA. The work group for the NPRM drafted a "white paper" entitled, "Scientific Information Concerning the Issue of Whether Prions Are a 'Pest' under the Federal Insecticide, Fungicide, and Rodenticide Act." The purpose of this paper was to provide the work group with sound knowledge about the scientific issues that are relevant to the proposed rule. Although the EPA's NPRM will be focused also on legal and policy matters and not exclusively on scientific discussion, EPA nevertheless presented this paper to the SAP for review as to the completeness and accuracy of its characterization of the scientific issues. EPA was not asking the SAP to interpret Congress' intent in drafting FIFRA.

In parallel with the NPRM, EPA is also developing guidance on a test protocol for measuring the performance of antimicrobial pesticides intended to reduce the infectivity of prions (TSE agents) on inanimate surfaces (hereafter referred to as a "prion-related claim"). This draft document is entitled, "EPA Guidance for Efficacy Test Methods for Products Bearing Prion-Related Claims." This guidance is needed because the proposed rule will require that antimicrobial products with prion-related claims be supported by valid efficacy data acceptable to EPA prior to registration. Although this guidance is not part of the rulemaking *per se*, it is important to the implementation of the rule, so EPA presented it to the SAP for comment on the scientific issues.

Dr. Steven Bradbury (Deputy Director, Office of Pesticide Programs, EPA) offered welcome and opening remarks at the meeting.

The agenda for this SAP meeting included presentations from the Antimicrobials Division (AD) in the OPP and the Office of Research and Development (ORD) in EPA. In addition, several presentations were made by scientists from the U.S Department of Agriculture and U.S. Food and Drug Administration.

PUBLIC COMMENTERS

Written statements were provided by:

Rebecca Humphries, Chair, Fish and Wildlife Health Committee and Director, Michigan Department Natural Resources

Greg Arthur, Deputy Director, Wyoming Game and Fish Department

Donald L. Montgomery, D.V.M., Ph.D., Dipl ACVP, Professor and Head, Department of Veterinary Science, University of Wyoming

Walter E. Cook, D.V.M., Ph.D., DACVPM, Wyoming State Veterinarian, Wyoming Livestock Board

SUMMARY OF PANEL DISCUSSION AND RECOMMENDATIONS

As charged by the EPA, the Panel reviewed the interagency White paper titled **"Scientific Information Concerning the Issue of Whether Prions Are a 'Pest' under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA),"** and discussed the nature of prions with respect to their potential classification as a "Pest" under FIFRA; guidance on testing issues for assessing efficacy of pesticides for reduction of prion infectivity; efficacy performance and labeling guidance for such pesticides; and lastly, scientific evidence for prion hierarchy in resistance to pesticide action. The prion terminology defined in the White paper will be used in these meeting minutes: "…'prion' refers to a misfolded isoform of any prion protein; 'prion protein' can refer to both the normal and misfolded isoforms."

1. Interagency White paper issue: Does the interagency White paper on prions adequately identify and summarize available, relevant scientific studies and do these support designation of prions as "Pests"? The White paper summarizing the prion literature with respect to description of transmissible spongiform encephalopathies (TSEs) and prions, key characteristics of prions, the prion hypothesis and normal prion protein, neurotoxic mechanisms in TSEs, TSE diseases, history of TSE diseases, transmission and routes of exposure for TSEs, occupational and safety procedures and decontamination methods was accepted as adequate by the majority of the Panel. However, many statements in the White paper need to be revised to reflect accurately what is definitively known about prions and TSEs and that which is reasonably hypothesized based on scientific observations.

A major issue discussed by the Panel was the prion hypothesis for the infective nature of TSEs. A majority of the Panel accepted the prion hypothesis for TSE infectivity with the caveat that cofactors may also be involved. The prion hypothesis, although considered unproven by some Panel members, provides a logical explanation of the infective nature of TSEs and the apparent lack of nucleic acid. The strength of the support for the prion hypothesis by experimental infection of transgenic mice with synthetic prion fibrils was questioned because spontaneous neurodegenerative disease in unexposed mice is also observed. A minority opinion contended that prion accumulation in TSEs is a pathologic change in response to an as yet unidentified TSE etiologic agent, possibly a virus.

The penultimate scientific question from the EPA to the Panel relative to the character and nature of prions is whether the science in the White paper supports the classification of prions as a "Pest" under the FIFRA. The regulatory basis (not reviewed by the Panel) on which this science question is assessed is whether prions are a form of a microorganism, i.e., prions are microorganisms other than bacteria or viruses, and whether prions are living entities. A majority of the Panel concluded that prions can be included in the category of "other microorganisms." A minority of Panel members contended on scientific basis that prions lack the significant features typical of microorganisms.

In conclusion, a majority of Panel members agreed that once revised as suggested by the Panel the White paper can adequately identify and summarize the scientific literature concerning prions. Furthermore, a majority of the Panel accept that prions are "other microorganisms" exhibiting some features of living entities.

2. Efficacy Guidance Test Method Issue: Are the specific test systems recommended in the EPA draft guidance document scientifically appropriate to support the registration of pesticide products with prion-related claims?

The consensus of the Panel was that animal bioassays are necessary to adequately assess reduction of prion infectivity by prion pesticide products. The Panel was asked to comment specifically on the three requested areas.

a. Carrier-based, animal infectivity assays for environmental surfaces (e.g., hard, nonporous surfaces).

The Panel had reservations about the appropriateness of application of carrier-based animal bioassays for assessing pesticide product reduction of prion infectivity. In the case of carrier-based assays, end-point titrations pose the problem of the practicality of doing serial dilutions of the treated surface, i.e., treated stainless steel wire or beads of various compositions, where the presentation of absorbed prions to the bioassay may be limited by the surface area of the carrier exposed to the brain. There may be substantial problems in quantifying reduction of infectivity in carrier-based models as well, because absorption of diluted brain homogenates on to the carrier may not proportionately reflect the infectivity present in the original sample. However, it was also suggested that disproportionate absorption can be assessed by use of a positive standard dose-response curve for carrier-bound infectivity.

b. Suspension-based, animal infectivity assays used in liquid media (e.g., wastewater).

The Panel agreed that the suspension-based end point dilution animal bioassay is the most reliable method to be used for final validation of reduction of prion infectivity. Furthermore, the Panel considered incubation interval animal bioassays as a screening method to better focus the range of test dilutions necessary for more definitive end point dilution animal bioassays, with the advantage of reducing cost and use of animals for testing; however, an end point animal bioassay is necessary to calculate directly a reduction in infectious titers and cannot be substituted by an incubation interval animal bioassay. Some Panel members supported designation of a standard animal bioassay such as a rodent-adapted scrapie strain in a rodent model system for use with all registrant prion pesticide testing, whereas others considered that testing needed to be conducted with native prion strains in relevant animal hosts. The Panel urged EPA to monitor closely the particulars of testing methods for uniformity of treatment of control and experimental animals, preparation of prion inocula, sufficiency of animal numbers used relative to the variance of the bioassay model system, and determination of the bioassay end points. Necropsies of all test animals for the presence of TSE disease based on Western blot detection of protease resistant prion and neuropathologic changes consistent with TSE are required.

c. Any other known test methods for evaluating the efficacy of pesticide products used on either environmental surfaces or in liquid media.

The majority of the Panel concluded that in vitro immunodetection assays or cell-based bioassays are not adequate for use in assessing reduction in infectivity for evaluating efficacy of pesticide products with prion related claims (hereafter referred as prion pesticide products). In the case of immunodetection assays, these do not measure infectivity; and in the case of cell-based bioassays, these have not been adapted for the application of assessing reduction in

infectivity. A minority contended that in vitro amplification immunodetection assays such as protein mis-folding by cyclical amplification (PMCA) and cell-based bioassays can serve as a complementary assay to animal bioassays allowing assessment of many more prion strains and prion substrates than is practical with animal bioassays.

The Panel recommends to EPA that end point dilution animal bioassays be used to assess reduction in infectious titer by treatment of samples with prion pesticide products.

2) **Efficacy Guidance Performance Criterion Issue:** Are the product performance criteria specified in the EPA draft guidance document to support the registration of pesticide products with prion-related claims scientifically sound? In particular, the draft efficacy guidance document recommends a target efficacy criterion of six (6) logs of reduction of infectivity in the treated versus untreated (control) groups.

The Panel was in general agreement that some widely used model systems, such as the 263K or Sc237 laboratory rodent-adapted scrapie prion strains have sufficiently high infectivity titers of brain tissue, making it possible to detect a six (6) log reduction in infectivity. However, several factors may reduce the dynamic range of detection for prions from particular prion strains or natural hosts to much less than six (6) logs. These factors include 1) limited volume of about 25μl for intracranial injection in laboratory rodents, 2) dilution of inoculum by inactivation or other reagents prior to animal bioassays, and 3) lower infectivity of naturally occurring prion strains from the natural host in the range of 10^5 to 10^8 ID_{50}/g resulting in a dynamic range for animal bioassays of three (3) or four (4) logs.

Prion pesticide registrant's claims of efficacious log reductions in infectivity will need to be accompanied by a statistical methodology, assessment of assay variability for a desired level of confidence, and regression analysis models for endpoint animal bioassays. Although suspension-based methods have a rational basis for calculating log reductions in prion infectivity, carrier-based methods provide further issues for calculating log reduction. Infectious brain material serially diluted and dried onto carriers may not retain infectivity proportional to that in the original sample thereby negating the endpoint animal bioassay calculation of log reduction in infectivity.

In addition, the target degree of reduction in infectivity may need to be considered in light of the proposed application or use, risk to human and animal health, and what is achievable and can be validated for a particular prion pesticide. A reduction of six (6) logs of infectivity in waste water may be adequate to reduce the infectivity compared to the risk posed by chronic wasting disease (CWD) prions to human health, whereas the efficacy of a six (6) log reduction for Creutzfeldt-Jakob disease (CJD) prions may not be adequate to reduce the potential that a human patient might contract CJD from a surgical instrument. Risk assessments may be available to help formulate expected standards of performance, but a one-size fits all product performance guidance may be neither valid nor desirable.

In conclusion, the Panel recommends that target reduction in infectivity be assessed in a relevant animal model using the naturally occurring prion strain from the natural host to support the specific label claim made by the manufacturer of the prion pesticide. To demonstrate target efficacy, the level of reduction should be set at the maximum measurable reduction with the

designated natural host prion strain in the designated model. No consensus was reached on acceptability of target efficacy of a six (6) log reduction in a widely used model such as the hamster-adapted 263K or Sc237 scrapie prion strains as the sole test for pesticide efficacy of reduction of infectivity for a target prion strain; however, there was consensus that a widely used model could be used in addition to the target prion strain to show the possibility of higher reduction levels of prion infectivity.

3) **Efficacy Guidance Labeling Claim Issue:** Are the labeling claims described in the EPA draft guidance document scientifically appropriate based on the recommended test systems and product performance standard? This guidance: "Has been demonstrated to reduce infectivity of prions (TSE agents) based on testing using (insert type of organism in which the prions were raised) (insert prion type)" is careful to avoid claims normally applied to microorganisms, e.g., "destroy," "mitigate," "eliminate," "control" which may be misleading when applied to prions."

The Panel agrees with EPA's proposal to frame the label claim statement as "reduce infectivity," but even with this relatively conservative claim, there are several caveats. From a scientific perspective, the state of "complete elimination of infectivity" is probably impossible to achieve in practice, because minimal infective doses of prions are not known, particularly for humans. In addition, experimental observations suggest that treatments which reduce TSE infectivity may lead to incubation periods that fall outside the linear range of animal bioassays. The phrase "reduce infectivity" is valid only as long as the method used to measure infectivity has an acceptable dynamic range of detection in a relevant animal bioassay system.

Care will need to be taken to state accurately prion pesticide product performance to guide user understanding and expectations for the prion pesticide product. Users in hospitals concerned about CJD may expect something closer to "elimination of infectivity" on environmental surfaces, whereas the veterinary and wildlife management community may be more comfortable with the "reduced infectivity" approach. EPA might require registrants to discuss the implications of the "reduce vs. elimination" approach in sufficient detail so that users can understand and appreciate what the product can and cannot do in relation to intended uses, e.g., decontamination of hospital environmental surfaces, decontamination of laboratory working surfaces, application on porous materials such as fences, etc.

With respect to specificity of the labeling, the Panel suggests that the prion strain and original host, bioassay animal host, and observed log reduction in infectivity be present in the label as well as the dynamic range of the animal bioassay used. Concerning whether a six (6) log reduction in infectivity is needed to receive approval as efficacious, it was noted that even a five (5) log reduction would be acceptable for a product with a prion-related claim under the above conditions specified on the label for prion strains and natural hosts for which five (5) log is the maximum attainable reduction in infective titer possible in the animal bioassay. EPA is urged to have scientific advisement to seek a consensus compilation of acceptable prion strain/animal bioassay models. In particular, this activity might help guide registrants away from choosing model systems with low levels of prion infectivity to test their product, thereby preventing inappropriate testing for prion pesticide products.

In conclusion, there was general consensus among the Panel members that the label give sufficiently detailed and accurate information to support the manufacturer's label claim for reduction in prion infectivity and to enable the product user to assess how efficiently, and under what usage, the prion pesticide product reduces prion infectivity.

4) **Efficacy Guidance Hierarchy Issue:** Do different prion types exhibit variation in the degree of resistance to inactivation by pesticide chemicals and can a hierarchy of resistance by prion type be reliably determined at this time?

There is clear scientific basis for distinguishing various prion strains based on distinct incubation time and lesion profiles in a given host as well as biophysical and chemical properties. However, there are limited data on the relative resistance of prions from various species and strains to chemical inactivation. Furthermore, a simplified discussion of chemical reduction of prions infectivity is complicated because different prion pesticide products may act by differing mechanisms causing reduction of infectivity in different and unpredictable ways. With the understanding that there may be variability in assessment of reduction in infectivity based on the prion strain and animal bioassay used by registrants, the Panel considered the advisability of using one animal bioassay or a combination of two animal bioassays to assess prion pesticide efficacy. For the combined assay approach, one of these animal bioassays could be specific to the samples proposed for specific use of the prion pesticide, whereas the other could be a standard, widely-used model, e.g. 263K scrapie prion in hamsters. The majority of the Panel members favored using only one animal bioassay in an end-point titration format. The single animal bioassay needs to be robust and specific to the specified decontamination objectives.

In conclusion, the consensus from the Panel is that no single hierarchy of strain resistance to prion pesticide action can be established at this time.

PANEL DISCUSSION AND RECOMMENDATIONS

Agency Charge

1. White Paper Issue: Whether EPA's draft review paper, "Scientific Information Concerning the Issue of Whether Prions Are a 'Pest' under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)," adequately identifies and summarizes available, relevant scientific studies.

In 2005, EPA established a Work Group to develop a Notice of Proposed Rulemaking (NPRM) that defines a prion as a "pest" under FIFRA. To assure that it considers key available scientific studies that are relevant to the issue of whether a prion is a "pest" under FIFRA, the Work Group drafted a White paper. While the paper received intra-Agency review, it was not subjected to peer review outside of EPA. Accordingly, EPA seeks the SAP's peer review of the attached, draft review paper (USEPA 2008). Some of the key references cited in the review paper have been provided to the SAP.

EPA wishes to point out that the NPRM will also focus on legal and policy matters that are not addressed in depth in the "white paper." EPA is presenting this paper to the SAP solely for review as to its characterization of the scientific issues, and is not asking the SAP to interpret legal/policy issues such as Congress' intent in drafting FIFRA.

- **Please comment on the accuracy of the characterization of the nature of prions, and the adequacy of the review of the relevant scientific information to support that characterization, as presented in EPA's draft paper, "Scientific Information Concerning the Issue of Whether Prions Are a 'Pest' under the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA)."**

Panel Response

The Panel discussed the nature of prions described in the interagency White paper for the accuracy of its scientific characterization of prions and adequacy of the scientific information cited. The White paper is divided into three Parts with Part I concerning EPA's reasoning for reviewing the prion literature with respect to declaring prions as "Pests" under FIFRA, Part II

summarizing the prion literature with respect to description of TSEs and prions, key characteristics of prions, the prion hypothesis and normal prion protein, neurotoxic mechanisms in TSEs, TSE diseases, history of TSE diseases, transmission and routes of exposure for TSEs, occupational safety procedures and decontamination methods, and Part III examining the science of prions in relation to the FIFRA definition of "Pests". The authors of the White paper have done a tremendous amount of work assembling this information and are commended for summarizing a great deal of prion literature. In general, the Panel found the White paper to be comprehensive and most of its content to be accurate. As a review of available scientific literature on TSEs and prions, the White paper was accepted as adequate by the majority of the Panel, pending incorporation of suggested revisions presented herein. It was noted by several Panel members that there are many statements in the White paper needing revision to accurately reflect what is known and what is reasonably speculated about prions and TSEs. One Panel member found the White paper to be strongly biased for the prion hypothesis relative to the viral hypothesis and lacking in relevant information.

The Panel questioned EPA representatives at the Meeting about Part **I. Introduction and Background** to better understand the EPA's intent for the Panel's review process. There are some inconsistencies in the material presented in this Part compared to Part III of the White paper. One Panel member concluded that Part I read like there is a presumption that prions will be recognized as "pests" with the assertion that prions share key characteristics with other microorganisms, but in Part III the authors of the White paper did not appear to be entirely convinced that their "pest" argument would withstand scientific scrutiny. This member thought that Part I might be more correctly titled "Prions and Consequences for Public Health."

For the peer review of White paper Parts **II. The Science of Prions** and **III. The Science of Prions as It Relates to the FIFRA Definition of "Pests"** the Panel's comments are provided as general comments immediately below and specific comments in the subsequent section.

General Comments

For Part **II. The Science of Prions**, one Panel member commented that in multiple areas of the White paper too much credence is given to the Legname paper as proof of the prion hypothesis (Legname, Baskakov et al. 2004). First, the synthetic fibrils accelerated neurodegenerative disease only in transgenic animals that overexpress (by 16-fold) the mutated prion transgene (PrP90-231), and not in wild type mice; the transgenic mice that were used become spontaneously ill even without any inoculation whatsoever, albeit at a slower rate. Moreover, this paper and the subsequent literature lacks the negative control most critical to establishing that the synthetic amyloid contained prions, i.e., proof that prions are not spontaneously accumulating in mock-inoculated control transgenic mice. Thus, it remains unclear whether the synthetic amyloid initiated, or merely accelerated, a spontaneous, ongoing pathogenic process that was due to massive overexpression of the transgene. Infectious agents, i.e. prions, should be initiators rather than mere accelerants of disease. A much more important study of the primacy of prion conversion in prion propagation that is not appropriately emphasized in the White paper is the Deleault study (Deleault, Harris et al. 2007) in which prions were amplified by PMCA containing normal prion protein (purified from brain) and polyanions, a study which strongly indicates the importance of non-prion polyanions, i.e., RNA or heparan sulfates, in prion replication.

A. Description is a good review of the "protein only" hypothesis. Although the "virus" hypothesis is mentioned, one Panel member thought that this needs to be discussed in more detail. Also, there was general agreement with the hypothesis that cofactors (polyanion/sugar/nucleic acids) play a critical role in the process and also need to be described. A Panel member suggested that Griffith (Griffith 1967), not Alper (Alper, Cramp et al. 1967) or Prusiner (Prusiner 1982), should be more prominently portrayed as the originator of the protein only hypothesis and that this section should be based on a more accurate history.

B. Key Characteristics

1. Prion Protein and Terminology

One Panel member suggested that including a brief summary of yeast prions could be beneficial to emphasize the more general scope of the prion phenomenon, and another thought that the definition of prion must emphasize infectivity rather than the mere misfolding of prion protein; many misfolded forms of prion protein are not (infectious) prions.

2. Current Status of "Protein Only" Hypothesis

One Panel member commented that the "protein only" hypothesis for prions, although it does provide a logical explanation for many of the characteristics of this agent, does not fit exactly with "infectivity" when the context of the discussion centers on the generation of increasing amounts of the abnormal isoform. The "recruitment and conversion" explanation appears to be a more logical description of the process. Another Panelist disagreed that the prion hypothesis is a logical or adequate explanation of TSE infectivity, but rather is circular reasoning.

3. Normal Prion Protein

One Panel member commented that the discussion of the expression (homozygosity vs. heterozygosity) at codon 129 of the prion protein is an important topic. This could be expanded with regards to vCJD, where there is good epidemiologic information from the United Kingdom on apparent susceptibility or resistance and its impact on the incidence of the disease in that population. It should also be expanded to scrapie, where it is well known that certain genotypes of sheep are more resistant or susceptible to developing scrapie. Another Panel member indicated that the physiological role of the prion protein is not discussed sufficiently (synaptic transmission, T cell activation, circadian rhythm, olfactory physiology).

4. Neurotoxic Mechanism

Although this topic is interesting, one Panel member agreed with the White paper authors that we need to learn more about the pathology of TSEs. Despite some in depth knowledge about important aspects of the neurotoxic mechanism, we have an incomplete understanding of the process as a whole. Also in this section, the authors exclusively talk about degradation of prions in "proteasomes", but do not mention "lysosomes" as important intracellular sites of prion degradation. In addition, it was noted that prion amyloid cannot be considered responsible for neurotoxicity as there is no correlation between prion amyloid plaques and neuronal death, and furthermore, one Panel member noted that the requirement for endogenous normal prion protein for toxicity has not been proven, as is implied in the White paper.

C. TSE Diseases

The Panel generally agreed that this is a good discussion and review of the known TSE diseases in animals and humans. However, one Panel member noted that the statement that most prion

diseases in humans are "acquired" needs to be rephrased. When one considers the epidemiology of CJD, the overwhelming majority of cases are identified as sporadic, with the next largest category being familial or inherited CJD. Rather, the statement can be clarified to say that certain TSEs (kuru and vCJD) appear to be "acquired" as a result of "exposure" via ingestion of the prions. There also needs to be some discussion about the period between exposure and the onset of symptoms in this section of the document.

D. History of TSE Disease

This section of the review presents a reasonable summary of the history of TSE diseases.

E. Transmission and the Potential Routes of Exposure

One Panel member felt that an important point missing from the discussion of transmission is the differing lengths of time between "exposure" and onset of symptoms as related to the portal of entry. Direct inoculation of prion-contaminated material into the central nervous system (CNS) results in a relatively shortened time interval, whereas there is a considerably longer interval between exposure and onset when the prions enter at a site considered as "peripheral."

F. Occupational Safety Procedures

One Panel member thought that this section needs to be discussed in more depth. This section also provides a good opportunity to discuss the "Chain of Infection," a concept that explains why infectious diseases occur "when and where" they do. For example, just because there is infectivity potentially present in some source at some time, this does not automatically mean that transmission will occur and infection will ensue. There is very little evidence to support the

notion that prions can be acquired from environmental surfaces. In spite of this, it is prudent to keep work areas and surfaces clean and decontaminated, especially if one is working with known infectious materials.

With regard to the six cases associated with neurosurgical instruments and devices, there is only strong evidence of prion transmission in those cases associated with the use of stereotactic depth electrodes. In the opinion of one Panel member, the four cases presumably associated with neurosurgical instruments are not very well described, and there are very few details on the methods used to clean and reprocess the instruments. It is also important to note that the last of the instrument or device cases was reported in 1976.

G. Decontamination Methods

One Panel member noted that the history of development of decontamination methods is interesting and provides a logical explanation of why experiments were designed as they were. Veterinary scientists attempted to deactivate prion infectivity while retaining immunogenicity such that these "disinfected" prion preparations could be used to immunize sheep against scrapie. Clearly, the objective was very different than modern day objectives of eliminating prion biomass by decontamination. This Panel member indicated that there is increasing interest in exploring prion decontamination methods that more closely resemble reprocessing methods currently used in modern hospitals and laboratories for which the objective of sterilization is to remove patient derived biomaterials and bioburdens as completely as possible, making medical instruments safe for use with subsequent patients. New technologies are being evaluated in this

regard, and some of these should be discussed here. An additional complicating factor is that strain diversity in natural populations and its impact on decontamination is not known.

It was noted that the susceptibility of humans to infection with CWD prions is not known. Currently, there is no epidemiological evidence to suggest that CWD has been transmitted to humans. Experimentally, CWD has not been successfully transmitted to transgenic mice expressing human prion protein. Human prion protein can be converted to a proteinase K resistant form *in vitro* by CWD prions; however, this conversion is much less efficient compared to species with known susceptibility to CWD. Finally, non-human primates are susceptible to intracerebral CWD infection. Recent evidence suggests that more than one strain of CWD may exist, complicating the interpretation of previous work involving human susceptibility to CWD infection.

The Panel discussed Part **III. Science of Prions as It Relates to the FIFRA Definition of a "Pest"** extensively and had a divided opinion about whether the scientific understanding of the nature of prions supports its classification as a "Pest" as specified in FIFRA Sections 2(t) or 25(c)(1). The major opinion was that there is sufficient scientific evidence to support this classification, whereas a minority of Panel members did not support this view.

A. How Do Prions Relate to Common Definitions of "Microorganisms"?

The Panel was in general agreement that prions are pathogenic and infectious, may have some similarity to viruses, are present in the environment resulting in human exposures, might have a

non-host encoded component, induce innate immune responses and are transmissible infectious agents.

A minority of Panel members concluded that prions do not possess features typical of microorganisms.

One Panel member agreed that prions are deleterious to animal and human health, but disagreed that prions can be classified as "other microorganisms." In order to do this, the White paper authors would need to emphasize the "unknown virus" hypothesis. This Panel member stated that prions do not reproduce and they do not grow. The normal prion protein exhibits turnover as a normal function, but prions accumulate. Are prions alive? This panel member thought the answer was "no."

Another Panel member suggested that whether scientific studies support classification of prions as pests depends on the interpretation of what "pests" are. In particular the second part of the definition of "pests" as "any other form of … virus, bacteria or other microorganism…." is problematic because prions lack the characteristics of viruses or bacteria and the meaning of "other microorganisms" implies a living microbe, which this discussant believed was not scientifically supported. Prions are primary proteinaceous substances and have some characteristics in common with self-assembling structural components of bacteria such as the multimeric extracellular curli protein of the bacteria *Escherichia coli* (Wang and Chapman 2008), but prions lack the overall multi-component complexity and organization that are hallmarks of microorganisms.

Instead of being microorganisms, prions could be classified as "infectious substances" under the definition of "biological agent" in Code of Federal Regulations Title 42 Public Health Section 73.1 (42CFR). Biological agents are defined as "any microorganism or infectious substance capable of causing death, disease, or other biological malfunction in a human, an animal, a plant, or another living organism; of deleterious alterations of the environment". "Infectious substances" of Title 42 consist of microbial, plant and animals toxins, and many of these, like prions, are entirely proteinaceous substances (7CFR). Protein toxins do many things that prions do. Protein toxins exhibit host and cell-type specificity of intoxication, use host cellular machinery for their intoxication mechanisms, and can resist endosomal protein degradation pathways (Watson and Spooner 2006). Some protein toxins are stable in the environment and can enter the host via ingestion (Wobeser, Baptiste et al. 1997). Protein toxins recruit normal host cell proteins to adopt different conformations in a way that causes injury to cells and organs (Fabbri, Travaglione et al. 2008). Ingestion of protein toxin infected tissues can result in transmission of infection to new susceptible hosts (Wobeser, Baptiste et al. 1997). Prions do not share extensive similarities to bacteria or viruses, but prions share many distinctive features of protein toxins. It appears that prions might be best classified as "infectious substances" rather than "other microorganisms". This Panel member concluded that attempts to classify prions as "other microorganisms" as a means to gain regulatory oversight is not supported scientifically.

A majority of Panel members supported the view that prions can be categorized as "other microorganisms."

One Panel member commented that when considering the issue of whether or not prions fit the definition of a microorganism, the White paper concludes that prions do not reproduce, adapt, grow or move. However, when viewed from the point of view of TSE biology, these conclusions are inaccurate. Research spanning the last 50 years leaves no doubt that the agents which cause prion disease can reproduce, adapt, grow and move within the host.

Another Panel member commented that replication of prions has been amply demonstrated by the fact that they can be titered and that animals infected with a low level of prion infectivity eventually accumulate exponentially higher levels of infectivity. This can only occur if prions can replicate themselves. As demonstrated by the isolation and careful characterization of multiple mouse and hamster-adapted sheep scrapie strains, prions can adapt to new species. Peripheral inoculation of TSE infectivity eventually leads to infectivity in the brain, something that can only occur if the infectious agent moves within the host. Finally, prions most definitely exhibit a stable steady state over a long period of time (maintenance). Established stocks of TSE infectivity can be maintained for decades with no change in either their titer or their biological characteristics.

A third Panel member said that defining bacteria and viruses as microorganisms does not depend upon the very different mechanisms they use to replicate, spread, and adapt. Likewise, defining TSE agents as microorganisms should not depend upon the mechanisms underlying their replication and spread. Rather, it should be based upon whether or not their general biological characteristics are consistent with that of a microorganism. It is only in trying to reconcile the proposed protein-only mechanisms underlying TSE pathogenesis with the universally accepted

nucleic acid based mechanisms of more conventional infectious organisms that defining prions as microorganisms becomes more complex.

Another Panel member suggested re-writing Part III to better reflect the characteristics of prions similarity to other microorganisms, such as viruses. Prions grow, replicate, adapt, maintain, move and are organized. The discussion should be centered around Koch's postulates, instead of a dictionary definition of microorganism or life.

Another Panel member stated that TSE agents, i.e., prions, are environmental pathogens that infect mammals, replicate virus-like, cause disease according to Koch's postulates, though the infectious agent has not been sufficiently purified.

One Panel member stated that the virus hypothesis of TSE infectivity is in accord with a classification of the TSE agent as a microorganism. It was suggested that Part III needs to discuss Koch's postulates as they relate to prions. Important considerations for FIFRA being:

- Infectivity: Kuru epidemic stopped with end of ritual cannibalism, United Kingdom BSE epidemic markedly decreased with removal of external source, endemic scrapie, & environmental CWD
- TSE agents as environmental pathogens, not spontaneous generation of infectivity
- Strains of TSE agents as:
    - Encode individual properties: obvious in controlled host genotype background
    - Classified by incubation time and by neuropathology (lesion profile)

- Agents often maintain strain identity even after passage in different species, e.g., sCJD to mice
- Most individual agents not separated by abnormal prion band profiles on blots

B. Are Prions Alive?

One Panel member concluded that instead of focusing so much on the vague question of whether prions are living microorganisms, it seems that the prions as pests discussion might be more fruitfully oriented towards the discrimination between prions as pathogens (which propagate in the host) or toxins (which don't propagate).

In conclusion, a majority of the Panel agreed that once revised to incorporate revisions suggested by the Panel the White paper will adequately identify and summarize the scientific literature concerning prions and that prions are "other microorganisms" exhibiting some features of living entities.

Specific Comments

With respect to **specific comments and editorial remarks** on the White paper, the Panel noted the following specifics:

1. Please add Castilla et al., 2005; Delault et al., 2007 and Sigurdson et al., 2009 to the literature list in section II, A:

   Castilla, J., P. Saa, C. Hetz, and C. Soto. 2005. In vitro generation of infectious scrapie prions. Cell 121:195-206.

Deleault, N. R., B. T. Harris, J. R. Rees, and S. Supattapone. 2007. Formation of native prions from minimal components in vitro. Proceedings of the National Academy of Sciences of the United States of America 104:9741-6.

Sigurdson, C. J., K. P. Nilsson, S. Hornemann, M. Heikenwalder, G. Manco, P. Schwarz, D. Ott, T. Rulicke, P. P. Liberski, C. Julius, J. Falsig, L. Stitz, K. Wuthrich, and A. Aguzzi. 2009. De novo generation of a transmissible spongiform encephalopathy by mouse transgenesis. Proc Natl Acad Sci USA 106:304-9.

2. Page 2, 1st paragraph:
   a. Delete "Alper et al., 1966, 1967" when referring to the prion-only hypothesis
   b. "bovine spongiform encephalitis" should be "bovine spongiform encephalopathy.
3. Page 2, 2nd paragraph:
   a. The "by-passing of the proteasome" does NOT produce TSE clinical features. Please rephrase.
   b. TSE clinical features depend on "species AND TSE strain used for infection." Please modify.
   c. The statement that "the property of infectivity appears to reside in the beta sheet-rich conformation" is a great oversimplification. In fact many beta-sheet-rich forms of prion have been generated that have no infectivity.
4. Page 3, 1st paragraph:
   a. The use of the word transcript in the sentence "misfolded transcripts recruit and convert normal prion transcripts" is incorrect. Prion protein would be more correct.
   b. "low level of recruitment and conversion..." This is incorrect.

c. It is not the "recruit and convert" mechanism that separates prions from other protein misfolding diseases; it is their relative transmissibility between individuals.

d. Plaques are not linked to cell death

5. Page 3, 2nd paragraph:

a. Replace plaque-forming isoform" by "aggregation-prone isoform"

b. Correct spelling of "plaque" in line 8

c. Take out sentence on Legname et al., 2005

6. Page 3, 3rd paragraph: There are still cases of bovine spongiform encephalopathy (BSE) in the UK. Wrong statement, that BSE "appears to have effectively ended."

7. Page 3, last paragraph: The apparent molecular weight of prion-associated fragments can range from ~17,000 to 37,000.

8. Page 4, first paragraph:

a. The term PrP^d is prion that is present in diseased animal and is not detected in uninfected animals using immunohistochemistry or immunoelectron microscopy.

b. PrP-res has been used to connote a "purified protein or recombinant polypeptide fragment" resistant to PK. This is incorrect.

c. It needs to be stated that "prion is infectious, not only "misfolded."

e. Replace "disease isoform" by "agent strain";

f.Replace "infectious conformation of transcripts" by "misfolded PrP" and also "prion" should refer to "infectious particle" whether it is constituted solely of prion or not

9. Page 4, 2nd paragraph:

a. Reference "Colby et al, 2007" is wrong

b. Wrong statement regarding Legname manuscript: Fibrils consisting of recMoPrP(89-230) were inoculated intracerebrally into transgenic (Tg) mice expressing MoPrP(89-231).

b. The explanation regarding the domains of interactions between PrP^{C} and PrP^{Sc} is very narrowly focused. Other studies, such as those by Horiuchi et al., 2001 and Chabry et al., 1998 on the localized interactions between the two prion isoforms should be included.

10. Page 4, 4th paragraph:

    a. The statement "The Prnp gene appears to be conserved across species from yeast to primates" is incorrect. Prnp is conserved among mammals and also to amphibians and reptiles. More distantly related proteins are present in fish. Yeast and fungal prions are unrelated to mammalian prions on a sequence level, but are so named because they share the characteristic of being able to adopt two different conformations, one of which leads to aggregation.

    b. The word "homology" is used incorrectly. This should be replaced with "sequence identity" or in the case with doppel to indicate if normal prion protein and doppel are homologous.

11. Page 5, 2nd paragraph: There is just as much evidence that copper inhibits prion protein conversion as there is that copper stimulates conversion.

12. Page 5, 4th paragraph: Prion function involves olfactory physiology (Le Pichon et al., 2009) etc.

13. Page 5, 5th paragraph:

    a. It is incorrect that prions kill neurons by disrupting the function of the normal prion protein.

b. Delete "development of prion diseases requires the presence of normal PrP"

14. Page 5, last paragraph and page 6, first 4 paragraphs:

a. The literature interpretation in relation with the neurodegenerative mechanism is incorrect. Plaques or immunohistochemically detectable prion aggregates are not neurotoxic. Several groups have shown that peptide models of prion (Haik et al. 1998) and small prion oligomers are neurotoxic (Kazlauskaite et al. 2005, Novitskaia et al. 2006, Simoneau et al. 2007) in the presence or absence of prion expression. Therefore the current view is that prion-related neurodegeneration might have two components: a loss of function of prion protein, and a gain of toxicity of prion oligomers (independent of neuronal prion expression).

b. In the three references cited (Brandner, Mallucci and Chesebro), the form of prion that was detected was large, non-toxic aggregates and does not demonstrate that the absence of prion protein expression is responsible for the absence of toxicity. In Brandner, the absence of prion expression by the mice prevented the generation of toxic prion oligomers. In Mallucci, prion was largely sequestered in astrocytes, and no reactive prion oligomers were generated, again because of the absence of prion expression by the mice. In Chesebro, prion plaques are not toxic, but again it has nothing to do with the absence of cell surface prion protein expression. It is suggested to remove these three references and cite the two currently accepted components of neurodegenerative mechanism, with the appropriate references.

15. Page 6, 4th paragraph: "GTI" should be changed to "GPI". It should also be noted that these mice have approximately 1 log lower brain titers and that the disease is altered not prevented.

16. Page 7, Table 1: The table should be divided into "infectious', "sporadic", and "inherited" prion diseases. Also, the disease is called "fatal familial insomnia, FFI", Chronic wasting disease of "deer, elk and moose."

17. Page 8, 1st paragraph:

    a. Please mention also transmissible mink encephalopathy (TME) outbreaks.

    b. Please add "France" to the other contaminated European countries. In France, 13 cases of BSE were already diagnosed in 1995.

    c. Canada and USA had their first indigenous cases in 2003 and 2004, respectively.

    d. Canada not only has imported but also indigenous cases of BSE. Should be deleted from last sentence.

18. Page 8, 2nd paragraph: The second US BSE animal was from Texas.

19. Page 9, 3rd paragraph:

    a. CWD was found in moose (Baeten et al., 2007).

    b. Epidemiology/Prevalence of cervids in captivity can be rather high (up to 90%).

20. Page 10, 1st paragraph: add "intralingual, intranasal and intrasciated" to the route of applications.

21. Page 10, 2nd paragraph:

    a. Consumption of contaminated food is generally not considered to be involved in transmission of scrapie. Mention that both vertical and horizontal routes are likely natural scrapie transmission.

b. "prionuria": remove Ligios et al., 2007 — add Gabizon and Gregori literature.

22. Page 10, 4th paragraph: Maternal transmission of BSE and CWD is rather questionable.

23. Page11, 1st paragraph:

a. The interpretation of the study by Heaton et al, 2008 is incorrect. The study concludes that the prevalence for the E211K variant is extremely low (less than 1:2000 cattle) and, therefore, additional atypical BSE cases with K211 in the US will be very low.

b. Citation "Chesebro, 2003" is incorrect. The Chesebro review actually states that it has been *suggested* that the feeding of scrapie or BSE-infected tissue to mink caused BSE. It was not a statement of fact.

24. Page 11, 3rd paragraph: So far, 4 cases of vCJD blood transfusion have been reported.

25. Page 12, 4th paragraph: The citation for the Guideline for Handwashing and Hospital Environmental Control is incorrect. This guideline has different authors and was released in 1985.

26. Page 14, 2nd paragraph: There is no evidence to support the statement "prions bind tightly to stainless steel and are most difficult to inactivate on this surface." Prions do indeed appear to bind tightly to stainless steel and are difficult to remove, but there are very limited comparative studies of other surfaces.

27. Page 14, last paragraph- to page 15: In the context of decontamination a limitation can be the intrinsic limited sensitivity of the bioassay model, rather than a problem of the lifespan of the animals.

28. Page 15, 3rd paragraph: BSE prions "readily" transmit to felines, but not to humans. So far, 210 people out of the millions that were presumably exposed to BSE have gotten vCJD.

29. Page 17, 1st paragraph: "solely of proteins" needs to be modified.

30. Page 18, 2nd paragraph: correct spelling of reference from "Leninger" to Lehninger.

31. Throughout manuscript the authors use regularly "mad cow disease"; they should use "Bovine Spongiform Encephalopathy, BSE" instead.

32. It is not clear if 263K or 301V recapitulate the scrapie and BSE agent, respectively.

33. In general, the word "plaque" is not used correctly. The generalization of TSEs as diseases where neuronal plaques are made is not precisely correct. In fact, plaques are not the primary phenotype in the majority of cases of the most common human TSE, sporadic CJD. It would be more accurate to define TSE diseases as diseases of protein misfolding where, depending upon the type of TSE, prion can be found in either diffuse "non-amyloid deposits" or in "amyloid plaques."

Agency Charge

2. Efficacy Guidance Test Method Issue: Whether the specific test systems recommended in the draft guidance document are scientifically appropriate to support the registration of pesticide products with prion-related claims.

The draft efficacy guidance document (USEPA 2009) recommends a carrier-based, animal infectivity test method, if the intended use of a product is for treating environmental surfaces, and a suspension-based, animal infectivity test method if the intended use of a product is for treating liquids. The draft efficacy guidance document also states that the test methods may either be end-point titration or incubation time interval assays. EPA is interested in knowing the SAP's opinion on whether these recommended test systems are scientifically sound and appropriate approaches to evaluating the efficacy of pesticide products with prion-related claims. EPA would also like to know whether the SAP recommends that other test methods be considered to evaluate the efficacy of pesticide products used either on environmental surfaces or in liquid media.

- **Please comment on the scientific appropriateness of:**

  a. Carrier-based, animal infectivity assays recommended by EPA's guidance for evaluating the efficacy of pesticide products used on environmental surfaces (e.g., hard, nonporous surfaces).

  b. Suspension-based, animal infectivity assays recommended by EPA's guidance for evaluating the efficacy of pesticide products used in liquid media (e.g., wastewater).

c. Any other known test methods for evaluating the efficacy of pesticide products used on either environmental surfaces or in liquid media.

Panel Response

a. Carrier-based, animal infectivity assays recommended by EPA's guidance for evaluating the efficacy of pesticide products used on environmental surfaces (e.g., hard, nonporous surfaces).

One Panel member commented that there are substantial problems in quantifying reduction of infectivity in carrier-based models. Wires (or other carriers) incubated in diluted brain homogenate may not effectively represent proportionately the dilution made. Proportional dilution would only occur if all components of brain homogenate had exactly the same affinity for the surface. If prions bind more efficiently than other components the endpoint would be an overestimate, if prions bind less efficiently the endpoint would be an underestimate. Thus, dilution of brain homogenate and absorption to a carrier cannot then be used as a basis for incubation time bioassay. Determinations of log reduction would therefore be invalid. According to this Panel member, there is one published stainless steel wire based endpoint titration (hamster 263K prions serially diluted in normal hamster brain) which gave an endpoint of 5.6 (five point six) log ID_{50} (Fichet, Comoy et al. 2004). Dr. David Asher, Laboratory of Bacterial, Parasitic & Unconventional Agents, Division of Emerging and Transfusion-Transmitted Diseases, FDA, reported at the open Panel meeting an endpoint of 7 (seven) log ID_{50} when 263K was diluted in calf brain paste and mentioned a difficulty in getting prions to stick to the wires when using PBS as diluent. This highlights the potential problem and could lead a potential applicant for EPA approval to choose a formulation for which it is easiest to reach a defined log reduction, but which is not valid. Conversely, it is not possible to do an endpoint

titration with a treated wire to determine remaining titer.

Another Panel member said that full end-point titrations, or at least titer reduction experiments including the end-point, are the test method of choice for both the suspension-based and the carrier-based assays. Indeed, it has been shown in several studies that various physical (e.g. heat) or chemical treatments of prion-infected material can increase the incubation period by bioassay (when compared to the untreated sample) disproportionately to the titer reduction measured by end-point titration. Therefore, incubation period assays can overestimate the log reduction of a given treatment (Somerville and Carp 1983; Taylor and Fernie 1996).

In the case of carrier-based assays, end-point titrations pose the problem of the practicality of doing serial dilutions of the treated surface (practically, the treated wire or beads) to which prions are attached. If the treated surface, once "inoculated" induces an incomplete attack rate, it means that the amount of infectivity corresponds to the end-point and the log reduction can be calculated directly. If not, the only available method so far is the glass adsorption and crushing method, published by Chen and validated for prions by Dr. Asher (Chen 1975). A standard dose-response curve for carrier-bound infectivity can be provided by the adsorption onto carriers of serially diluted brain material (positive control). This Panel member commented on the potential lack of proportionality of adsorption of diluted sample on to carriers. Although this theoretical possibility is worthwhile considering, there is so far no experimental evidence for this. More data are needed to clarify this point. In any case, this point can be overcome by performing the same carrier crushing methodology to generate the standard curve.

b. Suspension-based, animal infectivity assays recommended by EPA's guidance for evaluating the efficacy of pesticide products used in liquid media (e.g., wastewater).

The Panel agreed that suspension-based animal bioassays are the only validated and broadly accepted ways to determine infectious titer. Additionally, all agreed on the final requirement for a quantitative and direct animal bioassay of infectivity and for a test system specific for each of the different agents, e.g., sCJD, scrapie, CWD, vCJD, and BSE agents. The Panel discussed in detail the potential limitations of the incubation-time bioassay and the potential high cost and animal usage of the end-point bioassay.

One Panel member stated that an incubation time assay done at >5 readable (each one log-significant difference) points, including the end-point, would be most acceptable. This would reduce costs of a complete end-point dilution assay. A few animals (~5) inoculated with uninfected material from the same tissue type should also be included to rule out non-specific and toxic effects. In addition, clinical illness is not sufficient for readout because animals die from other causes, especially during prolonged end-point incubations. A full necropsy inspection of organs to rule out such non TSE causes of death, as well as western blotting or neuropathology of all animals, is required to confirm the infectious agent is causal. Such criteria are important for a statistically limited group of animals. Furthermore, the agent-type can be determined by the distribution of spongiform changes as well as prion staining in brain and spleen. Methods are available to evaluate both spongiform changes and prion deposits simultaneously (Manuelidis, Fritch et al. 1997).

Another Panel member disagreed stating that the end point dilution assay was the best method for final validation of any decontamination method, although the incubation interval assay could be used as a screening method to focus the dilution groups to be performed with end point dilution. This Panel member suggested that additional detail should be sought by EPA to provide guidance to registrants in testing their products.

The end point titration assay is the only means of directly calculating titer. The numbers of animals per group should be based on the expected variance in the parental strain that is tested. The time post inoculation the animals are monitored should be based on the incubation period of the strain examined and should be at least 50% greater than the incubation period of the highest dilution that causes disease. Clinical signs should be monitored a minimum of three times per week. The monitoring of clinical signs can be by subjective observation of clinical signs or quantification of behavioral changes and should be consistent between control and experimental groups. All animals in the study should be examined for evidence of infection (Western blot for PrPres and/or neuropathology). Only differences in calculated titers that are equal to or greater than one log are considered significant (Mould, Dawson et al. 1967).

The incubation time interval assay has serious limitations that need to be addressed. Please note that the incubation time interval assay provides an estimate of titer and is not a direct calculation of titer. Although the incubation time interval assay can yield similar results as endpoint titration, serious limitations of this assay have been reported. Several reports have indicated that treatment of prions with heat and/or detergent can alter the relationship between titer and incubation period resulting in large errors in estimating titer based on incubation period alone

(Dickinson and Fraser 1969; Lax, Millson et al. 1983; Somerville and Carp 1983; Taylor and Fernie 1996). Additionally, it has been shown that the relationship between the dose of the agent and incubation period can be tissue specific, precluding using incubation interval assays when comparing the dissimilar tissues (Robinson, Cheevers et al. 1990). Finally, there are numerous examples in the literature of 10-fold differences in titer of the agent that have similar incubation periods that reduce the precision of the incubation time interval assay (Kimberlin and Walker 1977; Kaeser, Klein et al. 2001; Kincaid and Bartz 2007). This is in contrast to endpoint dilution analysis that is accurate to 0.5 (zero point five) log ID_{50} and is highly reproducible (Mould, Dawson et al. 1967; Taylor, McConnell et al. 2000).

Inoculum preparation should be consistent between control and experimental groups with the only variable being the treatment. The biological matrix used should mimic the likely form the agent would be present for the intended use. It is important to note that brain homogenate and preparations that are enriched for infectivity can differ in the response to inactivation.

c. Any other known test methods for evaluating the efficacy of pesticide products used on either environmental surfaces or in liquid media.

Most of the Panel was not enthusiastic about using protease resistant prion quantification or amplification (PMCA) as a measure of infectivity because infectious titer in many cases does not correspond to protease resistant prion. Nevertheless, an applicant may wish to use such an approach in initial pilot studies to save time and reduce costs.

There are also stable tissue culture models that can be used to assess infectivity for a number of TSE agents, including vCJD, Japanese CJD, 22L, Chandler (RML) and ME7 scrapie (Manuelidis, Liu et al. 2009) (Arjona, Simarro et al. 2004; Nishida, Katamine et al. 2005; Mahal, Baker et al. 2007). These can speed the first choice of best pesticide reagents, especially for materials dried on stainless steel or glass surfaces. These treated surfaces can be cultured with susceptible cells for titer readout in as few as twenty-one days. Experiments with some of these agents grown in culture, such as Japanese CJD and 22L scrapie, have shown faithful reproduction of the original TSE by re-inoculation of animals. Such cultured cells carry higher brain-like titers of infectivity than most other cultures.

The majority of the Panel concluded that in vitro immunodetection assays or cell-based bioassays are not adequately developed for use in assessing reduction in infectivity for evaluation of efficacy of pesticide products with prion related claims because in the case of immunodetection assays these do not measure infectivity and in the case of cell-based bioassays these have not been adapted for the application of assessing reduction of infectivity. The Panel recommends to EPA that end point dilution animal bioassays be used to assess reduction in infectious titer by treatment of samples with prion pesticide products.

Agency Charge

3. Efficacy Guidance Performance Criterion Issue: Whether the product performance criterion specified in the draft guidance document to support the registration of pesticide products with prion-related claims is scientifically sound.

The draft efficacy guidance document recommends a target efficacy criterion of six (6) logs of reduction of infectivity in the treated versus untreated (control) groups. This criterion is widely used in the current scientific literature. EPA would like the SAP's comment on this proposed product performance criterion.

- **Please comment on the scientific soundness of the product performance criterion recommended in the draft guidance document to support the registration of pesticide products with a prion claim.**

Panel Response

The Panel was in general agreement that some widely used model systems, such as the 263K or Sc237 rodent-adapted prion strains, have titers up to ~10^{11} ID_{50}/g of brain tissue and make it possible to detect six (6) log reduction in infectivity. However, bioassays in rodents are usually performed by intracerebral inoculation of a small volume such as 25µl. For a 10% brain homogenate, 25µl represents 2.5 x 10^8 ID_{50}, making an 8 (eight) log reduction measurable for bioassays in rodents with model prion strains. The treatment methodology may further reduce the initial infectious dose, for example, mixing a brain homogenate with an inactivation reagent would dilute the level of infectivity. It may then be necessary to further dilute the sample to reduce any potential toxic effects of the inactivation reagent on the bioassay animals. In such cases it may not be possible to measure such a high reduction.

The Panel considered the important caveat that for naturally occurring prion strains from natural hosts, titers are much lower in the available animal bioassay models: only in the range of 10^5 to 10^8 ID_{50}/g. In such cases it may only be possible to measure a three (3) or four (4) log reduction in infectivity.

Furthermore, any claim of a log reduction in infectivity needs to be accompanied by a statistical methodology, and some understanding of assay variability, e.g., 95% confidence interval, in attaining such a given log reduction. End point animal bioassays should utilize Kaplan-Meier and/or Cox regression analysis models to account for observed censoring, e.g., from animals

dying prematurely, in the estimation of mean times to outcome, e.g., onset of clinical signs, death, etc. In this way, confidence intervals for these estimates can also be provided. In addition, specific tests (test for equality of appropriate Cox regression parameters, or non-parametric tests for equal survival curves via the Kaplan-Meier curves) and parameter/characteristic to be used for comparing controls to treatment groups need to be specified.

One Panel member added that carrier-based methods provide further issues for calculating log reduction in infectivity. Firstly, it is not possible to use endpoint based methods, with the possible exception of the ground glass surface (Chen 1975). This is not an established method for quantification of prion titer, although preliminary data were reported to the Panel by Dr. Asher of the FDA. Also, there is no basis to assume all non-porous surfaces are equal in terms of prion binding. One published value for stainless steel wires incubated in serially diluted 263K-infected brain homogenate gave an endpoint of 5.6 log ID_{50} (Fichet, Comoy et al. 2004). However, there is a possibility that carriers incubated in serially diluted brain homogenate do not accurately bind infectivity at the same proportion to the dilution. If this is the case, then the endpoint calculation is not valid. Conversely, concerns were also expressed about the incubation period animal bioassay, because it has been shown that estimation of titer of physically or chemically treated samples by this method does not always correspond with titer calculated by endpoint titration (Somerville and Carp 1983; Taylor and Fernie 1996).

Another Panel member noted that the target amount of reduction may need to be considered in light of the proposed application or use, risk and what is achievable and can be validated for a

particular chemical or physical treatment. For example, treatment of potentially CWD contaminated waste water from an area associated with deer housing or processing may have a different standard for reduction in infectivity compared to that for surgical instruments in a human hospital which have potentially been exposed to CJD. A reduction of six (6) log in infectivity of CWD in waste water may be adequate to reduce the infectivity compared to the risk posed by CWD prions to human health, whereas the efficacy of a six (6) log reduction for CJD may not be adequate to reduce the potential that a human patient might contract CJD from an instrument with a six (6) log reduction in CJD prions. Risk assessments may be available to help formulate expected standards of performance, but a one-size fits all approach may not be valid.

The Panel recommends that if it is not possible to measure to six (6) log reduction in a relevant model (one using the naturally occurring prion strain from the natural host for which the inactivation method is designed to protect against), then the maximum measurable reduction should be required to demonstrate target efficacy. However, it was not generally agreed whether this should also be accompanied by demonstration of at least a six (6) log reduction in a widely used model, such as the hamster-adapted scrapie prion 263K or Sc237 strains.

Agency Charge

4. Efficacy Guidance Labeling Claim Issue: Whether the labeling claim described in the draft guidance document is scientifically appropriate based on the recommended test systems and product performance standard.

The draft efficacy guidance document recommends a carefully worded labeling claim statement: "Has been demonstrated to reduce infectivity of prions (TSE agents) based on testing using (insert type of organism in which the prions were raised) (insert prion type)." EPA believes that claims that may normally be applied to microorganisms (e.g., "destroy," "mitigate," "eliminate," "control") may be misleading when applied to prions. Because currently available test methods can only measure a reduction in infectivity, and the total

elimination or destruction of prions cannot be measured, EPA believes that "reduce infectivity" is the only appropriate claim.

- **Please comment on the scientific appropriateness of the term "reduce infectivity" in a label claim to reflect the action of a pesticide on prions.**

Panel Response

The Panel agreed with the EPA's proposal to frame the label claim statement as "reduce infectivity." From a scientific perspective, the state of "complete elimination of infectivity" is probably impossible to achieve in practice, let alone from a probability standpoint. One Panel member asked why the EPA was hesitant to claim complete elimination of infectivity given that techniques to measure TSE infectious titer are similar to those of other infectious agents. The EPA replied that with other microorganisms the target reduction is also less than 100%, and they want to be consistent with recommendations from other Federal agencies, for example the recommendations of the FDA Transmissible Spongiform Encephalopathies Advisory Committee.

Another Panel member stated that no one knows what constitutes a minimal infective dose of prions and that there is no way to measure this in humans. Infective dose can also be influenced by the immune and other systems and how these systems react to different doses of prions is unknown. It is very difficult to estimate the dose of prions needed to transmit TSE infectivity to humans. As a result, there is great reluctance to claim that all TSE infectious doses have been removed following treatment with a prion decontaminating reagent. Additionally, one Panel member noted that due to the prolonged disease incubation times and the lack of understanding as to what constitutes an infectious dose, reduced infectivity is the only term that can be used to assess a solution used to decrease the impact of prion disease.

One Panel member noted that different expectations drive the reluctance to use a word like "inactivation". There was concern that registrants and the user communities will have differing expectations of the prion pesticide product's performance. Users in hospitals concerned about CJD may expect something closer to "elimination of infectivity" on environmental surfaces, whereas the veterinary and wildlife management community may be more comfortable with the "reduced infectivity" approach. One Panel member recommended that the label clearly indicate the implications of the "reduce vs. elimination" approach in sufficient detail so that the user can understand and appreciate what the product can and cannot do. Furthermore, the label of the registered product should follow current EPA labeling recommendations in providing use condition details for each general application of the product, e.g., decontamination of hospital environmental surfaces, decontamination of laboratory working surfaces, or application on porous materials such as fences, etc. The Panel asked EPA representatives if the label was intended to be understood by the general public, and an EPA representative confirmed this.

One Panel member noted that when considering the phrase "reduce infectivity" it is important to note 1) that it does not refer to the complete elimination of infectivity with absolute confidence and, 2) that any product has to be tested in a way that supports the labeling claim. Thus, the phrase "reduce infectivity" is valid only as long as the method used to measure infectivity demonstrates a log reduction in a valid model system. Use of the word reduce would therefore be model dependent. A claim to "reduce infectivity" also implies that the efficacy testing will involve a measurable endpoint, so the reduction can be quantified.

One Panel member stated that incubation time animal bioassays used to measure infectivity after treatment of prions may not be the best assays to use as there are several examples in the literature that suggest treatments which reduce prions may lead to incubation periods that fall outside the linear range of the assay . There was a general consensus among Panel members that endpoint titration animal bioassay is the best way to measure log reduction in TSE infectivity. The issue of whether or not to qualify the phrase "reduce infectivity" was discussed. The phrases "maximally reduced" or "maximally achievable reduction of infectivity" were introduced as was the idea that the label could note that infectivity was reduced to below the level of detection in a specific assay. An EPA representative noted that EPA was reluctant to put adjectives in front of "reduced."

It was suggested that, if the testing is based on a specific animal bioassay model, the details of that model be included in the label. One suggestion was to separate the description of the prion strain and host of origin from the species of host in which the prion strain had been assayed. Another suggestion was to precisely define the log reduction as well as the dynamic range of the assay (range of infective titer for which the bioassay yields precise and accurate results) so that the reader could understand whether the reduction of infectivity corresponded to an exact value or a lower limit. Two examples were read to the Panel:

Example 1: Has been demonstrated to reduce infectivity of prions (TSE agents) by five (5) logs or more based on bioassay of the bovine BSE strain in transgenic mice expressing the bovine prion gene in (specify transgenic mice). The dynamic range of the bioassay is five (5) logs.

Example 2: Has been demonstrated to reduce infectivity of prions (TSE agents) by six (6) logs based on bioassay of the hamster Sc237 strain in Syrian hamsters. The dynamic range of the bioassay is nine (9) logs.

Given the variety of different prion animal bioassay systems, the Panel discussed whether or not a specific log reduction of infectivity should be specified by EPA. It was brought out that even a six (6) log reduction would not be enough to completely inactivate high titer brain tissues such as a hamster brain infected with the Sc237 strain which could have as much as eleven (11) logs of infectivity per gram of brain. However, a six (6) log reduction is thought to be within the range of inactivation for human sporadic CJD or cattle BSE prions as these generally contain around six (6) logs of infectivity per gram of brain. One Panel member brought up the important caveat that limited sensitivity of these test models could account for the apparent lower infectivity TSE-infected human and bovine brain tissue, rather than a smaller number of infectious particles. One Panel member suggested that even a five (5) log reduction might be acceptable for a product with a prion-related claim under the condition that the labeling specified the infectivity reduction and the dynamic range of the animal bioassay.

Another Panel member was concerned with the notion that the registrant provide the agency with the details of the product testing methods, thereby presenting EPA with potentially a wide variety of methods requiring a great deal of time on EPA's part to review. Other Panel members raised concerns that registrants might choose a model system with low levels of TSE infectivity to test their product. It was suggested that EPA poll members of this SAP and/or members of the interagency TSE working group to review the literature and gather the details of various animal

bioassay designs, seek a consensus on these details and compile a list of acceptable prion strain/animal bioassay models. This would facilitate agency review of registrant methods by establishing an array of acceptable efficacy test methods.

There was general consensus among the Panel members that the label should give sufficiently detailed and accurate information to enable the product user to assess how efficiently, and under what usage, the prion pesticide product inactivates TSE infectivity.

Agency Charge

5. Efficacy Guidance Hierarchy Issue: Whether different prion types exhibit variation in the degree of resistance to inactivation by pesticide chemicals and whether a hierarchy of resistance by prion type can be reliably determined at this time.

Comparisons of different types of prions in a common animal infectivity assay indicate there may be significant differences with regard to their ability to resist inactivation by pesticide chemicals. For example, Peretz et al. (2006) compared the resistance of hamster scrapie and human CJD prions in transgenic mice expressing either hamster PrP or a chimeric mouse-human PrP transgene and found that human sCJD prion tested was 100,000 fold more difficult to inactivate than hamster Sc237 prion. Preliminary additional studies indicate that the cow BSE prion may be even more resistant to inactivation than the human CJD prion (Giles et al. 2006; 2008 in press).

- **Please comment on whether a hierarchy of resistance among prion types can be reliably demonstrated for different pesticide chemicals based on the available data.**

Panel Response

There is currently little data on the relative resistance to inactivation of prions from various species and strains, even though some studies report strain specific variations in resistance to inactivation (Peretz, Supattapone et al. 2006; Fernie, Steele et al. 2007; Giles, Glidden et al. 2008). However, it is clear that prions from different species and strains within the same species have distinct incubation time and lesion profiles in a given host as well as biochemical and

biophysical properties. Given the fact that different prion pesticide products might act in different ways, it is likely that strains may respond differently and unpredictably to inactivation procedures. Therefore, the Panel's consensus is that no single hierarchy of strains to inactivation procedures can be established at this time.

Efficiency of a particular prion inactivation procedure must be measured by a relevant and robust infectivity bioassay, depending on the prion agent for which the procedure is intended to be used. There was some debate among Panel members regarding the need relative to the prion strain hierarchy of resistance issue for use of two bioassay tests, with one of them done in a standard widely-used model, e.g. scrapie prion strain 263K in hamsters, and the other being specific to the samples under study. This will enable a more robust proof-of-concept validation and the possibility to compare results from different prion pesticide products. However, the limitation is that some procedures may not work as well in the standard assay and still be useful for specific applications as supported by an animal bioassay in a relevant species. After discussion, and based on practical considerations, the majority of the Panel members recommend using end-point titrations in a relevant and robust animal bioassay depending on the samples to be decontaminated. Although, there was some debate, the majority of the Panel members think that decontamination studies should be performed on tissues from the natural host and that rodent adapted models of natural prion disease are not reliable substitutes. The experiments should be done with the natural samples, e.g. CJD, scrapie, or CWD, in the host such as either wild type animals or transgenic that provides the best practical and dynamic range for the study. One Panel member expressed concern that transgenic mice overexpressing prion protein may not be a

more appropriate model to assess the problem than mice with wild type prion protein (J. Cell. Biochem. 106: 220-231; 2009).

The Panel recommends that the experimental protocol to study efficacy of a given procedure be reviewed in advance by a group of experts to decide the best animal model and sample preparation to conduct the study.

There was also discussion that experiments done with one source of material, e.g. one strain of sCJD, in one animal model, may not extrapolate to related strains of the agent, e.g. vCJD, fCJD or even other strains of sCJD (although one Panel member did not think there is substantial evidence of the existence of sCJD strains), so registration based on positive results with one strain may not guarantee that the product works even in related strains of the same species. One suggestion to approach this problem in a practical way was to complement results obtained in the in vivo infectivity assay with in vitro or cell based assays that can analyze a large diversity of strains.

In conclusion, the consensus from the Panel is that no single hierarchy of strain resistance to prion pesticide action can be established at this time.

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APPENDIX

In concluding remarks of the open meeting, Dr. Kempter, Senior Advisor, Antimicrobials Division, Office of Pesticide Programs, EPA invited Panel members to provide the Agency with a choice of information on the types of tests or materials that might be used in testing pesticides including additional references or narratives, even if not necessarily widely accepted by the entire Panel. Subsequent to the meeting, one Panel member did so. These references and narratives are placed herein as an appendix. Please note that they were not specifically discussed during the open Panel meeting.

1) Animal assays are the only validated and broadly accepted ways to determine titer. Additionally, the final requirement for a quantitative and direct animal assay of infectivity for a test system must be specific for each different agent, e.g., sCJD, scrapie, CWD and vCJD/BSE agents.

2) An incubation time assay done at >5 readable (each 1 log-significant difference) points, including the end-point, would be most acceptable. This would reduce costs of a complete end-point dilution assay. A minimum of 8 animals surviving through the prolonged incubation time should be used per assay point.

3) In general, a standard high titer infectious sample should be inoculated in parallel with the treated sample at the same dilutions, and at least 2 control dilutions made with carrier materials other than the decontaminating reagent. This should ensure that alterations in incubation time and end-point are not artifactual. A few animals (~5) inoculated with uninfected material from the same tissue type should also be included to rule out non-specific and toxic effects.

4) Clinical illness is not sufficient for readout because animals die from other causes. The agent-type can be determined by the distribution of spongiform changes as well as abnormal PrP staining in brain and spleen. Methods are available to evaluate both spongiform changes and abnormal PrP deposits simultaneously (1).

5) In some cases a target agent or material may not have sufficient starting titer to determine if >6 logs have been inactivated by the treatment, the minimal level generally agreed to be required for approval of product labeling. In such cases, other model systems may be used to support the application. The high titer 263K scrapie agent in hamsters (9.5 logs per gm of brain by endpoint in 6 different laboratories*) can be used. Since hamsters have their own species characteristics, it would also be advisable to use one high titer established mouse scrapie model such as standard normal mice infected with either the 22L scrapie agent or the Chandler (RML) scrapie agent used in many laboratories. These agents yield ~8.5 logs per gram of brain. These two models offer a standard for comparison, and the Agency may wish to include such a requirement to assure a reasonable degree of uniformity; it can also provide a database for improvement.

6) There are also stable tissue culture models that can be used to assess infectivity for a number of TSE agents, including vCJD, Japanese CJD, 22L scrapie, Chandler (RML) scrapie (2) (3). These can speed the first choice of best inactivating methods, especially for materials dried on sterile needles or glass surfaces. These treated surfaces can be cultured with susceptible cells for titer readout in 21 days. Experiments with some of these agents grown in culture, such as Japanese CJD and 22L scrapie, have shown faithful reproduction of the original TSE by re-inoculation of animals. Such cultured cells carry higher brain-like titers of infectivity than most other cultures (2, 4).

8) Since it was agreed that different animal models are needed for different agents, an FIFRA member asked for more guidance on specific models for each agent. Here are some suggestions, and others on the panel can better suggest the most relevant additional scrapie and CWD models. Although the use of normal mice allows one to compare many different agents in a constant background, several other rodent models in hamsters, or in mice with transgenic PrPs, can be advantageous in particular instances. However, it should be recognized that high transgenic copies of a foreign PrP sequence in a mouse can be problematic as they can induce artifactual spongiform lesions without infection, and lead to premature death (5).

Sporadic CJD (sCJD) has a stable high titer (8.5 logs) in hamster brain (assayed by both incubation time and endpoint >15 times) and hamster adapted sCJD brain homogenate has been successfully used for decontamination studies (6). More than 20 other classic sCJD cases have been transmitted to hamsters (Manuelidis et al.1988 Figures 1 and 2 on pages 120 and 122), and more than 6 of these primary transmissions have been serially passaged. They all yield the same short incubation time (~125 days) with widespread brain lesion profiles that suggest a common and relatively invariant sCJD agent. In contrast, mouse models of sCJD are less useful. Mice with human transgenic (Tg) versions of PrP that are inoculated with the sCJD agent typically have incubations of >250 days. These Tg mice have not been titered for endpoint infectivity to the best of our knowledge, and normal mice have a very prolonged incubation time and low titers with sCJD (7). There are other distinct CJD agents, and the geographically restricted Japanese CJD agent isolated from 2 patients (FU and YAM agents) has a high titer in both mice and murine cultures. In culture these isolates also show an agent-specific 13kd PrP marker (3). All experimental models of CJD must be used for FIFRA purposes because human TSE agents cannot be verified by re-inoculating humans.

Variant CJD (vCJD) from human brain has been serially passaged in standard normal mice and this agent also yields a reasonably short incubation time of ~140 days (3, 8). Endpoint dilutions have not been published but from this incubation time are likely to be >6.5 logs. This agent induces a marker PrP band of 19kd regardless of PrP species sequence differences; this band validates the specific presence of the vCJD agent. Notably, PrP humanized mice are *not* readily infected by the human adapted vCJD agent and show 100-200 day longer incubations than mice with murine PrP (3, 9, 10). Since the vCJD agent is the same as the BSE agent, and the cow agent from human brain also reproduces well in PrP bovinized mice, the use of both standard mice and bovinized PrP mouse models could be advantageous, especially if the bovinized PrP mice show a titer of >6 logs.

Chronic Wasting Disease (CWD). At least 2 laboratories have made mice with elk PrP (13,14), and at least one of these models shows a short incubation time of 90-120 days on serial passage of material from both infected elk and deer brain (14). Although not yet titered, this model would seem good for testing inactivation of the CWD agent.

Many different sheep scrapie agents have been established and well characterized by the UK group beginning with the first transmission of a scrapie agent to standard (PrP-A) mice by Chandler in 1963. The set of scrapie agents are all valuable models with typical endpoint titers of ≥7.5 logs per gram of brain, sufficient for decontamination confidence. The use of two or more of these scrapie models would seem most appropriate for testing scrapie agents. Notably, passage of scrapie agents in mice has not altered the unique nature of each agent, as first shown in sheep re-inoculation experiments by Zlotnik and Rennie (11). Studies in scrapie infected cultures have also shown that scrapie strains are stable and host-independent (12).

9) Although a lack of "commercial" models was raised, this is probably not an impediment. Any commercial company can easily contact or work with the labs with these models, and have help with the validation of their assays. In any case, there appear to be no commercialized animal assays, even in progress, that would be accepted by the majority of this committee as valid for final labeling by FIFRA. Nevertheless, the committee should remain open to new discoveries and assays that can further streamline quantitative agent titration.

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*e.g., Marsh, Carp, Diringer, Kimberlin, Bruce, Manuelidis. In contrast, Prusiner's publications cite 11-12 logs/gram

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