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Responses to Public Comments on Protocol for Testing the Efficacy of Disinfectants Used to Inactivate Hepatitis B Virus

I. Background

EPA has authority through the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) to register pesticide products, including antimicrobial pesticide products, for sale and distribution in the United States. FIFRA section 3(c)(5) requires that the composition of a pesticide product is such as to warrant the claims made for it, i.e., that a product work as claimed. Although registrants must maintain data demonstrating efficacy in their files and must submit these data to the Agency upon request, EPA does not routinely review efficacy data prior to registration of most insecticides, fungicides, herbicides and non-public health antimicrobial pesticides. However, for public health pesticide products (i.e., those that work against pests in situations where they pose public health threats) the Agency reviews efficacy data prior to registration. The Agency believes that the potential consequences of performance failure for public health products warrant this extra precautionary step in the review process. Moreover, for public health products intended to control bacteria, fungi and viruses, the user is typically unable to determine whether the product is working, due simply to the microscopic size of these organisms. Subdivision G of the Pesticide Assessment Guidelines describes the efficacy tests routinely used to validate the claims made by antimicrobial public health pesticide products. These guidelines are available from the National Technical Information Service, 5285 Port Royal Road, Springfield, VA 22161.

For the past several years, EPA has been engaged in a process to identify scientifically and statistically adequate test protocols for evaluating the efficacy of disinfectants used to inactivate human hepatitis B virus (HHBV). In 1986, (51 FR 19174), the Agency published a Notice of Amendment to Policy regarding certain virucidal claims. Specifically, the Notice stated that virucidal claims for HBV would be permissible only for sterilizer products until such time that acceptable protocols to demonstrate virus isolation and disinfectant product efficacy could be developed.

In 1990, the Agency received and approved a chimpanzee testing protocol to support HBV efficacy claims for hard, environmental surface disinfection products. While the data were being generated using the approved protocol, a GAO Report was issued (August 1990) that criticized the Agency for accepting test methods without criteria or a systematic review process. In response to this criticism, the Agency initiated a process whereby new protocols would undergo external review by scientific experts. In 1995, as a result of this change in process, the chimpanzee protocol was subjected to external review by experts working in various

scientific institutions, including Food and Drug Administration (FDA), Center for Disease Control (CDC), National Institutes of Health (NIH), and two university medical schools. The experts were asked to review the data generated using the EPA-approved protocol as well as similar data developed by Bond *et al.* 1983, at CDC. After careful review of all comments received, the Agency concluded that the chimpanzee data submitted by the applicant, when considered together with the data developed by Bond *et al.* 1983, were sufficient to support a label claim of disinfection against HBV.

During the 1995 external review process for the chimpanzee protocol, several experts urged the Agency to accept data developed using a surrogate virus, thus making available an alternative to chimpanzee testing. One expert stated that it would be unjustified to permit the use of any type of animal for germicidal testing and that such testing could be avoided though the use of properly designed *in vitro* methods . As a result of these concerns, the Agency began to seek alternative means of testing the product performance of disinfectant products intended for inactivation of HBV. One of the steps in this process was consultation with the FIFRA Scientific Advisory Panel (SAP) in September 1997. At that meeting the questions posed to the Panel were as follows:

> (1) If the Agency decides to replace the chimpanzee test used in testing the efficacy of disinfectants against human hepatitis B-type virus, what test methodologies could be used as a replacement? Two possibilities that have been proposed to the Agency are the duck hepatitis B Virus Test (DHVT) and the Morphological Alteration and Disintegration Test (MADT). Could one or both of these tests be used to test for efficacy against Human Hepatitis Virus B?
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> (2) If a surrogate test system (i.e., the DHVT) is found to be acceptable for efficacy testing using Hepatitis virus B, would the results be sufficient to allow the registrant to make a label claim that the product was efficacious against human hepatitis B virus, even though it was tested against a surrogate virus (i.e., duck hepatitis B virus) and not the human virus?

Briefly, the SAP's responses to these questions were as follows. The Panel concurred with the notion that it is unethical to continue to require testing using a species of primates, chimpanzees, where alternative methods are available, and observed that there is a long history of using surrogate microbes to assess the efficacy of disinfection/sterilization technologies against various classes of microorganisms. The Panel stated that the duck hepatitis B virus (DHBV) constitutes an appropriate HHBV surrogate and added that an advantage to this surrogate is that the DHBV can be utilized in both *in vivo* and *in vitro* settings. In particular, the Panel stated that the DHBV approach would allow for sufficient numbers of test samples to be used for each set of experimental conditions so that statistically significant results can be obtained. The Panel discussed the possibility that DHBV may be more resistant to germicidal chemical activity but, in essence, felt that even if this were true it was not a serious issue, given that hepatitis B-type viruses have been demonstrated to be sensitive to the activity of a wide spectrum of liquid chemical germicides including low level disinfectants. While the panel did not discuss the MADT alternative at great length nor exclude the possibility of its use, it did observe that the test is only subjective because it is a qualitative and not a quantitative measurement. The Panel stated its belief that registrants who use DHBV could make a label claim of product efficacy to either the

specific virus or in the alternative to perhaps the whole virus family as a group. The example of claims against *Mycobacterium tuberculosis* by testing against *Mycobacterium bovis* was cited as precedent for the use of a surrogate in disinfectant efficacy testing. If tests validate that a surrogate virus is less or equally susceptible to inactivation by disinfectants, then logically any product which demonstrates efficacy against the surrogate virus should be allowed a label claim against HHBV.

The responses of the SAP to these questions provided invaluable guidance to the Agency in its pursuit of scientifically adequate test protocols for evaluating the efficacy of disinfectants used to inactivate HHBV. The Antimicrobials Division of the Office of Pesticide Programs sponsored a workshop in July 1998 to discuss alternative models for testing disinfectants against HHBV. The workshop was attended by representatives from academia, research centers, testing laboratories, and industry. Presentations were given by experts in hepatitis on various animal models of HBV infection followed by technical presentations on *in vitro* and *in vivo* duck models of infection that might be used in testing disinfectants for use against HHBV. Presentations were followed by a discussion on criteria to be used in decision making about surrogate model(s) and proposed labeling claims of registered products. Many participants in the workshop proposed that EPA leave the label claim broad, such as "Effective against HBV" or "Hepadnavirucidal" and not add information about the test organism. Submitted protocols were evaluated and discussed by all participants. At the end of the workshop an outline was presented, showing the Agency's implementation plans for allowing products to be registered with HHBV label claims using surrogate animal models. Subsequently, the Agency published an FR Notice in December 30, 1998 (63 FR 34292) announcing the availability and requesting comments on two protocols for testing the efficacy of disinfectants against HHBV. These protocols were for an *in vitro* assay using duck hepatocytes and DHBV and an *in vivo* assay using ducklings and DHBV.

The Agency received 12 sets of comments in response to that Notice. Comments were received from consultants, an animal welfare organization, university scientists, the regulated industry, the California Department of Pesticide Regulation, and private organizations. These comments in their entirety are available in the public docket (OPP-00538A). Many of the comments were similar in content, and pertained to general issues concerning Agency policy or specific sections within the protocols themselves. To facilitate review and consideration of the comments, the Agency has grouped comments addressing similar issues together.

II. Responses to Comments

A. Use of DHBV as a surrogate for HHBV

Two commenters expressed concern that there are insufficient data to ascertain that a disinfectant which is efficacious against DHBV will be equally efficacious against HHBV. Another commenter asserted that the chimpanzee is the most suitable animal model for studying HHBV infection and prevention. Two commenters supported the use of surrogate test protocols using DHBV.

Agency Response

The scientific evidence indicates there are considerable similarities between isolates of human, duck and woodchuck HBV. Both the SAP members and the scientists who discussed these issues during the EPA sponsored workshop in July1998 have agreed that the duck, woodchuck and chimpanzee are all suitable animal models in which to test the effects of disinfectants on HBV. It has been well demonstrated that HHBV is sensitive to the activity of a wide spectrum of liquid chemical germicides (Bond *et al.*, 1983, Prince *et al*., 1993). Considerable research has been done comparing HHBV with other hepatitis B-like viruses. A strong case has been made that these viruses are very closely related and because of similarities in their structure, DNA homology and encoded protein components, they have been placed in the same virus taxonomic family "Hepadnaviridae" (Mason *et al.*, 1983). All Hepadnaviruses possess a lipid envelope which is highly sensitive to inactivation by a wide variety of disinfectants. Therefore, there is no credible scientific foundation for predicting that different Hepadnaviruses would exhibit variable resistance to a given disinfectant. Modern disinfectants are used at high concentrations which are many fold greater than that required to kill enveloped viruses (Favero and Bond, 1998). Additionally, disinfectants have multiple inhibitory effects, such as protein denaturation, protein cross linking, lipid removal and nucleic acid degradation, all of which are independently sufficient to inactivate enveloped viruses (Pugh *et al.*, 1999). Thus, the Agency concludes there is no scientific evidence of significant differences between human HBV and duck HBV in sensitivity to any disinfectant agent.

An in-depth search of the scientific literature on this subject has revealed the following findings:

The discovery of HBV in domestic ducks (DHBV), woodchucks (WHBV), and ground squirrels (GHBV) in the late 1970s provided useful alternative animal models to use of chimpanzees for HBV research (Marion, 1998). DHBV rapidly emerged as the model best suited for elucidating the replication pathway of HBV. Experimental infection of ducklings with DHBV results in high levels of virus in the blood approximately one week after infection (Mason *et al.*, 1983). In addition, primary duck hepatocyte cultures can be readily infected with DHBV in the laboratory (Tuttleman *et al., 1986*). Consequently, the duck model is especially amenable to infectivity studies, such as the testing of disinfectants against HBV (Murray *et al.*, 1991; Prince *et al.*, 1993).

DHBV and HHBV are remarkably similar in all features that are relevant to testing disinfecting agents. Both viruses share structural similarities in virion size (40 - 42 nm), morphology and DNA length (3.0 - 3.2 K base pairs). Both viruses replicate via reverse transcription and encode similar surface antigens and core proteins (Mason *et al.*, 1983). The differences that exist between DHBV and HHBV principally concern the difference between the hosts they infect and the nature of the disease they produce, and have no bearing on the ability of disinfectants to abolish infectivity of the viruses (Pugh *et al.*, 1999).

Although one commenter noted that in one study by Tsiquaye and Barnard (1993) the duck virus was twice as sensitive to sodium hypochlorite (NaOCL) than the human virus, this study is not relevant for a variety of reasons. First, in this study, the only comparison done was on the effect of two disinfectants, NaOCL and sodium dichloroisocyanurate (NaDCC), on the DNA polymerase activities present in crude concentrates of the two viruses. No comparison was made regarding the inactivation of whole infectious viral particles. Second, although the study did state that DHBV was less resistant than HHBV to NOCL, but it also reported that

HHBV was shown to be less resistant than DHBV to NADCC. Thirdly, the authors demonstrated that two minute exposure of minimal effective concentrations of NaOCL and NADCC totally inhibited DNA polymerase activity of both DHBV and HHBV.

In summary, the Agency has found no evidence that there are significant differences between HHBV and DHBV in sensitivity to any disinfecting agent (Marion, 1999). Several studies have shown the general lack of HBV resistance even to the least potent category of environmental germicides (Bond *et al.*, 1983; Kobayashi *et al.*,1984; Prince *et al.*, 1993) and suggest that all current guidelines for decontaminating medical instruments or environmental surfaces are adequate in terms of germicide selection, i.e., nothing "special" is needed to inactivate HBV (Favero & Bond, 1998).

Recently, investigators have used the DHBV model to evaluate the efficacy of disinfectants and sterilants of medical instruments such as angioscopes and have stated that "DHBV has similar biologic and structural characteristics to HHBV and has been adopted by these investigators as a suitable model for disinfectant testing" (Chaufour *et al.*, 1999). Other investigators have used the DHBV to test the efficacy of a hydrogen peroxide plasma sterilization system and concluded that the sterilization process completely inactivates DHBV, a representative of the hepadna group of viruses (Vickery *et al.*, 1999).

B. Does *in vitro* testing using DHBV adequately measure disinfectant efficacy?

Three commenters expressed a view that the *in vivo* DHBV model is more sensitive than the *in vitro* DHBV model. One of these commenters stated, " . . . it is also easier to remove disinfectant toxicity using the *in vivo* model than the *in vitro* model. I believe that the *in vivo* model should be used if the *in vitro* model cannot show at least a 5 log decrease in titre. Decreasing the titre only 3 or 4 logs from a starting point of 10^9 would still result in 100% infectivity." Another commenter stated, "This opinion is based on observations that it is more sensitive than any existing *in vitro* assay and that a non-subjective end point is possible. The end point of the *in vivo* assay involves determining the viral titer in duck sera by quantitative DNA hybridization techniques, with which viral titers can be determined by computer analysis of phosphorimager data. The end point of the *in vitro* assay to date is determining the presence or absence of immunostained cells by microscopic observation." The third commenter encouraged the Agency to require preferential use of the *in vitro* methodology and asserted that the *in vitro* method is not only equivalent but preferable as it "avoids the issue of HBV resistant ducklings."

Agency Response

These comments generally address the issue of the adequacy of the *in vitro* DHBV assay to measure disinfectant efficacy. There is enough evidence in the scientific literature to show that the *in vitro* duck assay is comparable in sensitivity to the *in vivo* duck assay and is adequate to test the efficacy of disinfectants against HBV. In a study done by Prince *et al.* 1993, the efficacy of three commercial disinfectants (two quaternary formulations and one phenolic compound) was evaluated against HHBV using the chimpanzee host assay and the morphological alteration and disintegration test (MADT). These disinfectants were also tested against

DHBV and their efficacy evaluated using the *in vitro* duck assay. Both HHBV and DHBV were nearly equally susceptible to disinfection by the low-level quaternary ammonium germicides, becoming non-infectious after 10 min contact time with the disinfectant.

Duck primary hepatocyte cultures were also established from ducklings congenitally infected with the duck HBV and plated onto feeder cell layers of irradiated human embryonic lung fibroblasts. The hepatocytes were shown to contain all the DNA intermediates found during DHBV replication as well as the DHBV structural proteins. This observation allows the conclusion that duck primary hepatocyte cultures support normal DHBV replication. The authors concluded that this cell culture model provides a convenient system to study the effects of conventional inhibitors of DHBV replication (Bishop *et al.*, 1990).

In a recent study (Eble and Corash, 1996), primary duck hepatocyte cultures infected with DHBV were used to detect the effect of photochemical inactivation in a highly specific *in vitro* infectivity assay using polymerase chain reaction (PCR) gene amplification to detect HBV. Culture results were confirmed by a sensitive *in vivo* duckling assay that indicated that 6.3 $\log_{10}$ infectious duck HBV had been inactivated by photochemical decontamination. These authors concluded that the hepatocyte cell culture assay was comparable to that of the *in vivo* duckling assay.

It is evident from these studies that an *in vitro* duck infectivity assay is comparable to an *in vivo* assay in measuring the effect of disinfectants on log reduction of viral titre of DHBV. In response to the comment "it is easier to remove disinfectant toxicity in the *in vivo* model than in the *in vitro* model," the Agency refers to the general guidelines for testing disinfectants for virucidal effect. These guidelines state that an *in vitro* assay requires that at least a 3 $\log_{10}$ reduction in viral titre must be demonstrated beyond any disinfectant dilutions which exhibit cell culture cytotoxicity [Subdivision G, 91-2 (f)]. In response to the comment that a 3- 4 $\log_{10}$ reduction of an original viral titre of 10^9 will still result in 100% infectivity, the Agency notes that all disinfectants claiming efficacy against HHBV-contaminated, inanimate surfaces must specify that the surface be pre-cleaned before the application of the disinfectant to the hard surface; such pre-cleaning will reduce the viral titre to about 10^5. The treatment of the pre-cleaned surface with a disinfectant followed by drying will achieve an additional 3-4 $\log_{10}$ reduction in the viral titre on inanimate surfaces.

C. Method Validation

Two commenters expressed concern that the Agency will allow products to be registered with human HBV claims during the method validation process. One commenter suggested that a 2-year, time limited registration should be allowed during the validation phase and extended if unexpected delays in the process of validation occur. The same commenter asked for specific information on the method validation process and clarification as to the statutory authority for a 2-year, time-limited registration during that validation process. Another commenter stated that the method should be validated in order to maintain the claim beyond two years.

Agency Response

The Agency understands the importance of registering public health products designed to control human pathogens only after those products have been proven to be effective. Thus, the Agency agrees that validation of efficacy test protocols, i.e., the accumulation of sufficient data suitable for ensuring test reproducibility, is needed prior to registration. To ensure that the *in-vitro* duck method has been adequately validated, data should be provided from at least two independent laboratories for each product tested (two batches per product). Validation of the protocol should also require the use of a common positive control disinfectant to be tested concurrently with all new products. This agent should serve as both an intra- and an inter-laboratory control and will be used for analyzing reproducibility of the efficacy data. It is critical for the Agency to know that a test method is repeatable; i.e., that there is an appropriately small standard deviation of log reduction (LR) values found when the test is repeated on different occasions in the same laboratory as well as when the test is conducted in different laboratories. The use of the common positive control and the generation of confirmatory data in a second testing lab will achieve a statistically justifiable approach to protocol validation and will provide an assurance that those products have satisfactorily met efficacy data requirements that are acceptable to the Agency. Because the protocol will, in effect, be independently validated for each product, there is no need to register such products with a time limitation. After sufficient data have been generated for a number of products from different chemical classes, the Agency will consider adopting the protocol as a standard guideline.

D. Permitting label claims for human HBV following testing with a surrogate virus

One commenter objected to label claims that "deny the public the right to know if a disinfectant has been proven effective against human, as opposed to duck, HBV." Another commenter suggested that label claims such as "effective against HBV or hepadnavirucidal" without adding a qualifying statement about the test organism would be misleading unless the surrogate virus is proven to be equally or more resistant to the specific disinfectant than the human HBV under the same test conditions. Four commenters expressed the view that label claims such as "Effective against hepatitis B virus (HBV)" or "Kills HBV" should be permitted, regardless of the efficacy testing method adopted. One of these commenters further stated that it should not be necessary to designate that the non-human virus was tested.

Agency Response:

Products meeting the Agency's virucidal performance standard using the *in vitro* DHBV protocol will be eligible to make claims against HBV or the virus family as a whole group, i.e., "Hepadnavirus." Surrogate models have been used previously to evaluate the efficacy of antimicrobial products. The Agency has already established precedent by allowing tuberculocidal claims for antimicrobial products utilizing a surrogate (*Mycobacterium bovis* for *Mycobacterium tuberculosis)* as the test organism. This issue was previously discussed at the SAP meeting held in September 1997 and the Agency HBV workshop held in July 1998. Both groups proposed that registrants who use the DHBV could make a label claim of product efficacy to either the specific virus or to the virus family as a whole group (i.e., "Hepadnavirus"). Scientific research has shown that the human HBV and its surrogate viruses are structurally related and because of their significant

similarities they have all been placed in the same virus taxonomic family "Hepadnaviridae." It should be noted that the DHBV already is accepted as a surrogate for the testing of disinfectant efficacy against HHBV by the Australian Health and Family Services, Therapeutic Goods Administration and label claims of virucidal activity against HBV are allowed. (Therapeutic Goods Order No. 54, 54A & Guidelines).

Based on the foregoing scientific discussion, the Agency is accepting disinfectant testing on DHBV as a surrogate for testing on HBV, and, therefore, the Agency will allow labeling claims against HBV. Moreover, a labeling disclaimer to indicate that the product has been tested only against DHBV is unnecessary.

E. Use of Animals for Testing

One commenter expressed concern about the continued use of the chimpanzee model and encouraged efforts to eliminate or minimize the use of chimpanzees for experimental purposes. A second commenter asked the EPA to place more emphasis on the validation of non-animal alternatives and to take a more active role in the validation of alternative methods and their incorporation into EPA regulatory requirements. A third commenter recommended that users be informed, via labeling, of any live animals used in the testing procedures.

Agency Response

The Agency is adopting, where possible, policies and data requirements that minimize animal testing. When animal testing must be conducted, the Agency is committed to reducing the number of animals needed for testing, reducing the pain and suffering of test animals. Whenever possible, i.e., based upon sound scientific principles, it will replace animal-based systems with validated, non-animal test systems. A fact sheet on animal welfare concerns and other information on Agency activities to reduce the use of animals can be found on the EPA Internet website, www.epa.gov/chemrtk.

By adopting the *in vitro* DHBV assay as the appropriate and preferred method for testing disinfectant efficacy against HBV, EPA has generally moved away from tests using live animals. The number of ducks sacrificed will be very minimal using the *in vitro* rather than the *in vivo* DHBV assay. The *in vivo* duck test method requires the sacrifice of approximately 122 ducks per product to provide statistically valid data while the *in vitro* test typically requires the sacrifice of only one duck per product, which the Agency believes to be justifiable based on the importance of the pathogen.

F. Products Already on the Market

One commenter asked whether existing products will be held to the same standard and conform to the new surrogate protocols and labeling language. Another commenter expressed a view that all HBV method protocols used should be validated including the chimpanzee method.

Agency Response

Consistent with EPA's commitment to reduce animal testing, the Agency will neither require further validation of the chimpanzee protocol, nor require retesting of products previously registered on the basis of chimpanzee data.

G. Protocols

1. Protocol Modifications for Different Product Forms

One commenter mentioned that " . . . there (are) other product forms that need to be incorporated into both of the protocols. These include, but are not limited to, saturated towelettes, impregnated towelettes, and solid products not intended to be diluted prior to using, e.g., absorbents for use with blood/body fluids and other forms which may subsequently (be) tested."

Agency Response

The Agency believes that the protocol may be modified to allow for testing of a variety of antimicrobial product types, including towelettes and solid products. Applicants interested in testing and registering such products should contact the Agency's Office of Pesticide Programs, Antimicrobials Divisions for further guidance. After consultation, the registrant will be required to submit a modified protocol for revision and acceptance.

2. "Sephadex Gel Filtration"

One commenter stated that most disinfectants can be neutralized by methods other than the use of sephadex gel filtration. *In vivo* toxicity can usually be eliminated by diluting the virus/disinfectant mixture 1/100 before animal inoculation while sephadex gel filtration in the *in-vitro* method may lead to possible loss of virus onto the column.

Agency Response

In the *in vitro* assay, the virus/disinfectant mixture dilutions are first passed through a small sephacryl column and then added to the hepatocytes culture. The loss in virus concentration is minimal and usually a 4 $\log_{10}$ reduction in virus titer or a 3 $\log_{10}$ reduction in titer beyond the virus-disinfectant dilution that shows cytotoxicity in the wells can be demonstrated.

3. HBV Antibodies in DHBV- Negative Ducklings

One commenter stated "Only ducklings obtained from a DHBV negative flock should be used for disinfectant testing if the *in vivo* model is used. DHBV negative ducklings from a DHBV positive flock have antibodies to DHBV which reduce the infectivity of DHBV. Up to 75% of day-old ducklings with maternal antibodies to DHBV were protected from infection when given 100 ID_{50} doses of DHBV. For most studies

this does not matter as excess DHBV is given. However, when conducting disinfectant testing, a low number of viable viral particles may be present. The antibodies present in these ducklings are able to neutralize low levels of virus."

Agency Response

The Agency agrees with this comment, and believes it lends further support for use of the *in vitro* assay. In the *in vitro* assay, only a duck that tests negative for DHBV is used for primary hepatocyte culture preparation.

4. Use of Liver Samples

One commenter stated, "Liver samples should be obtained from each duckling 2 to 4 weeks post inoculation. The liver should be used for DHBV analysis not serum. Not all DHBV positive ducklings become viraemic (even by PCR). In addition, some ducklings with low level of viral inoculum maybe viraemic on only one day (personal observation). Alternatively, serum samples could be tested for DHBV and then the liver from non-viraemic ducks analyzed."

Agency Response

This is a valid observation. However, the Agency has determined that the *in vivo* duck assay will not be the preferred protocol; rather, the hepatocyte cell culture assay (i.e., the *in vitro* duck assay) will be the preferred protocol.

5. Collection of Samples

One commenter stated, "The following comments address the *in vivo* protocol submitted by ViroMed Biosafety Laboratories: (a) Serum samples can be collected easily and with far less stress to the animals by venipuncture rather than cardiac puncture, which requires anesthesia at many institutions such as Stanford University; and (b) Liver samples should be collected at the end of an efficacy study and those from animals which have failed to become viremic should be analyzed for the presence of viral DNA. Most bleeding schedules used miss viremia in approximately 5% of the animals, but infection is readily apparent in the site of viral replication: the liver."

Agency Response

The Agency concurs with the commenter, however, as noted above, the Agency has determined that the *in vivo* duck assay will not be the preferred protocol. It has determined that the hepatocyte cell culture assay will be the preferred protocol. The latter assay is equally as capable as the *in vivo* assay and reflects the Agency's commitment to reduce the number of animals needed for testing, reduce the pain and suffering of test animals, and whenever scientifically-defensible, replace animals with validated non-animal test systems.

III. Conclusions

After the Agency reviewed the comments, it reached three conclusions:

1. It is the Agency's position that duck HBV serves as an adequate surrogate for human HBV and that the *in vitro* assay is sufficiently sensitive to preclude the need for any *in vivo* testing. The Agency is adopting, where possible, policies and data requirements that minimize animal testing, and when animal testing must be conducted, EPA is committed to reducing the number of animals needed for testing, reducing the pain and suffering of the test animals, and whenever scientifically-defensible, replacing animals with validated non-animal test systems. Therefore, relying heavily on the recommendations of the SAP, the Agency expects to rely on the use of the *in vitro* duck protocol as the method for evaluating the efficacy of disinfectants used to inactivate HHBV. Notwithstanding its commitment to maximize the reduction or elimination of animal testing where feasible, the Agency recognizes that some testing may already have been initiated or completed using the duck *in vivo* methodology as of the date of this Notice. On a case-by-case basis, the Agency will generally accept these data, if deemed valid, to support a registration.

2. Label claims against either the Hepadnavirus family or, more specifically, HHBV will be permitted when supported by adequate efficacy claims as described below. In addition, the following label claim language will be deemed acceptable: "effective against HBV." The Agency believes that these label claims can be supported by appropriate DHBV efficacy tests, since the surrogate DHBV has been shown to be a reliable predictor of resistence to chemical disinfection for the Hepadnavirus family as a whole.

3. To ensure that the *in-vitro* duck method has been adequately validated, data should be provided from at least two independent laboratories for each product tested (two batches per product per laboratory). The validation of a protocol requires the use of a common positive control disinfectant to be tested concurrently with all new products. The recommended control is alkyldimethylammonium chloride (BTC-835, Onyx Chemical Co.) (AOAC Official Methods of Analysis, Chapter 6, p. 136, 15th Edition, 1990). This agent should serve as both an intra-laboratory and an inter-laboratory control and should be used for analyzing the reproducibility of the efficacy data results for that particular protocol. In order to obtain the necessary inter-laboratory data, all submissions must additionally be subjected to confirmatory testing, with the common positive control, at a second laboratory test facility. It is critical for the Agency to know that a test method is repeatable; i.e., that there is an appropriately small standard deviation of log reduction (LR) values found when the test is repeated on different occasions in the same laboratory as well as when the test is conducted in different laboratories. The use of the common positive control and the generation of confirmatory data in a second testing facility will achieve these goals. A more detailed document outlining the criteria for validation is available electronically under the section entitled "Related Documents" section of the electronic version of this Notice ("Protocol for Testing the Efficacy of Disinfectants Used to Inactivate Hepatitis B Virus"). This document may also be requested by mail directly from the Agency (refer to "For Further Information Contact" section of this Notice).

IV. Non-Binding Statement

The guidance discussed in this notice is intended to provide guidance to EPA personnel and to pesticide applicants and registrants. This notice is not binding on EPA, applicants and registrants, and EPA may depart from the guidance where circumstances warrant and without prior notice. Registrants and applicants may propose alternatives to the protocols described in this notice and the Agency will assess them on a case-by-case basis.

V. Literature Cited

1- Bishop, N *et al.*, 1990. Antiviral strategies in chronic hepatitis B virus infection: I. Establishment of an *in vitro* system using the duck hepatitis B virus model. J Med Virol 31:82-9.

2- Bond, WW *et al.*, 1983. Inactivation of hepatitis B virus by intermediate-to-high level disinfectant chemicals. J Clin Microbiol 18: 535-38.

3- Chaufour, X, *et al.*, 1999. Evaluation of disinfection and sterilization of reusable angioscope with the duck hepatitis B model. J Vas Surg 30: 277-82.

4- Eble, BE; Corash L.,1996. Photochemical inactivation of duck hepatitis B virus in human platelet concentrates: a model of surrogate human hepatitis B virus infectivity. Transfusion 36:406-18.

5- Favero, MS and Bond, WW. 1998. Disinfection and Sterilization. In Viral Hepatitis. Edited by Zuckerman, AJ and Thomas HC. Churchill Livingstone Publisher, 2nd edition.

6-Kobayashi, H *et al.*, 1984. Susceptibility of hepatitis B virus to disinfectants and heat. J Clin Microbiol 20: 214-16.

7- Marion, PL, 1998. Use of animal models to study hepatitis B virus. Prog Med Virol 35: 43-75

8- Marion, PL, 1999. Personal Communication.

9- Mason WS, *et al.*, 1983. Experimental transmission of duck hepatitis B virus. Virology 131: 375-84.

10- Murray SM, *et al.*, 1991. Duck Hepatitis B virus: a model to assess efficacy of disinfectants against hepadnavirus infectivity. Epidemiol Infect 106: 435-43.

11-Prince, DL, *et al.*,1993. Methodological approaches to disinfection of human hepatitis B virus. J. Clin Microbiol 31:3296-304.

12-Pugh, JC, *et al.*,1999. Use of surrogate models for testing efficacy of disinfectants against hepatitis B virus. Amer J Infect Control 27: 375-76.

13-Tsiquaye, KN and Barnard, J,1993. Chemical disinfection of duck hepatitis B virus: a model for inactivation of infectivity of hepatitis B virus. J Antimicrob Chemotherapy 32: 313-23.

14-Tuttleman J, *et al.,* 1986. In vitro experimental infection of primary duck hepatocyte cultures with duck hepatitis B virus. J Virol. 58:17-25.

15- Vickery, K, *et al.,* 1999. Inactivation of duck hepatitis B virus by a hydrogen peroxide gas plasma sterilization system: Laboratory and "in use" testing. J Hosp Infect 41: 317-22.