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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

January 16, 2003

MEMORANDUM

Subject: Efficacy Review for EPA Reg. No. 72977-G / Axen 30
DP Barcode: D286172

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Applicant: ETI H_2O, Inc.

Formulation From Label:

PC072501

Active Ingredient(s)	% by wt
Silver	0.003
Citric Acid	4.840
Inert Ingredient(s)	95.157
Total	100.000

I BACKGROUND: ETI H_2O, Inc., has submitted a set of antimicrobial efficacy studies to support the registration of their product, "Axen 30". The MRID Numbers are 457572-02 thru 457572-10. The studies were conducted by Nelson Laboratories, Inc., and AppTec Laboratory Services.

These studies were primarily reviewed by the EPA contractor, DynCorp Systems & Solutions, LLC. The studies and the DynCorp report were briefly reviewed by EET/PSB/AD scientists to assure that they met Agency guidelines.

Axen® 30 (EPA Reg. No. 72977-G), is a new, as yet unregistered, ready-to-use product. The applicant requested to register the spray product as a disinfectant (bactericide, virucide, fungicide) for use on hard, non-porous, inanimate surfaces, including for use in homes, hospitals, restaurants, schools, and offices. The applicant indicated that the product is a "me-too" end-use product. The label claims that the product is effective against certain microorganisms in the presence of organic soil (1% or 5%). The label further claims that the product "remains effective in eliminating bacteria from hard surfaces up to 24 hours after application." Studies were conducted at Nelson Laboratories located at 6280 South Redwood Road in Salt Lake City, Utah 84123-6600 and AppTec Laboratory Services located at 2540 Executive Drive in St. Paul, Minnesota 55120.

This data package contained EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-35 (Data Matrix), nine studies (MRID Nos. 457572-02 through 457572-10), Statements of No Data Confidentiality Claims for all studies, and the proposed label.

The reports describe studies conducted on the product, Axen® (EPA Reg. No. 72977-2; 0.0012% silver) and state that the test material was a 30 ppm solution of Axenohl® (EPA Reg. No. 72977-1; 0.24% silver). Axenohl® is also referred to as the 2400 or 2410 ppm concentrate. Nothing in the reports or other parts of the data package actually states that 72977-G/Axen 30 is a 30 ppm solution of Axenohl. As there was a question of the test material identity, the study sponsor, Dolana Blount, was contacted. She faxed a letter to the study reviewer specifically stating that each of the test materials used in the reports submitted (MRID Numbers are 457572-02 thru 457572-10), once diluted, was equivalent to Axen 30. The concentrate used in these studies was Axenohl, 72977-1. (That letter is attached to this review.)

II Use Directions

The product is designed to be used for disinfecting hard, non-porous, inanimate surfaces such as walls, floors, counter tops, sinks, toilets, cabinets, tubs, showers, doorknobs, lights switch covers, telephones, appliances, stove tops, bed frames, wheelchairs, over-bed tables, examination tables and waste containers, tables, and chairs. Directions on the proposed label provided the following information

regarding preparation and use of the product as a disinfectant: Pre-clean surfaces that are heavily soiled. Completely wet surfaces with the product for the label-specified contact time (which varies depending upon the organism being targeted). Wipe surfaces dry with a clean towel.

The proposed label directions also included special instructions for cleaning and decontaminating against HIV-1 on pre-cleaned surfaces or objects previously soiled with blood/body fluids.

III Agency Standards for Proposed Claims

Confirmatory Efficacy Data Requirements - "Me-Too" Applications

A "me-too" application involves an old chemical (i.e., active ingredient) that has been previously registered for use as a pesticide and that is also the active ingredient present in the product proposed for registration (i.e., the product for which the current application is being submitted). DIS/TSS-5 states that products proposed for registration that are merely dilutions of a product already registered require only documentation of this identity and specific references to the supporting data developed for the original product. DIS/TSS-5 also states that confirmatory data must be produced when the test methodology used in support of the original supporting efficacy data (i.e., for the old chemical) are modified to include additional elements (e.g., organic soil load, shorter contact time). For hospital disinfectants, 10 carriers on each of two samples representing 2 different batches of product must be tested against *Salmonella choleraesuis* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442) using either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products Test. Killing on all carriers is required. The above Agency standards are presented in DIS/TSS-5.

Note: The proposed label for the product, Axen® 30, indicates a 30 second contact time for *Salmonella choleraesuis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The last accepted label (dated June 21, 2001) for the product, Axen®, indicates a 10 minute contact time for these three microorganisms.

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments (Additional Microorganisms)

Effectiveness of disinfectants against specific microorganisms other than those named in the AOAC Use-Dilution Method, AOAC Germicidal Spray Products Test, AOAC Fungicidal Test, and AOAC Tuberculocidal Activity Method, but not including viruses, must be determined by either the AOAC Use-Dilution Method or the AOAC Germicidal Spray Products Test. Ten carriers must be tested against each specific microorganism with each of 2 product samples, representing 2 different batches. To support products labeled as "disinfectants" for specific microorganisms (other than

those microorganisms named in the above test methods), killing of the specific microorganism on all carriers is required. In addition, plate count data must be submitted for each microorganism to demonstrate that a concentration of at least 10^4 microorganisms survived the carrier-drying step. These Agency standards are also presented in DIS/TSS-1.

Virucides

The effectiveness of virucides against specific viruses must be supported by efficacy data that simulates, to the extent possible in the laboratory, the conditions under which the product is intended to be used. Carrier methods that are modifications of either the AOAC Use-Dilution Method (for liquid disinfectants) or the AOAC Germicidal Spray Products Test (for spray disinfectants) must be used in developing data for virucides intended for use upon dry inanimate, environmental surfaces (e.g., floors, tables, cleaned dried medical instruments). To simulate in-use conditions, the specific virus to be treated must be inoculated onto hard surfaces, allowed to dry, and then treated with the product according to the directions for use on the product label. One surface for each of two different batches of disinfectant must be tested against a recoverable virus titer of at least 10^4 from the test surface for a specified exposure period at room temperature. Then, the virus must be assayed by an appropriate virological technique, using a minimum of four determinations per each dilution assayed. The calculated viral titers must be reported with the test results. For the data to be considered acceptable, results must demonstrate complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a 3-log reduction in titer must be demonstrated beyond the cytotoxic level. These Agency standards are presented in DIS/TSS-7.

Disinfectants for Use as Fungicides (Against Pathogenic Fungi)

The effectiveness of liquid disinfectants against specific pathogenic fungi must be supported by efficacy data derived from each of 2 product samples representing 2 different batches using the AOAC Fungicidal Test. The highest dilution that kills all fungal spores is the minimum effective concentration. In addition, the method indicates that conidia of required resistance survive a 10 minute exposure at 20°C to phenol dilution of 1:70, but not to one of 1:60. These Agency standards are presented in DIS/TSS-6 and AOAC Method 955.17.

Alternatively, the AOAC Use-Dilution Method may be modified to conform with the appropriate elements in the AOAC Fungicidal Test. If the product is intended to be used as a spray product, the AOAC Germicidal Spray Products Test must be employed. The inoculum in the test must be modified to provide a concentration of at least 10^6 conidia per carrier. Ten carriers on each of 2 product samples representing 2 different batches must be employed in the test. Killing of the specific pathogenic fungi on all carriers is required. These Agency standards are also presented in DIS/TSS-6.

IV Comments on the Submitted Efficacy Studies

1 MRID 457572-02: "AOAC Use Dilution - Carrier Confirmation," by Shelli A. Baxter. Study conducted at Nelson Laboratories, Inc. Study completion date – January 10, 2002. Lab Number – 197155. Protocol Number – 200135303-01.

This study was conducted against *Staphylococcus aureus* (MRSA) (ATCC 700698), *Enterococcus faecium* (VRE) (ATCC 700221), *Listeria monocytogenes* (ATCC 19111), and *Escherichia coli* OH157 (ATCC 43888). Two lots (Lot Nos. 2001-042-001 and 2001-005-001) of the product, Axenohl (EPA Registration Number 72977-1), were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. A 30 ppm solution of Axen® was prepared by diluting the 2410 ppm concentrate with 5% (w/w) citric acid in purified water. Equine blood serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel penicylinder carriers were immersed in a 48-54 hour old suspension of the test organism for 15 minutes, removed and shaken to remove excess culture, and dried for 40±2 minutes at 37±2°C. The carriers were exposed to 10 mL of the use solution at 20±0.5°C. Carriers were exposed for 30 second, 1 minute and 2 minute intervals. Following the exposure intervals, the carriers were removed from the use solution, shaken to remove residual product, transferred to tubes containing LETH, and shaken thoroughly. The culture tubes were incubated at 37±2°C for 2 days and then observed for the presence or absence of visible growth. Controls included neutralization verification, growth promotion of recovery media, and dried carrier counts.

Note: Although the report states that the test material was a dilution of Axen, information from the sponsor states that the test material was actually a dilution of Axenohl, 72977-1.

2 MRID Number 457572-03: "AOAC Use Dilution - Carrier Confirmation," by Shelli A. Baxter. Study conducted at Nelson Laboratories, Inc. Study completion date – January 9, 2002. Lab Number – 194972. Protocol Number 200126906-02.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), and *Salmonella choleraesuis* (ATCC 10708). Two lots (Lot Nos. 2001-042-001 and 2001-005-001) of the product, Axenohl®, were tested using the AOAC Use-Dilution Method as described in the AOAC Official Methods of Analysis, 15th Edition, 1990. For both lots, 15 ppm, 20 ppm and 30 ppm solutions of Axenohl were prepared by diluting the 2410 ppm concentrate with 5% (w/w) citric acid in purified water. Equine blood serum was added to the culture to achieve a 5% organic soil load. Ten (10) stainless steel cylinder carriers were immersed in a 48-54 hour old suspension of the test

organism for 15 minutes, removed and shaken to remove excess culture, and dried for 40±2 minutes at 37±2°C. The carriers were exposed to 10 mL of the use solution at 20±0.5°C. Carriers were exposed for different intervals as follows:

Organism	Exposure Times		
	15 ppm Use Solution	**20 ppm Use Solution**	**30 ppm Use Solution**
Staphylococcus aureus	1, 5, and 10 minutes	1, 2, and 5 minutes	30 seconds, 1 and 2 minutes
Pseudomonas aeruginosa	1, 5, and 10 minutes	1, 2, and 5 minutes	30 seconds, 1 and 2 minutes
Salmonella choleraesuis	1, 5, and 10 minutes	1, 2, and 5 minutes	30 seconds, 1 and 2 minutes

Following the exposure intervals, the carriers were removed from the use solution, shaken to remove residual product, transferred to tubes containing Letheen Broth, and shaken thoroughly. The culture tubes were incubated at 37±2°C for 2 days and then observed for the presence or absence of visible growth. Controls included phenol resistance, neutralization verification, growth promotion of recovery media, and dried carrier counts.

3 MRID Number 457572-04: "Fungicidal Activity of a Disinfectant" by Shelli Baxter. Study conducted at Nelson Laboratories, Inc. Study completion date – January 11, 2002. Laboratory Sample ID – 197157. Protocol Number – 200124703-03.

This study was conducted against *Trichophyton mentagrophytes* (ATCC 9533). Two lots (Lot Nos. 2001-042-001 and 2001-005-001) of the product, Axenohl, were tested using the Fungicidal Activity of Disinfectants Method as described in the AOAC Official Methods of Analysis, 17th Edition, 2000. A 30 ppm solution of Axenohl was prepared by diluting the 2410 ppm concentrate with 5% (w/w) citric acid in purified water. Five mL of use solution was placed into test tubes. A volume of 0.5 mL of the conidial suspension was placed in the first tube of use solution and shaken. After 30 seconds, 0.5 mL of the conidial suspension was added to a second tube. This was repeated at 30 second intervals until all tubes were inoculated. After 30 second, 1, 2, 5, and 10 minute intervals, a sample from each tube was removed and placed into 20 mL of glucose broth. The culture tubes were incubated at 27-29°C for 10 days and then observed for the presence or absence of visible growth. Controls included phenol resistance, neutralization verification, growth promotion of recovery media, and sterility.

4 MRID Number 457572-05: "Evaluation of Axen® for Residual Activity " by Shelli Baxter. Study conducted at Nelson Laboratories, Inc. Study completion date – February 8, 2002. Lab Sample ID – 197158. Protocol Number 200132009-02.

This study was conducted against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442), and *Salmonella choleraesuis* (ATCC 10708). Two lots (Lot Nos. 2001-042-001 and 2001-005-001) of the product, Axenohl, were tested. The report referenced the AOAC Germicidal Spray Products as Disinfectants Method as described in the AOAC Official Methods of Analysis, 16th Edition, 1995. A 30 ppm solution of Axenohl was prepared by diluting the 2410 ppm concentrate with 5% (w/w) citric acid in purified water. Eighteen glass slides per organism, per lot, per time point were prepared. One mL of the use solution was applied to each glass slide. The use solution was spread over the entire slide with a clean towel. At 0, 1, 6, and 24 hours, 0.01 mL of the test culture was transferred onto the sterile test slides and spread uniformly over an approximate one inch by one inch area. Three slides per organism, per lot of product, were held for 30 seconds, 1 minute, and 2 minutes. After the exposure intervals, the slides were transferred into a bottle of lethene. One slide per organism, per time point, was extracted by shaking manually for 1 minute. A plate count was performed in triplicate. The remaining bottles and all plates were incubated for 48-54 hours at 37±2°C and observed for the presence or absence of visible growth. Controls included neutralization verification, growth promotion of recovery media, and initial counts.

Note: This study was conducted to support the claim that the product "remains effective in eliminating bacteria from hard surfaces up to 24 hours after application." In this study, the test material was applied **before** the application of the bacterial.

Note: Data were provided in triplicate (i.e., 3 data points) for both product lots, for each organism, and for each exposure interval. Only qualitative results (i.e., reported as growth or no growth) were provided for testing of the product against Lot No. 2001-005-001. Qualitative results also were provided for 2 of the 3 data points for testing of the product against Lot No. 2001-042-001. Quantitative results were provided for 1 of the 3 data points for testing of the product against Lot No. 2001-042-001 (i.e., the data point for the slide that was subjected to extraction).

5 MRID Number 457572-06: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Axen® 30, by Mary J. Miller. Study conducted at AppTec Laboratory Services. Study completion date – January 17, 2002. Project Number – 12465.

This study was conducted against Influenza A virus, Hong Kong strain (ATCC VR-544), using Rhesus monkey kidney cells (obtained from ViroMed Laboratories, Inc., Minneapolis, Minnesota) as the host system. Two lots (Lot Nos. 2001-042-001 and 2001-005-001) of the product, Axenohl, were tested according to an AppTec Laboratory Services protocol (Protocol No.

IMS01121301.FLU, copy not provided). The product was received, ready to use, from the applicant, and was identified as the 30 ppm use dilution of Axenohl®, a 2400 ppm concentrate. The stock virus titer contained a 1% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 20.1°C in a relative humidity of 46% for 20 minutes. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution at 22°C. After 10 minutes of exposure, the plates were scraped with a cell scraper to re-suspend the contents and the virus-disinfectant mixture was passed through a Sephadex column. The filtrate was then titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 1% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL Fungizone. Rhesus monkey kidney cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included dried virus counts, cytotoxicity, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

6 MRID 457572-07: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Axen® 30, by Mary J. Miller. Study conducted at AppTec Laboratory Services. Study completion date – December 20, 2001. Project Number – 12305.

This study was conducted against Human Immunodeficiency Virus Type 1, Strain HTLV-III_B; (obtained from Advanced Biotechnologies, Inc., Columbia, Maryland), using MT-2 cell cultures (human CD4+ lymphocytes; propagated in-house; originally obtained from the National Cancer Institute, Frederick, Maryland) as the host system. No ATCC Number was provided for this strain of HTLV-III. Two lots (Lot Nos. 2001-042-001 and 2001-005-001) of the product, Axenohl, were tested according to an AppTec Laboratory Services protocol (Protocol No. IMS99111501.HIV, copy not provided). The product was received, ready to use, from the applicant, and was identified as the 30 ppm use dilution of Axenohl®, a 2400 ppm concentrate. The stock virus titer contained a 5% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were air-dried at 17°C for 20 minutes, then incubated at 36-38°C for an additional 30 minutes. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution at 17°C. After 30 seconds of exposure, the plates were scraped with a cell scraper to re-suspend the contents and the virus-disinfectant mixture was passed through a Sephadex column. The filtrate was then titered by serial dilution in RPMI 1640 supplemented with 15% (v/v) heat-inactivated fetal bovine serum, 2 mM L-glutamine, and 50 µg/mL gentamicin. MT-2 cells were inoculated in

quadruplicate with 0.2 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 8 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included cytotoxicity, dried virus controls, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

7. MRID 457572-08: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Axen® 30, by Mary J. Miller. Study conducted at AppTec Laboratory Services. Study completion date – February 12, 2002. Project Number 12609.

   This study was conducted against Herpes simplex virus type 1 (ATCC VR-733, F(1) Strain), using rabbit kidney cells (obtained from ViroMed Laboratories, Inc., Minneapolis, Minnesota) as the host system. Two lots (Lot Nos. 2001-042-001 and 2001-005-001) of the product, Axen®, were tested according to an AppTec Laboratory Services protocol (Protocol No. IMS01011002.HSV, copy not provided). The product was received, ready to use, from the applicant, and was identified as the 30 ppm use dilution of Axenohl®, a 2400 ppm concentrate. The stock virus titer contained a 1% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of six separate sterile glass Petri dishes. The virus films were dried at 10°C in a relative humidity of 50% for 25 minutes. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution at 24°C. After both 1 minute and 10 minute exposures, the plates were scraped with a cell scraper to re-suspend the contents and the virus-disinfectant mixtures were passed through a Sephadex column. The filtrate was then titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL Fungizone. Rabbit kidney cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included cytotoxicity, dried virus controls, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

8. MRID Number 457572-09: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Axen® 30, by Mary J. Miller. Study conducted at AppTec Laboratory Services. Study completion date – February 12, 2002. Project Number – 12608.

   This study was conducted against Poliovirus type 2 (ATCC VR-1002, Strain Lansing), using Vero cells (obtained from ViroMed Laboratories, Inc., Minneapolis, Minnesota) as the host system. Two lots (Lot Nos. 2001-042-001

and 2001-005-001) of the product, Axenohl, were tested according to an AppTec Laboratory Services protocol (Protocol No. IMS01011002.POL, copy not provided). The product was received, ready to use, from the applicant, and was identified as the 30 ppm use dilution of Axenohl, a 2400 ppm concentrate. The stock virus titer contained a 1% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of six separate sterile glass Petri dishes. The virus films were dried at 10°C in a relative humidity of 50% for 25 minutes. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution at 23°C. After both 1 minute and 10 minute exposures, the plates were scraped with a cell scraper to re-suspend the contents and the virus-disinfectant mixtures were passed through a Sephadex column. The filtrate was then titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 5% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL Fungizone. Vero cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 36-38°C in a humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included cytotoxicity, dried virus controls, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

9 MRID Number 457572-10: "Virucidal Efficacy of a Disinfectant for Use on Inanimate Environmental Surfaces" for Axen® 30, by Mary J. Miller. Study conducted at AppTec Laboratory Services. Study completion date – February 12, 2002.

This study was conducted against Rhinovirus type 37 (ATCC VR-1147, Strain 151-1), using MRC-5 cells (human embryonic lung cells; obtained from ViroMed Laboratories, Inc., Minneapolis, Minnesota) as the host system. Two lots (Lot Nos. 2001-042-001 and 2001-005-001) of the product, Axenohl, were tested according to an AppTec Laboratory Services protocol (Protocol No. IMS01010402.R37, copy not provided). The product was received, ready to use, from the applicant, and was identified as the 30 ppm use dilution of Axenohl®, a 2400 ppm concentrate. The stock virus titer contained a 1% organic soil load (fetal bovine serum). Films of virus were prepared by spreading 0.2 mL of virus inoculum uniformly over the bottoms of three separate sterile glass Petri dishes. The virus films were dried at 10.1°C in a relative humidity of 51% for 25 minutes. For each lot of product, separate dried virus films were exposed to 2.0 mL of the use solution at 22°C. After 10 minutes of exposure, the plates were scraped with a cell scraper to re-suspend the contents and the virus-disinfectant mixture was passed through a Sephadex column. The filtrate was then titered by serial dilution in Eagles minimal essential medium (E-MEM) supplemented with 10% heat-inactivated fetal bovine serum, 10 µg/mL gentamicin, 100 units/mL penicillin, and 2.5 µg/mL Fungizone. MRC-5 cells were inoculated in quadruplicate with 0.1 mL of each dilution and incubated at 31-35°C in a

humidified atmosphere of 5-7% CO_2. The plates were scored periodically for 7 days for the absence or presence of unspecified cytopathic effects, cytotoxicity, and viability. Controls included cytotoxicity, dried virus controls, and neutralization. Viral and cytotoxicity titers were calculated by the method of Spearman Karber.

V Results

Table 1. Antibacterial Activity of Axen Against *Staphylococcus aureus* (MRSA), *Enterococcus faecium* (VRE), *Listeria monocytogenes* and *Escherichia coli* 0157:H7.

MRID Number	Organism	Exposure Time	No. Exhibiting Growth/Total No. Tested		Dried Carrier Counts (CFU/carrier)
			Lot No. 2001-042-001	Lot No. 2001-005-001	
457572-02	*Staphylococcus aureus* (MRSA)	30 sec.	9/10	9/10	1×10^5
		1 min.	7/10	7/10	
		2 min.	0/10	0/10	
	Enterococcus faecium (VRE)	30 sec.	0/10	2/10	1×10^4, 1×10^5
		1 min.	1/10	1/10	
		2 min.	0/10	0/10	
	Listeria monocytogenes	30 sec.	0/10	0/10	1×10^4
		1 min.	0/10	0/10	
		2 min.	0/10	0/10	
	Escherichia coli 0157:H7	30 sec.	1/10	0/10	1×10^4, 1×10^5
		1 min.	0/10	1/10	
		2 min.	0/10	0/10	

Table 2. Test of 30 ppm Dilution of Axenohl Against Three Species of Bacteria

Axenohl Against Three Species of Bacteria					
MRID Number	Organism	Exposure Time	No. Exhibiting Growth/Total No. Tested		Microbes Initially Present (CFU/mL) at 30 ppm
			Lot No. 2001-042-001	Lot No. 2001-005-001	
457572-03	*Staphylococcus aureus*	30 sec.	0/10	0/10	1×10^5, 1×10^6
		1 min.	0/10	0/10	
		2 min.	0/10	0/10	
	Pseudomonas aeruginosa	30 sec.	0/10	0/10	1×10^4, 1×10^5
		1 min.	0/10	0/10	
		2 min.	0/10	0/10	
	Salmonella choleraesuis	30 sec.	0/10	0/10	1×10^5
		1 min.	0/10	0/10	
		2 min.	0/10	0/10	

Note: Results were also reported for a 15 and 20 ppm use solution of the product for *Staphylococcus aureus, Pseudomonas aeruginosa*, and *Salmonella choleraesuis*. See MRID No. 457572-03.

Table 3. Test of a 30 ppm Dilution of Axen Against *Trichophyton mentagrophytes*

Axen Against *Trichophyton mentagrophytes*					
MRID Number	Organism	Exposure Time	Number Exhibiting Growth/ Total Number Tested		Viability (conidia/mL)
			Lot No. 2001-042-001	Lot No. 2001-005-001	
457572-04	*Trichophyton mentagrophytes*	30 sec	59/60	60/60	1.2×10^7
		1 min.	58/60	60/60	
		2 min.	54/60	57/60	
		5 min.	11/60	10/60	
		10 min.	0/60	0/60	

Table 4. MRID Number 457572-05: Residual Bactericidal Activity of Axen

Organism	Exposure Time	% Reduction/ Lot No. 2001-042-001				Microbes Initially Present (CFU/mL) at 0, 1, 6, 24 Hours
		0 Hour	1 Hour	6 Hours	24 hours	
Staphylococcus aureus	0.5 min.	99.7	99.8	99.993	99.6	7.7×10^3 7.7×10^3 3.2×10^4 3.2×10^4
	1 min.	99.99	99.94	99.997	99.96	
	2 min.	99.99	99.97	99.96	99.99	
Salmonella choleraesuis	0.5 min.	64	99.4	81	83	1.1×10^5 1.1×10^5 3.4×10^4 3.4×10^4
	1 min.	99.5	99.997	99	93	
	2 min.	99.99	99.996	99.7	>99.997	
Pseudomonas aeruginosa	0.5 min.	99	99.7	74	>99.998	5.7×10^4 5.7×10^4 4.2×10^4 4.2×10^4
	1 min.	99.8	99	99.93	99.990	
	2 min.	99.99	99.9	99.97	>99.998	

Table 5. Virucidal Efficacy of Axen at 30 ppm.

MRID Number	Organism	Results			Dried Virus Control
			Lot No. 2001-042-001	Lot No. 2001-005-001	
457572-06	Influenza A virus, strain Hong Kong	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{5.25}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457572-07	Human Immuno-deficiency Virus Type 1	10^{-1}	Cytotoxicity present	Cytotoxicity present	$10^{5.25}$ $TCID_{50}$/0.2 mL
		10^{-2} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	
		$TCID_{50}$/0.2 mL	$\leq 10^{1.5}$	$\leq 10^{1.5}$	
		Log reduction	$\geq 3.75\ log_{10}$	$\geq 3.75\ log_{10}$	
457572-08 Contact Time: 1 min.	Herpes simplex virus type 1	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.0}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457572-09 Contact Time: 10 minutes.	Poliovirus type 2, strain Lansing	10^{-1} to 10^{-8} dilutions	Complete inactivation	Complete inactivation	$10^{6.5}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	
457572-10	Rhinovirus type 37, strain 151-1	10^{-1} to 10^{-7} dilutions	Complete inactivation	Complete inactivation	$10^{5.0}$ $TCID_{50}$/0.1 mL
		$TCID_{50}$/0.1 mL	$\leq 10^{0.5}$	$\leq 10^{0.5}$	

VI Conclusions

1 MRID Number 457572-02: The submitted efficacy data support the use of Axen 30 (EPA File Symbol 72977-G) as a disinfectant of hard, inanimate, non-porous surfaces contaminated with Methicillin-Resistant *Salmonella aureus* (MRSA) (ATCC #700698), Vancomycin-Resistant *Enterococcus faecium* (VRE) (ATCC 700221), *Escherichia coli* 0157:H7 (ATCC #43888) and/or *Listeria monocytogenes* (ATCC #19111) when used with a ten-minute exposure in the presence of a 5% organic soil load at 20°C. (The report lists the strain of *E. coli* tested as OH157. The correct strain for ATCC # 43888 is 0157:H7.) The product was effective against all tested strains of bacteria after a two-minute exposure. Although these studies were not conducted using the AOAC Germicidal Spray Products Test, they are acceptable because this product is not an aerosol spray.

2 MRID Number 457572-03: The submitted data support the use of Axen 30 as a disinfectant of hard, non-porous, inanimate surfaces that have been contaminated with *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442) or *Salmonella choleraesuis* (ATCC 10708) when used with a ten-minute exposure in the presence of a 5% organic soil load at 20°C.

3 MRID Number 457572-04: The submitted data support the use of Axen 30 as a fungicide on hard, non-porous, inanimate surfaces that have been contaminated with *Trichophyton mentagrophytes* (ATCC #9533), when the product is used with a 10-minute exposure at 20°C in the presence of a 5% organic soil load. Both lots of the test material were effective against *Trichophyton mentagrophytes* (ATCC #9533) after a 10-minute exposure.

4 MRID Number 457572-05: This study is not acceptable. Bacteriostatic claims are permitted only against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria) in the presence of moisture, but not against microorganisms of public health concern. All data in support of residual self-sanitizing efficacy data should include a wear component commensurate with what is expected to be encountered during actual product use.

5 MRID Number 457572-06: The submitted efficacy data support the use of the product, Axen®, at a dilution of 30 ppm, as a virucide when tested against Influenza A virus, Hong Kong strain, (ATCC VR-544) on hard, non-porous, inanimate surfaces with a 1% soil load and a 10-minute exposure period at room temperature.

6 MRID Number 457572-07: The submitted efficacy data support the use of the product, Axen®, at a dilution of 30 ppm, as a virucide when tested against Human Immunodeficiency Virus type 1, Strain HTLV-III_B, on hard, non-porous, inanimate surfaces with a 5% organic soil load and a 30-second exposure period at room temperature.

7 MRID Number 457572-08: The submitted efficacy data support the use of the product, Axen®, at a dilution of 30 ppm, as a virucide when tested against Herpes simplex virus, type 1, (ATCC VR-733) on hard, non-porous, inanimate surfaces with a 1% organic soil load with one and ten minute exposures at room temperature.

8 MRID Number 457572-09: The submitted efficacy data support the use of the product, Axen®, at a dilution of 30 ppm, as a virucide when tested against Poliovirus type 2, Strain Lansing (ATCC VR-1002) on hard, non-porous, inanimate surfaces with a 1% organic soil load with a ten minute exposure at room temperature.

9 MRID Number 457572-10: The submitted efficacy data support the use of the product, Axen®, at a dilution of 30 ppm, as a virucide when tested against Rhinovirus type 37, strain 151-1, on hard, non-porous, inanimate surfaces with a 1% organic soil load with a ten minute exposure at room temperature.

VII Recommendations

1 We ask that whenever the registrant submits data/studies/reports to PSB/AD for review, they properly identify the test material used. It is understood that registrants often have products tested before a final product name is decided on. However, it wastes much valuable time for reviewers to have to determine the proper test material identity when it is something that should be one of the most basic parts of a data submission. If the name of the test material used in the report is not exactly the same as the registration product, the registrant may state the relationship of the test material (the same product with a different name, a dilution of the test material, etc.) in a cover letter submitted with the test material.

2 The request to add labeling claims of Axen 30 being an effective disinfectant of hard, non-porous, inanimate surfaces contaminated with Methicillin-Resistant *Salmonella aureus* (MRSA) (ATCC #700698), Vancomycin-Resistant *Enterococcus faecium* (VRE) (ATCC 700221), *Escherichia coli* 0157:H7 (ATCC #43888) and/or *Listeria monocytogenes* (ATCC #19111) is approved. The submitted label lists the strain of *E. coli* as being "OH157". The statement also abbreviates the nomenclature of the organism. This should be changed from "*E. coli* OH157" to "*Escherichia coli* 0157:H7".

3 The request to add label claims that Axen 30 is an effective disinfectant against *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442) or *Salmonella choleraesuis* (ATCC 10708) is approved. Axen 30 is approved as a hospital disinfectant.

4 The request to add labeling claims that Axen 30 is an effective fungicide on hard, non-porous, inanimate surfaces that have been contaminated with *Trichophyton mentagrophytes* (ATCC #9533) is approved.

5 The request to add labeling claims that Axen 30 is a bacteriostatic agent, or, that it will inhibit, check, eliminate or otherwise stop the growth of bacteria that are introduced to a surface that has been pre-treated with Axen 30 is denied. Again, bacteriostatic claims are permitted only against microorganisms identified as causing economic or aesthetic problems (e.g., odor-causing bacteria) in the presence of moisture, but not against microorganisms of public health concern. **No label claims stating that this product provides residual activity against bacteria are allowed on the product label.** No labeling claims stating that this product continues to kill or eliminate bacteria after application are allowed on the product label. Please refer to Subdivision G, §91-2, (m).

6 The request to add labeling claims that Axen 30 is an effective virucide against Influenza A virus, Hong Kong strain (ATCC VR-544), is approved. PSB/AD recognizes that the lab tested the product with a 1% soil load. Soil loads are typically included in antimicrobial efficacy studies to obtain the designation of being effective in the presence of organic soil. An antimicrobial agent identified as a "one-step" cleaner-disinfectant, cleaner-sanitizer, or one intended to be effective in the presence of organic soil must be tested for efficacy by the appropriate method(s) which have been modified to include a representative organic soil such as 5% blood serum. However, the registrant may retain the statement that this product was evaluated against Influenza A virus, Hong Kong strain (ATCC VR-544), in the presence of 1% organic soil.

7 The request to add labeling claims of Axen 30 being an effective virucide against Human Immunodeficiency Virus type 1, Strain $HTLV\text{-}III_B$, in the presence of an organic soil load with a 10-minute exposure is approved.

8 The request to add labeling claims of Axen 30 being an effective virucide against Herpes simplex virus, type 1, ATCC Number VR-733, is approved. The registrant may retain the statement that this product was evaluated against Herpes simplex virus, type 1, ATCC Number VR-733, in the presence of 1% organic soil.

9 The request to add labeling claims of Axen 30 being an effective virucide against Poliovirus, type 2, ATCC VR-733, is approved. The registrant may retain the statement that this product was evaluated against Poliovirus, type 2, ATCC VR-733, in the presence of 1% organic soil.

10 The request to add labeling claims of Axen 30 being an effective virucide against Rhinovirus, type 37 (ATCC VR-1147, Strain 151-1), is approved. The registrant may retain the statement that this product was evaluated against Rhinovirus, type 37, ATCC VR-1147, Strain 151-1, in the presence of 1% organic soil.

11 Page 2 of the submitted label states that Axen 30 "... is ideal for use on ... contamination." Such statements are considered to be superlatives and are not allowed on the labels of EPA registered pesticides.

12 Page 2 of the submitted label states: "Proven to eliminate bacteria, fungus and viruses, ...contamination." This statement should refer to the table under General Information that lists the organisms that this product have been proven effective against.

13 Page 3 of the submitted label states: "For general disinfection and elimination of bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella choleraesuis* and *Listeria monocytogenes*, the surface must be completely wet with **Axen 30 Disinfectant, Fungicidal & Virucidal Spray** for 30 seconds." The EPA does not allow claims of 30-second disinfections. The product label must state that for disinfection, the treated surfaces must remain wet for **two minutes**.

14 The submitted label describes Axen 30 as being disinfectant, fungicidal and virucidal. According to Subdivision H, § 101-3, g, the unqualified label claim "virucidal" is not generally acceptable. The claim "virucidal" must be qualified by designating each specific virus against which the product has been tested and shown to be effective.

15 Statements claiming that this product kills or eliminates bacteria in 30-seconds are not allowed on the product label. This statement has not been proven for all species of bacteria that this product has been tested against.

16 The statement "disinfect with confidence" is not allowable. This could be taken to imply that one might not have confidence in other products. EPA/OPP/AD does not allow comparative labeling statements.

17 The names of all bacterial species listed on product labels should be italicized.

18 The statements "eliminates" or "kills 99.99999% of bacteria in 30 seconds", and, "eliminates" or "kills 99.9999% of bacteria in seconds" are not allowable. These

statements must be removed from the product label. One reason for this is that Axen 30 was not always able to eliminate 99.9999% bacteria in 30 seconds.

19 The label states: Eliminates bacteria, fungus and virus. This statement is not allowable. The statement may be used if it refers to the specific species/strains of bacteria, fungi and/or viruses that this product was shown to eliminate.