UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
PREVENTION, PESTICIDES
AND TOXIC SUBSTANCES

November 18, 2002

MEMORANDUM:

Subject: Efficacy Review for EPA Reg. No.: 9402-RN,"Kleenex® Brand Anti-Viral* Tissue #2"
DP Barcode: D284618
Case No: 072433

From: Emily Mitchell, M.S., Team Leader Emily Mitchell 11/18/02
Efficacy Evaluation Team
Product Science Branch
Antimicrobials Division (7510C)

To: Adam Heyward PM34/Drusilla Copeland
Regulatory Management Branch II
Antimicrobials Division (7510C)

Applicant: Kimberly-Clark Corporation
2100 Winchester Road
Neenah, WI 54957

Formulation From Label:

Active Ingredient(s)	% by wt.
Citric Acid	7.51%
Sodium Lauryl Sulfate	2.02%
Inert Ingredient(s)	90.47%
Total	100.00%

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I BACKGROUND

The product, Kleenex Brand Anti-Viral Tissue #2 (EPA Reg. No. 9402-RN), is a new "end-use" product. The applicant requested to register this virucidal facial tissue for use in hospitals, schools, churches, day care facilities, and physicians' offices. The environmental "surface" on which the product is intended to be active is the tissue itself. All laboratory studies were conducted at Hill Top Research, Inc., located at Main and Mill Streets in Miamiville, Ohio 45147.

This data package contained EPA Form 8570-4 (Confidential Statement of Formula), five laboratory studies (MRID Nos. 457138-11 through 457138-15), Statements of No Data Confidentiality Claims for all five laboratory studies, one confidential consumer survey study (MRID No. 457229-01), and the proposed label.

II USE DIRECTIONS

No specific directions for use (including the surface to be disinfected) are provided in the label section "Directions for Use." The proposed label directions state:

"Use to help prevent the spread of viruses*."
"Complete inactivation of viruses* within 15 minutes after contact."
"Dispose of used tissues in a normal fashion. Do not reuse empty container."

III AGENCY STANDARDS FOR PROPOSED CLAIMS

Impregnated Towelettes for Hard Surface Disinfection – Single-Use Towelette

The complete product, as offered for sale, should be tested according to directions for use to ensure effectiveness in disinfecting hard surfaces. Basic efficacy data requirements in DIS/TSS-1 are required. Additionally, a modification of the AOAC Germicidal Spray Products Test should be employed, using one towelette to wipe the surface of each glass slide carrier, subculturing the slides, expressing the liquid from the used towelette, and subculturing the expressed liquid. Sterile gloves should be used to handle the towelette, and the towelette should be rotated between each wipe so as to expose a maximum amount of towelette surface area. Supplemental recommendations in DIS/TSS-2 should be met, and data reporting requirements of DIS/TSS-3 should be met. These standards are provided in "Efficacy Data Requirements: Pre-Saturated or Impregnated Towelettes for Hard Surface Disinfection."

Disinfectants for Use on Hard Surfaces in Hospital or Medical Environments

The effectiveness of disinfectants for use on hard surfaces in hospital or medical environments must be substantiated by data derived using the AOAC Use-Dilution Method (for water soluble powders and liquid products) or the AOAC Germicidal Spray

Products Test (for spray products). Sixty carriers must be tested with each of 3 product samples, representing 3 different batches, one of which is at least 60 days old, against *Salmonella choleraesuis* (ATCC 10708), *Staphylococcus aureus* (ATCC 6538), and *Pseudomonas aeruginosa* (ATCC 15442). To support products labeled as "disinfectants," killing on 59 out of 60 carriers is required to provide effectiveness at the 95% confidence level. The above Agency standards are 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, four replicates per dilution. 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.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 457138-11 "Virucidal Efficacy of Facial Tissue For Treated and Untreated Tissue Against Rhinovirus 1A, ATCC VR-1364" for Kleenex Brand Anti-Viral Tissue #2, by Kathleen A. Baxter. Study conducted at Hill Top Research, Inc. Study completion date – June 13, 2002.

This study was conducted against Rhinovirus 1A (ATCC VR-1364) using WI-38 cells (source not identified) as the host system. The study protocol followed a modification of ASTM Method E 1053-97, Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces; a major modification

was the use of 1-inch-square "disks" of tissue as the carrier in lieu of glass slides. The carriers were inoculated with undried virus (presence of organic soil load not indicated), and held for the indicated contact time. Three (3) lots of product were tested (Lot Nos. 3-7-02-4A, 3-7-02-4B, 3-7-02-4C (60 Day Stability Sample)) and compared against untreated tissue control disks (Lot No. 3-7-02-4D). Two disks punched out of individual tissue sheets prior to inoculation with test virus were placed side by side in a sterile 60 mm glass Petri plate in a laminar flow hood at 24±3°C. Each disk was inoculated with 100 µL of test virus distributed uniformly in a spiral fashion from the center of the disk. After 15 minutes exposure at 24±3°C, the product was neutralized by flooding with 5.0 mL of neutralizer (bovine serum albumin/HEPES buffer/sodium hydroxide solution). Contents of the Petri plate were transferred to a test tube and vortexed 30-40 seconds. Ten-fold serial dilutions (diluent not specified) were prepared and WI-38 cells were inoculated according to current Hill Top Research SOP 11-DISF-20-0028. The cell plates were incubated at 33±1°C for 3 days ± 4 hours in 5±1 CO_2. The cells were examined for unspecified cytopathic effect. Controls included virus controls, neutralizer effectiveness, and cytotoxicity. Viral and toxicity titers were calculated by the method of Reed and Muench (1938).

2. MRID 457138-12 "Virucidal Efficacy of Facial Tissue For Treated and Untreated Tissue Against Rhinovirus 2, ATCC VR-482" for Kleenex Brand Anti-Viral Tissue #2, by Kathleen A. Baxter. Study conducted at Hill Top Research, Inc. Study completion date – June 13, 2002.

This study was conducted against Rhinovirus 2 (ATCC VR-482) using WI-38 cells (source not identified) as the host system. The study protocol followed a modification of ASTM Method E 1053-97, Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces; a major modification was the use of 1-inch-square "disks" of tissue as the carrier in lieu of glass slides. The carriers were inoculated with undried virus (presence of organic soil load not indicated), and held for the indicated contact time. Three (3) lots of product were tested (Lot Nos. 3-7-02-4A, 3-7-02-4B, 3-7-02-4C (60 Day Stability Sample)) and compared against untreated tissue control disks (Lot No. 3-7-02-4D). Two disks punched out of individual tissue sheets prior to inoculation with test virus were placed side by side in a sterile 60 mm glass Petri plate in a laminar flow hood at 24±3°C. Each disk was inoculated with 100 µL of test virus distributed uniformly in a spiral fashion from the center of the disk. After 15 minutes exposure at 24±3°C, the product was neutralized by flooding with 5.0 mL of neutralizer (bovine serum albumin/HEPES buffer/sodium hydroxide solution). Contents of the Petri plate were transferred to a test tube and vortexed 30-40 seconds. Ten-fold serial dilutions (diluent not specified) were prepared and WI-38 cells were inoculated according to current Hill Top Research SOP 11-DISF-20-0028. The plates were incubated at 33±1°C for 3 days ± 4 hours in 5±1 CO_2. The cells were examined for unspecified cytopathic effect. Controls included virus controls.

Note: The MRID referenced a neutralizer effectiveness study conducted under HTR Study No. 02-120089-106 using the same cell line (i.e., WI-38 cells) and the same product. This HTR study is MRID No. 457138-11 (see above).

3. MRID 457138-13 "Virucidal Efficacy of Facial Tissue For Treated and Untreated Tissue Against Influenza A, ATCC VR-1469" for Kleenex Brand Anti-Viral Tissue #2, by Kathleen A. Baxter. Study conducted at Hill Top Research, Inc. Study completion date – June 12, 2002.

This study was conducted against Influenza A (ATCC VR-1469) using MDCK cells (source not identified) as the host system. The study protocol followed a modification of ASTM Method E 1053-97, Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces; a major modification was the use of 1-inch-square "disks" of tissue as the carrier in lieu of glass slides. The carriers were inoculated with undried virus (presence of organic soil load not indicated), and held for the indicated contact time. Three (3) lots of product were tested (Lot Nos. 3-7-02-4A, 3-7-02-4B, 3-7-02-4C (60 Day Stability Sample)) and compared against untreated tissue control disks (Lot No. 3-7-02-4D). Two disks punched out of individual tissue sheets prior to inoculation with test virus were placed side by side in a sterile 60 mm glass Petri plate in a laminar flow hood at 24±3°C. Each disk was inoculated with 100 µL of test virus distributed uniformly in a spiral fashion from the center of the disk. After 15 minutes exposure at 24±3°C, the product was neutralized by flooding with 5.0 mL of neutralizer (bovine serum albumin/HEPES buffer/sodium hydroxide solution). Contents of the Petri plate were transferred to a test tube and vortexed 30-40 seconds. Ten-fold serial dilutions (diluent not specified) were prepared and MDCK cells were inoculated according to current Hill Top Research SOP 11-DISF-20-0037. The plates were incubated at 33±1°C for 7±1 days in 5±1% CO_2. The cells were examined for unspecified cytopathic effect. After 7±1 days, a hemagglutination assay was conducted. Controls included virus controls, neutralizer effectiveness, and cytotoxicity. Viral and toxicity titers were calculated by the method of Reed and Muench (1938).

4. MRID 457138-14 "Virucidal Efficacy of Facial Tissue For Treated and Untreated Tissue Against Influenza B, CDC ID# 2001701156" for Kleenex Brand Anti-Viral Tissue #2, by Kathleen A. Baxter. Study conducted at Hill Top Research, Inc. Study completion date – June 12, 2002.

This study was conducted against Influenza B (CDC ID# 2001701156) using MDCK cells (source not identified) as the host system. The study protocol followed a modification of ASTM Method E 1053-97, Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces; a major modification was the use of 1-inch-square "disks" of tissue as the carrier in lieu of glass slides. The carriers were inoculated with undried virus (presence of organic soil load not indicated), and held for the indicated contact time. Three (3) lots of product were tested (Lot Nos. 3-7-02-4A, 3-7-02-4B, 3-7-02-4C (60 Day Stability Sample)) and compared against

untreated tissue control disks (Lot No. 3-7-02-4D). Two disks punched out of individual tissue sheets prior to inoculation with test virus were placed side by side in a sterile 60 mm glass Petri plate in a laminar flow hood at 24±3°C. Each disk was inoculated with 100 µL of test virus distributed uniformly in a spiral fashion from the center of the disk. After 15 minutes exposure at 24±3°C, the product was neutralized by flooding with 5.0 mL of neutralizer (bovine serum albumin/HEPES buffer/sodium hydroxide solution). Contents of the Petri plate were transferred to a test tube and vortexed 30-40 seconds. Ten-fold serial dilutions (diluent not specified) were prepared and MDCK cells were inoculated according to current Hill Top Research SOP 11-DISF-20-0038. The plates were incubated at 33°C for 7±1 days in 5±1% CO_2. The cells were examined for unspecified cytopathic effect. After 7±1 days, a hemagglutination assay was conducted. Controls included virus controls.

Note: The MRID referenced a neutralizer effectiveness study conducted under HTR Study No. 02-120048-106 using the same cell line (i.e., MDCK cells) and the same product. This HTR study is MRID No. 457138-13 (see above).

5. MRID 457138-15 "Virucidal Efficacy of Facial Tissue For Treated and Untreated Tissue Against Respiratory Syncytial Virus, ATCC VR-26" for Kleenex Brand Anti-Viral Tissue #2, by Kathleen A. Baxter. Study conducted at Hill Top Research, Inc. Study completion date – June 13, 2002.

This study was conducted against Respiratory Syncytial Virus (ATCC VR-26) using LLMK2 cells (source not identified) as the host system. The study protocol followed a modification of ASTM Method E 1053-97, Standard Test Method for Efficacy of Virucidal Agents Intended for Inanimate Environmental Surfaces; a major modification was the use of 1-inch-square "disks" of tissue as the carrier in lieu of glass slides. The carriers were inoculated with undried virus (presence of organic soil load not indicated), and held for the indicated contact time. Three (3) lots of product were tested (Lot Nos. 3-7-02-4A, 3-7-02-4B, 3-7-02-4C (60 Day Stability Sample)) and compared against untreated tissue control disks (Lot No. 3-7-02-4D). Two disks punched out of individual tissue sheets prior to inoculation with test virus were placed side by side in a sterile 60 mm glass Petri plate in a laminar flow hood at 24±3°C. Each disk was inoculated with 100 µL of test virus distributed uniformly in a spiral fashion from the center of the disk. After 15 minutes exposure at 24±3°C, the product was neutralized by flooding with 5.0 mL of neutralizer (bovine serum albumin/HEPES buffer/sodium hydroxide solution). Contents of the Petri plate were transferred to a test tube and vortexed 30-40 seconds. Ten-fold serial dilutions (diluent not specified) were prepared and LLMK2 cells were inoculated according to current Hill Top Research SOP 11-DISF-20-0041A. The plates were incubated at 37±1°C for 10 days ± 4 hours in 5±1% CO_2. The cells were examined for unspecified cytopathic effect. Controls included virus controls, neutralizer effectiveness, and cytotoxicity. Viral and toxicity titers were calculated by the method of Reed and Muench (1938).

6. MRID 457229-01 "Consumer Survey: Facial Tissue Life Study, Project FACT (Flu and Cold Tissue)," Author, Completion Date, and Performing Laboratory Not Applicable

This study was conducted to obtain information about facial tissues. The applicant is claiming the nature of this study and its results as confidential. This 10-page document describes the study and presents the results. The applicant intends to use the information in developing a marketing strategy for the product, Kleenex Brand Anti-Viral Tissue #2.

V RESULTS

		Reduction of viral titer			
MRID Number	**Organism**	**Lot No. 3-7-02-4A**	**Lot No. 3-7-02-4B**	**Lot No. 3-7-02-4C**	**Cytotoxicity**
457138-11	Rhinovirus 1A	complete inactivation	complete inactivation	complete inactivation	No cytotoxicity observed
	Avg. titer control ($TCID_{50}$/0.1 mL)	4.2 log_{10}	4.2 log_{10}	3.7 log_{10}	
457138-12	Rhinovirus 2	complete inactivation	complete inactivation	complete inactivation	No cytotoxicity data provided
	Avg. titer control ($TCID_{50}$/0.1 mL)	3.6 log_{10}	3.6 log_{10}	3.6 log_{10}	
457138-13	Influenza A	complete inactivation	complete inactivation	complete inactivation	No cytotoxicity observed
	Avg. titer control ($TCID_{50}$/0.1 mL)	4.9 log_{10}	4.9 log_{10}	3.9 log_{10}	
457138-14	Influenza B	complete inactivation	complete inactivation	complete inactivation	No cytotoxicity data provided
	Avg. titer control ($TCID_{50}$/0.1 mL)	4.9 log_{10}	4.9 log_{10}	>4.8 log_{10}	
457138-15	Respiratory Syncytial Virus	complete inactivation	complete inactivation	complete inactivation	No cytotoxicity observed
	Avg. titer control ($TCID_{50}$/0.1 mL)	5.5 log_{10}	5.5 log_{10}	4.9 log_{10}	

VI CONCLUSIONS

1. The submitted efficacy data (MRID Nos. 457138-11 and 457138-13 through -15) appear to support the use of the product, Kleenex Brand Anti-Viral Tissue #2, as a virucide when tested against the following microorganisms for a contact time of 15 minutes:

Influenza A
Influenza B
Respiratory Syncytial Virus
Rhinovirus 1A

Complete inactivation was observed for all dilutions assayed. [The laboratory used a >3-log reduction in titer as the performance standard.] No cytotoxicity was observed in studies against Rhinovirus 1A, Influenza A, or Respiratory Syncytial Virus. Cytotoxicity data were not provided for the study Influenza B (MRID No. 457138-14); rather the MRID referenced cytotoxicity data that had been developed for the same cell line and the same product in support of another study (MRID No. 457138-13). The Agency also notes that there was no specific description of the characteristics of the cytopathic effects produced by each of the test viruses in the cell monolayer – quantitation using cytopathic effect, which can be subjective, is less desirable than objective techniques such as immunofluorescence. Finally, DIS/TSS-7 indicates that the recoverable virus titer must be at least 10^4. One of three product lots tested against Rhinovirus 1A (MRID No. 457138-11) and Influenza A (MRID No. 457138-13) did not meet that quality control requirement. For virucidal efficacy claims, however, only two product lots need be tested.

2. The submitted efficacy data (MRID No. 457138-12) do not appear to support the use of the product, Kleenex Brand Anti-Viral Tissue #2, as a virucide when tested against Rhinovirus 2 for a contact time of 15 minutes. Although complete inactivation was observed for all dilutions assayed, the recoverable virus titer was not at least 10^4 for any of the product lots tested.

3. The Agency reviewed the confidential results of the consumer survey (MRID No. 457229-01) and found no reason to adjust the conclusion presented above.

VII RECOMMENDATIONS

Despite the favorable efficacy study results, the proposed label claims are not currently acceptable regarding the use of the product, Kleenex Brand Anti-Viral Tissue #2, as a virucide against Rhinovirus 1A and 2, Influenza A and B, and Respiratory Syncytial Virus for a contact time of 15 minutes. As noted previously, the study against Rhinovirus 2 did not meet the DIS/TSS-7 requirement that the recoverable virus titer must be at least 10^4. Furthermore, the Agency standards for impregnated towelettes for hard surface disinfection do not apply to this impregnated facial tissue.

- EPA's standards for environmental surfaces are hard, inanimate surfaces, either porous or non-porous. Based on the study methodology, the tissue itself (as opposed to a human face or nose) is the surface of interest. A facial tissue is porous and is also soft.

- The product has not been registered as a disinfectant (i.e., successfully tested against bacteria *Salmonella choleraesuis* and *Staphylococcus aureus*, with or without *Pseudomonas aeruginosa*). Although three product lots (including a 60-day-old lot) were tested in the virucidal studies provided in this data package, the Agency will register a product tested merely as a virucide without having met basic disinfectant claims in DIS/TSS-1.

- An organic soil load was not mentioned in any of the studies. Although viral inoculum is frequently accompanied by some naturally-occurring organic soil (e.g., serum), in actual use, the product would be challenged with 100 percent organic soil load (e.g., nasal mucus, eye discharge, phlegm, and/or sputum). The applicant must provide data about the nature and concentration of the organic soil load challenge (if any) employed in these studies.

- The proposed label claims indicate that the product can be used in hospital settings. A mere virucide can not make that claim. The applicant has not provided efficacy data against *Pseudomonas aeruginosa*, which is an Agency requirement for a hospital disinfectant claim.

- The claim for 15 minutes is questionable. A tissue is usually disposed of in 1 or 2 minutes after use. A more appropriate contact time would be 30 seconds.

- What are the benefits of this product?

PM Note: As noted earlier, directions for use, a requirement for a pesticide, are not clear. Consumers, however, are likely to know how to use a facial tissue. PSB is not certain what should be included in the directions for use, but at a minimum, the probable source of the viral load (e.g., nose blowing) and the surface being treated (the tissue itself) need to be mentioned. In addition, the current instruction "Dispose of used tissues in a normal fashion" might be better phrased as "Dispose of used tissues promptly."

Other proposed label wording issues:

- Other questionable phrases [see page 2 of the label], implying "newness," include: "NOW with" and "Introducing a revolution in facial tissues."

- The PM should review the repeated use of percent kill (99.9%) on the proposed label [see page 2 of the label]; although accurate, this is not the Agency's virucidal performance standard in the absence of cytotoxicity.

- The proposed label repeatedly indicates the product's ability to "kill 99.9% of virus*" [see page 2 of the label]; this is an incomplete phrase and is not acceptable. However, "kill virus* on the used facial tissue in 15 minutes" may be more acceptable.
- Optional terms suggested on the proposed label [see page 3 of the label] include "clinically" testing efficacy; only non-clinical (i.e., no human subject) efficacy study reports were provided with this data package.