Antimicrobial Program Branch
EFFICACY REVIEW-EETMS Section

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EPA Reg. No. or File Symbol # 10492-4

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Type Product (s) Hospital Disinfectant(Saturated Towellete)

Data MRID NO. 414914-02, 414914-01, 415215-02, 415215-01,

Product Manager 31

Prod.Name Isotex 70 disinfecting Towelettes

Company Name Palmero Sales Company, Inc.,

Submission Purpose An amendment to add claims against S.aureus, S. choleraesuis, Ps. aeruginosa, T. mentagrophyes, M. tuberculosis, HIV-1(AIDS Virus), Poliovirus-Type 1, and Herpes simplex Type II.

Chemical & Formulation Single-use disposable towelette saturated with ready-to-use liquid enclosed in a dispenser

Active Ingredients	%
n-Alkyl (C14, 60%; C16, 30%; C12, 5%; C18, 5%) dimethyl benzyl ammonium chlorides	0.12
n-alkyl (C12, 50%; C14, 30%; C16, 17%, C18, 3%) dimethyl ethylbenzyl ammonium chlorides	0.12
Isopropyl alcohol	63.00

09/04/91

Reviewed by: Blue H. Wan

Signature 9/6/91

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200.0 Introduction

200.1 Use(s)

Refer to the subject product proposed label "Isotex 70 Disinfecting Towellete".

200.2 Background Information

The current submission received 07-11-91 is an amendment which in include efficacy data generated against S. auresus, S. choleraesuis, Ps. aeruginosa, T.mentagrophytes, M. tuberculosis, HIV-1(AIDS virus), Poliovirus Type 1 and Herpes simplex Type II in support of the product proposed label claims. A proposed label was also included with the proposed label claims.

201.0 Data Summary(MRID Nos.) 414914-02, 414914-01, 415215-02, and 415215-01.

201.2 Brief Description of Tests

A. Bactericidal Studies, Shaldra Biotest Inc, W. Bethesda, MD, by Robin A. Smith & Kyle Sibinovich.
The Data for Required Test Organisms developed against Ps. aeruginosa, S. aureus and S. choleraesuis by the Official Methods of the AOAC, 14th ed., 1984. (MRID #414914-01)

B. Fungicidal Tests, Shaldra Biotest, Inc, W. Bethesda, MD, by Robin A. Smith, data developed by the AOAC Fungicidal Test, 14th Ed., 1984, against Tri. mentagrophytes (MRID # 414914-01).

C. Tuberculocidal Test(Quanitative Logarithmic Reduction TB Test). Shaldra Biotest, Inc. W. Bethesda, MD 20817.
Official Methods of the AOAC, chapter 4, 15th ed., 1990 (Basic Data Reports)(MRID #414914-01). Tester: Robin Smith.

D. Tuberculocidal Test(Quantitative Log., Reduction Method). Reports performed by Nelson Laboratories, Inc, 4535 South 2300, Salt Lake City, UT 84117. (MRID #415215-01).
(Validation Data). Reports by Dennis Ransom & Jerry R. Nelson.

E. Virucidal Test (AIDS Virus) USEPA Pesticide Assessment Guidelines (Sub-Div. G, Product Performance, 1982, Section 91-30, pp 72-76). Testing performed by Southern Research Institute, Birmingham, AL 35255, SRI Project #7042. (MRID # 415215-02). Reports by: Bonnie J. Bowdon.

F. Virucidal Tests(EPA Test Method, H. simplex II & Poliovirus I) Reports by Intregrity Bioservices, Rockville, MD 20852, and followed the guidelines specified in DIS/TSS-7, Nov., 12, 1981. (MRID # 414914-02) Reports by: Philip R. Roane.

201.2 Test Summaries:

A. Disinfection(Microbiological Reports):

1. Method: AOAC Use-Dilution Test, 14th ed., 1984
2. Modifications: Tested with a 5% horse serum(DIS/TSS-2, item 4)

3. Samples:
Efficacy studies were reported to be conducted on the same product whether identified as Isotex 70, as Ideal Disinfecting Towelette, and/or as Decide. All samples were reported to be manufactured on the same day and reported to have the same lot number.

	Mfd date	Test date	Completion Date
Decide (A199-1)	01-19-90	02-12-90	02-14-90
Decide (B280-1)	02-28-90	03-07-90	03-09-90
Decide (D139-1)	04-13-89	02-14-90	02-16-90

4. Dilution Tested: Tested undiluted (as provided by the mfd.).

5. Test Bacteria:
Pseudomonas aeruginosa (ATCC 15442)(Phen. Res=1:90)
Staphylococcus aureus (ATCC 6538)(Phen. Res=1:60)
Salmonella choleraesuis (ATCC 10708) (Phen. Res=1:90)

6. Exposure Time: Four(4) minutes; Exposure Temp. = 20°C.
Neutralizer Time: 10 miuntes at 20°C.
Types of Carriers: Stainless Steel Penicylinders(SS)

7. Subculture Medium: Synthetic broth(as per chapter 4, Section 4.001)
The synthetic broth is not an acceptable subculture medium.

8. Incubation: 48 hours at 37°C.

9. Results:

Test Organism	Test Sample	Exposed	No. of Pos./Tested Pri	Sec	Avg CFU Recovered per cylinder
Ps. aeruginosa	A199-1	60	0/60	(no data)	(no data)
" "	D139-1	60	0/60	(no data)	(no data)
" "	B280-1	60	0/60	(no data)	(no data)
S. choleraesuis	A199-1	60	0/60	(no data)	(no data)
" "	D139-1	60	0/60	(no data)	(no data)
" "	B280-1	60	0/60	(no data)	(no data)
S. aureus	A199-1	60	0/60	(no data)	(no data)
" "	D139-1	60	0/60	(no data)	(no data)
" "	B280-1	60	0/60	(no data)	(no data)

*

Neutralization data:
No data were submitted to support the residual effects of the active ingredient as per item 7 of DIS/TSS-2 enclosure.
Control survival studies:
No data were submitted to support the recovery of surviving organisms after drying as specified in DIS/TSS-2, item #4 & #6.

10. Conclusions: Unsatisfactory performance for all three test organisms in the presence of 5% horse serum; no carrier drying conditions or times submitted (this information is critical to determine efficacy level); also the incorrect subculture medium was utilized. The applicant needs to provide secondary cultures as apecified in item 7 of DIS/TSS-2 enclosure and control survival studies as indicated in items 4 & 6 of DIS/TSS-2. Also, the generated data must utilize the applicable subculture media as listed in the Official Methods of Analysis of the AOAC(14th or 15th editions).

B. Disinfection: (Pathogenic fungi)
Shaldra Biotest, Inc. By Robin A.Smith.
1. Method: AOAC Fungicidal Test, 14th ed., 1984.
2. Modifications: Tested in 5% horse serum as per DIS/TSS-2, item 4.
3. Dilution tested: Undiluted(not diluted).
4. Samples: Decide (A199-1) & Decide (D139-1).
5. Test Organism: Trichophyton mentagrophytes, ATCC 9533,
(Phenol Resistance =1:70 at 10 min. at 20°C...
... Phenol Sensitivity=1:60 at 10 min. at 20°C.)
6. Subculture Medium: Glucose Broth 2% (as per chapter 4, Section 4.019).
Type of Carriers: Stainless Steel Penicylinders(SS).
7. Exposure: 4 minutes at 20°C. see next page
8. Neutralizer: Letheen Broth with Tween 80
9. Incubation: 10 days at 25°C to 30°C.
10. Results

Organism	Test Sample	No. Exposed	No growth (0)/Growth(+) at 10 min. with 5% horse serum
Tri. mentagrophytes	A199-1		
	(Subculture 1)	10	0/10
	(Subculture 2)	10	0/10
Tri. mentagrophytes	D139-1		
	(Subculture 1)	10	0/10
	(Subculture 2)	10	0/10

	Exposed	No showed growth
Viabilty control in neutralizer	2	2/2
" " "		

Phenol:	5 min*	10 min.*	15 min*
1:60	5/5	0/5	0/5
1:70	5/5	4/5	3/5

*Number of carriers showing growth/Total Number of Carriers.
Conidia count: 6.0×10^6/ml.

11. Conclusions: Raw data need to be submitted for review to determine satisfactory performance for the undiluted test product as a fungicide against Trichophyton mentagrophytes for a 4 minute exposure time.

C. Disinfection(Tuberculocidal Tests):

(Basic Tuberculocidal Data).
(MRID 414914-01) Shaldra Biotest, Inc., By Kyle H. Sibinovic.

1. Method: AOAC Use-Dilution Test Method, 15th ed., 1990.
(EPA Logarithmic Reduction Method)
The Quantiative Logarithmic Reduction Test Method was used to generate the tuberculocidal data and to determine product effectiveness as a tuberculocide.
2. Modifications: None reported.
3. Test Samples:

	mfd	test date
Decide A199,	01-19-89	03-09-90
Decide D139,	04-13-89	03-09-90

4. Dilution: product tested undiluted.
5. Exposure time: 0.50, 1.0, 1.5, 2.0 and 3.0 minutes.
6. Test Organism: Mycobacterium bovis ATCC 35743 (1028); see the results under attachment #1 for phenol resistance.
7. Test carriers: None, a bacterial test suspension was used.
8. Subculture Media/Neutralizer: Middlebrook 7H11 Agar.
Neutralizer: Letheen broth with Tween 80
9. Incubation: 21 days at 37^0C.
10. Exposure Temp: 20^0C.
11. Results:

Test Sample	Time (minutes)	Dilution Tested 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}
Decide (A199)	0.5	TNTC*	64**	7	Not done	Not done(ND)
"	1.0	36	1	0	ND	ND
"	1.5	3	0	0	ND	ND
"	2.0	0	0	0	ND	ND
"	3.0	0	0	0	ND	ND
(D139)	0.5	TNTC	34	4	Not done(ND)	ND
"	1.0	69	2	0	ND	ND
"	1.5	10	0	0	ND	ND
"	2.0	0	0	0	ND	ND
"	3.0	0	0	0	ND	ND

* TNTC= Too numerous to count
** Counts are average of duplicates:

Positive Control	10^{-1},	10^{-2},	10^{-3},	10^{-4},	10^{-5},	10^{-6}
			TNTC	TNTC	36	7
Static Control	10^{-1},	10^{-2},	10^{-3},	10^{-4},	10^{-5},	10^{-6}
			TNTC	52	2	0

		Time in Minutes		
		10	20	30
	10^{-4}	TNTC	TNTC	53/48
Phenol	10^{-5}	62/51	22/17	7/4
Control	10^{-6}	7/4	3/1	1/0
CFU/ml		$5.5x\ 10^{-6}$	$2.0x10^{-6}$	$5.5x10^{-5}$

Phenol control appear to meet the criteria of no less 0.5 $\log_{10}$ and no more than 1.0 $\log_{10}$ kill or reduction in 20 minutes.

12. Conclusions: The submitted tuberculocidal data developed by the logarithmic quantitative reduction method appear to show adequate performance for an exposure period of 5 minutes at 20^0C for 2 batches of product; however, the tuberculocidal data reports are deficient. The submitted data are refective only of one study per exposed test period. The reports are not reflective of an average of 4 separate independent studies for each exposed test period and for each test lot as per protocol requirements. This deficiency makes the conclusions invalid. In addition, it is not clear what neutralizer was used for the test. The neutralizer used as per the submitted protocol (2% sodium bisulfite) is inappropriate. Also, the submitted; summary data are not sufficient for review.

D. Disinfection(Tuberculocidal Tests):
(Validation data).
(MRID 415215-01) Nelson Labs., Performed by Dennis Ransom.

1. Method: AOAC Use-Dilution Test Method, data were generated by the logarithmic reduction test method.
2. Modifications: None reported.
3. Test samples: Lot #119-91(Ideal disinfecting towelletes) (60 day sample).
   Lot #510-91(Isotex 70 disinfecting towelletes)
4. Exposure time: 0.5, 1.0, 1.5, and 2.0 minutes.
5. Dilution tested: Product tested undiluted
6. Test organism: Mycobacterium bovis BCG.
7. Test carriers: None, bacterial suspension was used.
8. Subculture Media/Neutralizer: Letheen Broth
9. Exposure time: 20^0C
10. Results:

Test Samples	Time (minutes)	Dilution Tested (tested undiluted) Colony Forming Units(CFUs) (Duplicate 1)	(Duplicate 2)
Lot #510-91	0.50	0	0
"	1.00	*	0
"	1.50	0	0
"	2.00	0	0

*Contaminated

b

		(Duplicate 1)	(Duplicate 2)
Lot #119-91	0.50	0	0
"	1.00	0	0
"	1.50	0	0
"	2.00	0	0

Initial Titer: Organism is 9.27 x 10^6 cfu/ml

**Too numerous to count.

	Plate Count Data 10^4,	10^{-5},	10^{-6}
----	TNTC**	85	13
----	TNTC	95	8
----	TNTC	98	9

Phenol Exposure:

10^4,	10^5,	10^6
115,	12,	0
110,	10,	0
118,	5,	0

Average = 1.14 x 10^6 cfu/ml
Log_{10} reduction = 0.91 of initial titer.

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12. Conclusions: The submitted validation data appear to be adequate and are supportive of the product effectiveness as a tuberculocide against M. tuberculosis when used the product is used undiluted; however, the acceptance is pending the clarification or submission of acceptable basic tuberculocidal efficacy data for an efficacy determination.

E. Disinfection: (Viruses)

1. Method: Virucidal Tests: Tests were conducted in accordance with the virucidal requirements specified in DIS/TSS-7 enclosure and EPA Modified(AOAC), dated 12 November 1981.
2. Modification: Tested in the presence with 10% FCS.
3. Test samples: Isotex (A189-1) and Isotex(A199-2).
4. Exposure time: 3 minutes at 20°C.
5. Virus: Herpes simplex Type 2
6. Cell line & hosts: Propagation cell line/media: Hep II/EME 90% & 10% FBS. Recovery cell line/media: Vero/EMEM 98% + 2% FBS.
7. Type of carrier: 60 mm petri dishes
8. Neutralizer: Letheen broth
9. Incubation time for virus: 7 days at 37°C.

General Test Procedure: Virus coated petri dishes were dried at 2 hrs. at 20°C. After drying, the petri dishes were treated with germicide solution, then exposured time, then with the neutralizer solution, and followed by incubating an aliqiuot of the mixture on the applicable cell line for evidence of CPE.
Virucidal activity was enumerated/determined by the cytopathic effect(CPE) produced in cell culture. All reports included virus-disinfectant control, cell control, toxicity control, and virus-neutralization control.

E. Disinfection -Poliovirus Type 1-

10. Results:

	Herpes simplex 2 (A189-1)	Herpes simplex 2 (A199-2)
Virus + disinfectant $TCID_{50}$	< 3.50	3.50
Virus control, $TCID_{50}$	> 7.50	> 7.50
Virus toxicity, $TTLD_{50}$	3.50	3.50
Log. reduction in titer	> 4.00	> 4.00

11. Satisfactory performance versus Herpes simplex Type 2 in the presence of 10% FCS for an exposure time of 3 minutes.

Except for the cell lines used to propagate and recover poliovirus type 1, the remaining test conditions are the same as indicated on the previous page for H. simplex 2.

For the sake of brevity, the same conditions, materials, methods, etc... will not be listed for Poliovirus Type 1.

The EPA Virucidal Test Method as per DIS/TSS-7 enclosure was used to generate the data for (Poliovirus Type 1)

(a) Propagating cell line utilized was BGM/EMEM 90% & FBS 10%, and the recovery cell line was BGM/EMEM 90% & 2% FCS.

(b) Virus: Poliovirus Type 1 (Brunhilde-VR-ATCC 58).

(c) Sample: Isotex (A189-1) and Isotex (A199-2).

(d) For "Materials", "Methods", "Test Procedure", refer to the comments made for Herpes simplex Type 2 under 201.2-Test Summary- Item E.

(e) Results:

	A189-1	(A199-2)
Virus + disinfectant, $TCID_{50}$	$\leq$ 3.50	$\leq$ 3.50
Virus control, $TCID_{50}$	$\geq$ 7.50	7.50
Virus toxicity, $TLTD_{50}$	3.50	3.50
Log* reduction in titer	4.00	4.00

(f) Conclusions: The tests for herpes and polio viruses are inadequate because they were not inspected by a Quality Assurance unit and: the complete test procedure and data were not submitted; the volume placed on carrier (0.5 ml) should be 0.2 ml in order to achieve adequate drying, and the drying conditions (2 hr at 20°C) are not in compliance with the approved protocol.

F.

201.1 Test Summary(cont'd)

201.1 Disinfection(AIDS virus)(MRID 415215-02):

"Virucidal Efficacy for Palmero Sales Company's Isotex-70 " Project No. 7042 by Bonnie J. Bowdon, performing laboratory is Southern Research Institute, Birmingham, AL 35255. The report was initiated on 03-29-90 and completed on April 5, 1990.

201.2 Test Summary

1. Method: EPA product Performance Guidelines and the specifics in "Southern Research Institute Test Protocol approved by EETMS Efficacy, APB, RD, dated 08-15-89.

2. Test Virus: Human immunodeficiency virus Type 1 ($HTLV\text{-}III_{RF}$). Modifications: Tested in the presence of 5% serum.

3. Virus Inoculum & Drying Procedure: Two-tenths ml of virus pool in RPMI-1640, containing 5% heat-inactivated fetal bovine serum, was dried on the bottom surface of glass petri dishes (28 cm^2 area) at 23^0C until visibly (approx. 45 minutes), and then incubated at $35\text{-}37^0C$ in a dry-air oven for an additional 30 minutes.

4. Exposure: 2.0 ml of disinfectant was applied to dried virus film for a total exposure period of 1.0 minute at approximate (23^0C).

5. Test samples: Batch # A199-1 and # D139-1.

6. Use Dilution: Not diluted(undiluted).

7. Preparation of virus film, treatment of virus film with disinfectant, treatment of virus control film, cytotoxicity control, and cell control measures are indicated in attachment #1.

8. Refer to infectivity assays under the attached materials/methods and note attachment #1.

   Host Infection & Virus Assay:

   MT2 T-cells were indicator cells for the infectivity assay. The MT2 cells were treated with polybrene(2 ug/ml) for 30 minutes at 37^0C, collected by centrafugation and plated in 96-well culture plates at approximately 1 x 10^4 cells per well in 0.15ml per well in 0.15ml of medium, inoculated and incubated for 7 7 days at 37^0C for virus infection.

   Primary detection of virus was scored by lytic cytopathic effect (CPE) after 7 days of incubation at 37^0C.

9. Results:

9

Test Sample	Disinfectant Temperature	Exposure Time	Organic Soil	Hard Water	ID-50/LD50 (-Log 10) A199-1	D139-1
Virus Control	NA	NA	5% Serum	NA	7.00	7.00
Non-virucidal level of Disinfectant	23°C (RT)	1 min.	5% serum	NA	5.70	6.00
Virus + Disinfectant	NA	1 min.	5% serum	NA	$\leq$3.5	$\leq$ 3.5
Toxicity Control ...	NA	NA	NA	NA	3.5	3.5
Log Reduction	23°C	1.0 Minutes	5% serum	NA	$\geq$ 3.50	$\geq$ 3.50

NA= Not applicable.

11. Conclusions: The data meet the requirements for demonstrating virucidal efficacy of the product against HIV-1 in the presence of a moderate amount of 5% fetal (bovine serum) when used undiluted for a contact contact time of 1 minute at room temperature (23°C).