Summary of Test Data for 1.75% Iodine Disinfectant/Sanitizer Premise Disinfectant

Disinfectant Testing for Bacteria

Organism:	Staphylococcus aureus ATCC No. 6538
Test Method:	Use Dilution
Dilution:	1:213 (3 oz to 5 gal) 75 ppm iodine
Soil Load:	5% serum
Water:	400 ppm AOAC hard water
Temperature:	20°C
Exposure:	10 minutes
Results:	
Carriers exposed	Number of carriers showing growth

<u>Carriers exposed</u>	<u>Number of carriers showing growth</u>		
	Primary	Secondary	
120	0	0	

Phenol Resistance of Organism:

Exposure time

Phenol Dilution	<u>5 minutes</u>	<u>10 minutes</u>	<u>15 minutes</u>
1:60	+	-	-
1:70	+	+	+

Organism:	Salmonella chloraesuis ATCC No. 10708
Test Method:	Use Dilution
Dilution:	1:213 (3 oz to 5 gal) 75 ppm iodine
Soil Load:	5% serum
Water:	400 ppm AOAC hard water
Temperature:	20°C
Exposure:	10 minutes
Results:	
Carriers exposed	Number of carriers showing growth
	Primary <u>Secondary</u>
120	0 0

Phenol Resistance of Organism:

Exposure Time

Phenol Dilution	<u>5 minutes</u>	<u>10 minutes</u>	<u>15 minutes</u>
1:90	+	-	-
1:100	+	+	-

Disinfectant Testing for Bacteria 1.75%, continued

Organism:	Pseudomonas aeruginosa ATCC No. 15442		
Test Method:	Use Dilution		
Dilution:	1:213 (3 oz to 5 gal) 75 ppm iodine		
Soil Load:	5% serum		
Water:	400 ppm AOAC hard water		
Temperature:	20°C		
Exposure:	10 minutes		
Carriers exposed	Number of carriers showing growth		
	<u>Primary</u> <u>Secondary</u>		
240	0 0		

Phenol Resistance of Organism:

Exposure time

Phenol dilution	<u>5 minutes</u>	<u>10 minutes</u>	<u>15 minutes</u>
1:80	+	+	-
1:90	+	+	+

Organism:	Escherichia coli ATCC 8739
Test Method:	Use Dilution
Dilution:	1:213 (3 oz to 5 gal) 75 ppm iodine
Soil Load:	5% serum
Water:	400 ppm AOAC hard water
Temperature:	20°C
Results:	
<u>Carriers</u> 20	<u>Treated Number Showing Growth</u> 0
20	Neutralization Challenge Showing Growth 20

Disinfectant Testing for Bacteria 1.75%, continued

Organism:	<i>Escherichia coli</i> , 0157:H7 Strain (hemorrhagic)
Test Method:	Modified Germicidal and Detergent Sanitizer Test
Dilution:	1:213 (3 ounces to 5 gallons)
Soil Load:	5% serum
Water:	400 ppm AOAC synthetic hard water
Temperature:	25°C
Exposure:	15, 30, 60 seconds
Results:	

Percent Kill After

15 seconds	<u>30 seconds</u>	60 seconds*	<u>Results</u>
>99.9999%	>99.999%	>99.999%	Pass

*Calculated as percent reduction over numerical control

Sanitizer Testing for Bacteria 1.75%

Organism: Test Method:	<i>Staphylococcus aureus</i> ATCC No. 6538 ''Efficacy of Sanitizers Recommended for Inanimate Non-Food Contact Surfaces''
Dilution:	1:640 (1 oz to 5 gal) 25 ppm iodine
Water:	400 ppm AOAC hard water
Temperature:	5°C
Exposure Time	% Kill After Exposure
5 minutes	>99.999
Organism:	<i>Salmonella typhi</i> ATCC 6539
Test Method:	''AOAC Available Chlorine Equivalent Concentration Test''
Dilution:	1:640 (1 oz to 5 gal) 25 ppm iodine
Water:	400 ppm AOAC hard water
Temperature:	20°C
	Chlorine Equivalency >200 ppm

The Chlorine Equivalency test is a relative comparison of the test product to various concentration of chlorine.

Organism:	Listeria monocyt	ogenes ATCC 7644	
Test Method:	"Available Chlorine in Disinfectant: Germicidal Equivalent		
	Concentration T	'est''	
Dilution:	1:640 (1 oz to 5 g	gal) 25 ppm iodine	
Water:	Deionized		
Temperature:	20°C		
Control:			
	Dilution		
NaOCl	200 ppm	5 of 10 lots with growth	
	100 ppm	8 of 10 lots with growth	
	50 ppm	8 of 10 lots with growth	
Test Product:			
	4 (40)		
	1:640 ppm	0 of 10 lots with growth	
	1:640 ppm	0 of 10 lots with growth	

The Chlorine Equivalency test is a relative comparison of the test product to various concentration of chlorine.

Disinfectant Testing for Viruses 1.75%

Organism: Test Method: Dilution: Water: Temperature: Exposure: Soil Lood:	Polio 1 virus Virucide Assay, EPA method 1:213 (3 oz to 5 gal) 75 ppm iodine 400 ppm AOAC hard water 20°C 10 minutes
Soil Load:	5% serum
Results:	No virus recovered with at least a 3 log decline. TCID ₅₀ Virus Control $\geq 10^{-6.5}$ Change in Log ≥ 4.0 TCID ₅₀ Virus Disinfectant $\leq 10^{-2.5}$ % inactivation >99.99
Organism:	Vaccinia virus

Test Method:
Dilution:
Water:
Temperature:
Exposure:
Soil Load:

Results:

Vaccinia virus	
Virucide Assay, EPA method	
1:213 (3 oz to 5 gal) 75 ppm iodine	
400 ppm AOAC hard water	
20°C	
10 minutes	
5% serum	

No virus recovered with at least a 3 log decline. TCID₅₀ Virus Control $\geq 10^{-6.5}$ Change in Log ≥ 3.0 TCID₅₀ Virus Disinfectant $\leq 10^{-3.5}$ % inactivation >99.99

Organism:

Herpes Simplex Type 1 virus

Test Method:	Virucide Assay, EPA method			
Dilution:	1:213 (3 oz to 5 gal) 75 ppm iodine			
Water:	400 ppm AOAC hard water			
Temperature:	20°C			
Exposure:	10 minutes			
Soil Load:	5% serum			
Results:	No virus recovered with at least a 3 log decline.			
	TCID ₅₀ Virus Control $\geq 10^{-6.5}$ Change in Log ≥ 3.0			
	TCID ₅₀ Virus Disinfectant $\leq 10^{-3.5}$ % inactivation >99.99			

Disinfectant Testing for viruses, 1.75%, continued

Organism: Test Method: Dilution Water: Organic Soil: Cell Cultures:	HIV 1 (the virus associated with AIDS) EPA Carrier Method via Good Laboratory Practices 1:213 (3 oz to 5 gal) 75 ppm iodine Tap water 10% Fetal Calf Serum H-9
Cell method:	Dilute in 10 fold increments in RPMI [*] medium (no other suitable diluent) containing 20% calf serum. <u>Note</u> : The serum serves as a non-specific ''quencher'' or ''neutralizer'' of cytotoxicity without itself being injurious to the host cells.
	Administer the diluted disinfectant to host cell cultures at a ration of 1:10 at the frequency of 4 cultures per dilution.
	Observe cultures daily for the above signs and calculate the TCLD $_{50}$ by the Reed and Muench.

*Roswell Park Medical Institute

Test Method: The **HIV 1** pool containing 10% serum prepared as per test methodology was dried on the surface of a glass petri dish. The test disinfectant was then applied following label directions.

Results:

Dilution of virus from surface into host	Virus disinfectant recovery from treated surface	Virus control titration from untreated slide	Tox control disinfectant w/o virus into host
10 ¹	1234	1 2 3 4	1 2 3 4
10 ²	0000	+ + + +	0000
10 ³	0000	+ + + +	0000
10 ⁴	0000	+ + + +	0000
10 ⁵		+ + + +	
106		0 0 0 0	
	<i>TCID</i> ₅₀ <u><</u> 10 ^{-1.0}	TCID ₅₀ =10 ^{-5.5}	TCID ₅₀ ≤10 ^{-1.0}

Conclusion:

 $\begin{array}{ll} \text{ID}_{50} \text{ Virus Control} & 10^{-5.5} \text{ Change in } \text{Log} \geq 4.5 \\ \text{ID}_{50} \text{ Virus-disinfectant} & \leq 10^{-1.0} \text{ Change in } \text{Log} \geq 99.9\% \end{array}$

Product passes test.

Disinfectant Testing for viruses 1.75%, continued

Organism:	Influenza A ₂ J305
Test Method:	Virucide Assay, EPA method
Dilution:	1:213 (3 oz to 5 gal) 75 ppm iodine
Water:	400 ppm AOAC hard water
Temperature:	20°C
Exposure:	10 minutes
Soil Load:	5% serum
Results:	No virus recovered with at least a 3 log decline. EID ₅₀ Virus Control $\geq 10^{-6.5}$ Change in Log ≥ 5.5 EID ₅₀ Virus Disinfectant $\leq 10^{-1}$ % inactivation >99.99
Organism:	Canine Parvovirus
Test Method:	Virucide Assay, EPA method
Dilution:	1:213 (3 oz to 5 gal) 75 ppm iodine
Water:	400 ppm AOAC hard water
Temperature:	20°C
Exposure:	120 minutes
Soil Load:	5% serum
Results:	No virus recovered with at least a 3 log decline. TCID ₅₀ Virus Control = $10^{-5.0}$ Change in Log = 3.5 TCID ₅₀ Virus Disinfectant = $10^{-1.5}$ % inactivation >99.99

Disinfectant Testing for Fungus 1.75%

Organism:	Trichophyton mentagrophytes
Test Method:	Pathogenic fungi, alternative test, DIS/TSS-6
Dilution:	1:213 (3 oz to 5 gal) 75 ppm iodine
Water:	400 ppm AOAC hard water
Temperature:	20°C
Exposure:	10 minutes
Soil Load:	5% serum
Results:	
Carriers exposed	Number of carriers showing growth

Carriers exposed	Number of carriers showing growth		
	<u>Primary</u>	Secondary	
20	0	0	

Phenol Resistance of Organism

Exposure time

Phenol dilution	<u>5 minutes</u>	<u>10 minutes</u>	<u>15 minutes</u>
1:60	+	+	-
1:70	+	+	-

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Jean Richard VP and General manager West Penetone Inc. 10900 Secant Street Ville d'Anjou Quebec H1J 1S5

November 8, 2004

Dear Mr. Richard,

Enclosed find my final report regarding the trial I conducted at the University of Minnesota Swine Disease Eradication Center research farm on the efficacy of Premise Disinfectant against PRRSV-contaminated surfaces and transport vehicles.

In my opinion, when used properly, Premise Disinfectant is a highly efficacious disinfectant against PRRSV-contaminated surfaces and trailers. I encountered no negative effects of the product during handling or following exposure to the skin. I would highly support its use to disinfect PRRSV-contaminated fomites and premises found on swine farms. To insure the proper effect, it is important (as in all cases of disinfectant use) that users pay close attention to concentration, temperature and contact time.

I thank you for the opportunity to work with your company.

Sincerely

Scott Dee DVM MS PhD Dipl; ACVM Associate Professor

PRRSV: Premise Disinfectant summary

Objective 1: Surface testing

The goal of this experiment was to compare the efficacy of 2 different concentrations of Premise Disinfectant against PRRSV across a variety of farm-related surfaces and temperatures.

Variables

Surfaces: pieces of wood, plastic, concrete and metal Temperatures: -20°C, 10°C, 20°C and 30°C Concentrations: 75 ppm and 150 ppm

Procedure

- 1. Various surfaces were inoculated with 5 x 10⁵ TCID₅₀ PRRSV isolate MN-30100
- 2. Surfaces were stored at designated temperatures.
- 3. Premise Disinfectant was applied using low pressure foamer.¹
- 4. Swabs were collected at 0, 30, 60, and 120 minutes post-treatment.
- 5. Swabs were frozen at -20° C immediately post-collection.
- 6. Swabs were tested for the presence of PRRSV RNA by PCR.

^{1:} At -20^oC, all disinfectants were diluted in 40% methanol instead of water to control freezing. Dilution rates remained the same.

Controls

Synergize control (1:128 dilution) Virus control (stock virus, no disinfectant applied Negative control (virus-free cell culture fluid)

Conclusions

Effect of temperature: Premise Disinfectant performed best between at -20° C to 20° C. **Effect of concentration:** The efficacy of the product against PRRSV is enhanced at the higher (150 ppm) concentration versus the lower concentration (75 ppm).

Effect of surface: Premise Disinfectant was effective across all surfaces between -20° C to 20° C

Effect of time: Premise Disinfectant was most effective following a minimum contact period of 120 minutes.

Summary: At a 150 ppm concentration and with a 120 minute contact time, the efficacy of Premise Disinfectant was equal to that of Synergize at temperatures ranging from -20° C to 20° C across all surfaces.

Objective 2: Trailer testing

The purpose of this experiment was to assess the efficacy of 2 different concentrations of Premise Disinfectant in PRRSV-contaminated scale model trailers.

Variables

Time: 0, 30, 60 and 120 minutes post-inoculation **Temperature:** 10^oC and 20^oC **Concentration:** 75 ppm and 150 ppm

Procedure

- 1. Trailer models were inoculated with 5 x 10⁵ TCID₅₀ PRRSV isolate MN-30100
- 2. Trailers were housed at 10° C or 20° C.
- 3. Trailers were washed using cold $(20^{\circ}C)$ water for 72 seconds
- 4. Premise Disinfectant was applied using low pressure foamer.
- 5. Swabs were collected at 0, 30, 60 and 120 minutes post-treatment.
- 6. Swabs were frozen at -20° C immediately post-collection.
- 7. Swabs were tested for the presence of PRRSV RNA by PCR.
- 8. A total of 10 replicates were conducted.

Controls

Synergize control (1:128 dilution) Virus control (stock virus, no disinfectant applied Negative control (virus-free cell culture fluid) Drying control (PRRSV-positive trailers allowed to dry for 8 hours)

Summary

At a 150 ppm concentration and with a 120 minute contact time, the efficacy of Premise Disinfectant against PRRSV in model trailers was equal to that of Synergize when trailers were housed at 10^{9} C.

At a 150 ppm concentration and with a 60 minute contact time, the efficacy of Premise Disinfectant against PRRSV in model trailers was equal to that of Synergize when trailers were housed at 20° C.

PREMISE DISINFECTANT RAW DATA

OBJECTIVE 1: SURFACE TRIALS

30 ° C				
WOOD	<u>o min</u>	30 MIN	60 MIN	120 MIN
150 ppm	POS	POS	POS	POS
75 ppm	POS	POS	POS	POS
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG	NEG	NEG	NEG
PLASTIC				
150 ppm	POS	POS	POS	POS
75 ppm	POS	POS	POS	POS
synergize	POS	POS	POS	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG	NEG	NEG	NEG
CONCRT				
150 ppm	POS	NEG	NEG	NEG
75 ppm	POS	NEG	NEG	NEG
synergize	POS	NEG	NEG	NEG
virus ctri	POS	POS	POS	POS
neg ctrl	NEG	NEG	NEG	NEG
METAL				
150 ppm	POS	POS	POS	POS
75 ppm	POS	POS	POS	POS
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG '	NEG	NEG	NEG
-				
10 º C				
WOOD	0 MIN	30 MIN	<u>60 MIN</u>	<u>120 MIN</u>
150 ppm	POS	POS	NEG	NEG

10000	<u>o mina</u>	<u>oo muu</u>	00 11114	
150 ppm	POS	POS	NEG	NEG
75 ppm	POS	POS	POS	NEG
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctri	NEG	NEG .	NEG	NEG
PLASTIC				
150 ppm	POS	POS	POS	NEG
75 ppm	POS	POS	POS	POS
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctri	NEG	NEG	NEG	NEG
9820				

CONCRT				
150 ppm	POS	POS	NEG	NEG
75 ppm	POS	POS	POS	NEG
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG	NEG	NEG	NEG
METAL				
150 ppm	POS	POS	POS	NEG
75 ppm	POS	POS	POS	POS
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG	NEG	NEG	NEG

OBJECTIVE 2: TRAILER TRIALS

10 ° C					
TRAILER	0 MIN	30 MIN	60 MIN	120 MIN	<u>8 HR</u>
150 ppm	10/10 pos	10/10 pos	4/10 pos	0/10 pos	NT
75 ppm	10/10 pos	10/10 pos	8/10 pos	0/10 pos	NT
synergize	10/10 pos	6/10 pos	0/10 pos	0/10 pos	NT
dry	10/10 pos	NT	NT	0/10 pos	0/10 pos
virus ctrl	10/10 pos	10/10 pos	10/10 pos	10/10 pos	NT
neg ctrl	0/10 pos	0/10 pos	0/10 pos	0/10 pos	NT

20	0	С
20	u	С

20 0						
TRAILER	<u>0 MIN</u>	<u>30 MIN</u>	60 MIN	120 MIN	<u>8 HR</u>	
150 ppm	10/10 pos	6/10 pos	0/10 pos	0/10 pos	NT	
75 ppm	10/10 pos	8/10 pos	5/10 pos	2/10 pos	NT	
synergize	10/10 pos	3/10 pos	0/10 pos	0/10 pos	NT	
dry	10/10 pos	NT	NT	0/10 pos	0/10 pos	
virus ctrl	10/10 pos	10/10 pos	10/10 pos	10/10 pos	NT	
neg ctrl	0/10 pos	0/10 pos	0/10 pós	0/10 pos	NT	
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20 ⁰ C				
WOOD	0 MIN	30 MIN	60 MIN	120 MIN
150 ppm	POS	NEG	NEG	NEG
75 ppm	POS	NEG	NEG	NEG
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG	NEG	NEG	NEG
PLASTIC				
150 ppm	POS	NEG	NEG	NEG
75 ppm	POS	NEG	NEG	NEG
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG	NEG	NEG	NEG
CONCRT				
150 ppm	POS	NEG	NEG	NEG
75 ppm	POS	POS	POS	NEG
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG	NEG	NEG	NEG
J	-	1 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2		
METAL				
150 ppm	POS	NEG	NEG	NEG
75 ppm	POS	NEG	NEG	NEG
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG '	NEG	NEG	NEG
-20 ⁰ C				
WOOD	<u>0 MIN</u>	<u>30 MIN</u>	60 MIN	<u>120 MIN</u>
150 ppm	POS	NEG	NEG	NEG
75 ppm	POS	NEG	NEG	NEG
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctri	NEG	NEG .	NEG	NEG
PLASTIC				
150 ppm	POS	NEG	NEG	NEG
75 ppm	POS	POS	POS	POS
synergize	POS	NEG	NEG	NEG
virus ctrl	POS	POS	POS	POS
neg ctrl	NEG	NEG	NEG	NEG
~				

<u>CONCRT</u>					
150 ppm	POS	NEG	NEG	NEG	
75 ppm	POS	POS	POS	NEG	
synergize	POS	NEG	NEG	NEG	
virus ctrl	POS	POS	POS	POS	
neg ctrl	NEG	NEG	NEG	NEG	
METAL					
150 ppm	POS	POS	POS	NEG	
75 ppm	POS	POS	POS	NEG	
synergize	POS	NEG	NEG	NEG	
virus ctrl	POS	POS	POS	POS	
neg ctrl	NEG	NEG	NEG	NEG	

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