



**renalin<sup>®</sup>**

**Renalin<sup>®</sup> Dialyzer Reprocessing Concentrate**

This document is intended to be a supplement to the “Renalin<sup>®</sup> Directions for Use.”

It is important that you read and understand the “Directions for Use” prior to using Renalin<sup>®</sup> Cold Sterilant.



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# EFFECTIVENESS

## In Vitro Bacteria Kill

The first tests that were performed to show that Renalin will be effective against bacteria were comparative tests with formaldehyde. Three organisms were selected; *Pseudomonas aeruginosa*, *Bacillus subtilis* and non-tuberculous *Mycobacteria* species (chelonae-like).

The cultures were grown and then transferred to test tubes. A pre-diluted solution of Renalin was then added to the culture. The dilution was such that the culture media plus the pre-dilution gave an end concentration of .5% of the concentrated Renalin. The tubes were then capped, vortex mixed and allowed to stand at room temperature.

For the formaldehyde, 1.8ml of 37% formaldehyde solution was added to a test tube containing 33ml of the culture yielding a 2% solution. The tubes were capped, vortex mixed and allowed to stand at room temperature.

The analysis of these culture tubes was conducted using membrane filtration technique as defined by *Standard Methods for the Examination of Water and Waste Water*, 15th edition. Samples were taken from the culture disinfectant tubes at 1/20, 8, 24 and 36 hours. Prior to sampling the tubes were vortex mixed.

The samples were diluted in sterile buffered distilled water per Standard Methods. The resultant dilution were filtered through a .45 micron membrane. The filter membranes were aseptically placed on standard method agar petri plates and incubated at 35°C. The results are shown in the Table which follows.

<b>Inactivation of Various Microorganisms by Renalin and Formaldehyde Controls</b>					
Controls	Time (hours)*				
	0	1/20	8	24	36
<b>P. aeruginosa</b>					
Water	4.5x10 <sup>7</sup>	4.4x10 <sup>7</sup>	4.5x10 <sup>7</sup>	4.6x10 <sup>7</sup>	5.1x10 <sup>7</sup>
Broth	6.0x10 <sup>8</sup>	6.5x10 <sup>8</sup>	9.8x10 <sup>8</sup>	6.0x10 <sup>7</sup>	4.8x10 <sup>7</sup>
<b>B. Subtilis in water</b>					
Mycobacterium	6.0x10 <sup>3</sup>	5.3x10 <sup>3</sup>	5.8x10 <sup>3</sup>	5.9x10 <sup>3</sup>	5.2x10 <sup>3</sup>
in water	1.6x10 <sup>5</sup>	1.3x10 <sup>5</sup>	1.0x10 <sup>5</sup>	1.0x10 <sup>5</sup>	1.0x10 <sup>5</sup>
<b>Renalin (0.5%)</b>					
<b>P. aeruginosa</b>					
Water		<1	<1	<1	<1
Broth		>10 <sup>6</sup>	>10 <sup>6</sup>	3.8x10 <sup>7</sup>	2.8x10 <sup>7</sup>
<b>B. subtilis in water</b>					
Mycobacterium		3.5x10 <sup>2</sup>	<1	<1	<1
in water		2.9x10 <sup>2</sup>	<1	<1	<1
<b>Formaldehyde (2%)</b>					
<b>P. aeruginosa</b>					
water		<1	<1	<1	<1
Broth		2.8x10 <sup>1</sup>	<1	<1	<1
<b>B. subtilis in water</b>					
Mycobacterium		4.8x10 <sup>3</sup>	<1	<1	<1
in water		1.3x10 <sup>5</sup>	<1	<1	<1

**NOTE:** \*Data is in colony forming units per milliliter (cfu/1ml)

All test samples were shown to be reduced to less than 1 colony forming unit per milliliter within 8 hours. The one exception to this was the *pseudomonas* in broth, which showed no signs of inactivation. This was caused by the inactivation of the Renalin due to the organic load. This will be discussed later when we talk about Renalin's stability.

## Contaminated Dialyzer Testing

The next test performed to show the effectiveness of Renalin was an in vitro test on artificially contaminated dialyzers. Dialyzers were contaminated with *Pseudomonas aeruginosa* and *Bacillus subtilis* endospores and atypical non-tuberculous mycobacteria species (chelonei-like) bacteria. The dialyzers were then treated with .5% Renalin and 4.0% formaldehyde.

Twenty-four used dialyzers were selected, 4 dialyzers from each of 6 patients. Following use these dialyzers were cleaned and Disinfected with a Renatron 3-Chemical System.

Prior to contaminating the dialyzers, the blood compartment was flushed with 1 liter of sterile saline and the dialysate compartment with 10-12 liters of water. Since these dialyzers had been previously cleaned and filled with formaldehyde by a Renatron 3-Chemical System, the need to rinse the formaldehyde disinfectant from the dialyzers before proceeding was obvious.

Following the rinsing, 150ml of the microbial culture was perfused through both the blood and dialysate compartments. Eight dialyzers were contaminated with each of the 3 microbial cultures. The dialyzers were then allowed to stand for 18 hours at room temperature.

After the 18 hour incubation period, the culture contained within the dialyzer was sampled. One liter of the respective disinfectant was then pumped through the blood compartment. Disinfectant was not introduced directly into the dialysate compartment: it had to be diffused across the membrane.

Half of the dialyzers were analyzed at 2 hours and the other half at 24 hours. The analysis was performed as follows: 1 liter of sterile buffered distilled water was perfused through the blood compartment of the dialyzer and collected. Both the disinfectant in the blood compartment of the dialyzer and the 1 liter of sterile water were filtered through a sterile .45 micron membrane filter. The blood compartment volume was 60ml, thus the resultant readings are in colony forming units (cfu) per 60ml. The filter was then rinsed with 100ml of sterile water. Next 20-30ml of solution were removed from the dialysate compartment and also filtered through a sterile .45 micron filter membrane and rinsed with about 100ml of sterile water. The same technique was also used to analyze the 24 hour samples. The membrane filters were then placed on sterile standard method agar contained on petri plates and incubated at 35°C (25°C for Mycobacterium) for 48 hours (72-96 hours for Mycobacterium) and the microbial levels on the membranes were read and recorded.

**Dialyzers Contaminated with Pseudomonas Aeruginosa**

Control, cfu* in Contaminated Dialyzers at 18 hrs.	Renalin Treatment (0.5%)		Formaldehyde Treatment (4.5%)	
	2 hrs.	24 hrs.	2 hrs.	24 hrs.
2.0x10 <sup>5</sup> /ml (Blood)	<1 cfu/60ml			
8.2x10 <sup>4</sup> /mi (Dialysate)	<1 cfu/20-30m			
2.1x10 <sup>5</sup> /ml (Blood)		<1 cfu/60ml		
1.0x10 <sup>5</sup> /ml (Dialysate)		<1 cfu/20-30ml		
1.2x10 <sup>5</sup> /ml (Blood)			<1 cfu/60ml	
1.1x10 <sup>5</sup> /ml (Dialysate)			<1 cfu/20-30ml	
5.7x10 <sup>5</sup> /ml (Blood)				<1 cfu/60ml
2.6x10 <sup>5</sup> /mi (Dialysate)				<1 cfu/20-30 ml
1.1x10 <sup>6</sup> /ml (Blood)	<1 cfu/60mi			
7.9x10 <sup>5</sup> /ml (Dialysate)	<1 cfu/20-30ml			
2.3x10 <sup>6</sup> /ml (Blood)		<1 cfu/60ml		
1.5x10 <sup>6</sup> /ml (Dialysate)		<1 cfu/20-30ml		
1.2x10 <sup>6</sup> /ml (Blood)			<1 cfu/60ml	
1.3x10 <sup>6</sup> /ml (Dialysate)			<1 cfu/20-30ml	
4.2x10 <sup>6</sup> /ml (Blood)				<1 cfu/60mi
2.6x10 <sup>6</sup> /mi (Dialysate)				<1 cfu/20-30ml

\*colony forming units

**Dialyzers Contaminated with Bacillus subtilis**

Control, cfu* in Contaminated Dialyzers at 18 hrs.	Renalin Treatment (0.5%)		Formaldehyde Treatment (4.5%)	
	2 hrs.	24 hrs.	2 hrs.	24 hrs.
8.8x10 <sup>4</sup> /ml (Blood)	<1 cfu/60ml			
5.0x10 <sup>4</sup> /ml (Dialysate)	<1 cfu/20-30ml			
6.6x10 <sup>4</sup> /ml (Blood)		<1 cfu/60ml		
2.7x10 <sup>4</sup> /ml (Dialysate)		<1 cfu/20-30ml		
2.4x10 <sup>4</sup> /ml (Blood)			<1 cfu/60ml	
4.9x10 <sup>4</sup> /ml (Dialysate)			138 cfu/20-30ml	
1.2x10 <sup>5</sup> /ml (Blood)				<1 cfu/60ml
6.6x10 <sup>4</sup> /ml (Dialysate)				<1 cfu/20-30ml
1.5x10 <sup>5</sup> /ml (Blood)	<1 cfu/60ml			
4.9x10 <sup>4</sup> /ml (Dialysate)	<1 cfu/20-30ml			
2.1x10 <sup>5</sup> /ml (Blood)		<1 cfu/60ml		
8.4x10 <sup>4</sup> /ml (Dialysate)		<1 cfu/20-30ml		
6.9x10 <sup>4</sup> /ml (Blood)			<1 cfu/60ml	
1.0x10 <sup>4</sup> /ml (Dialysate)			172 cfu/20-30ml	
8.6x10 <sup>4</sup> /ml (Blood)				<1 cfu/60ml
9.0x10 <sup>3</sup> /ml (Dialysate)				<1 cfu/20-30ml

# Effectiveness

**— Dialyzer, Contaminated with Atypical Mycobacterium —**

Control, cfu* in Contaminated Dialyzers at 18 hrs.	Renalin Treatment (0.5%)		Formaldehyde Treatment (4.5%)	
	2 hrs.	24 hrs.	2 hrs.	24 hrs.
2.6x10 <sup>4</sup> /ml (Blood)	<1 cfu/60ml			
2.7x10 <sup>4</sup> /ml (Dialysate)	<1 cfu/20-30ml			
1.6x10 <sup>4</sup> /ml (Blood)		<1 cfu/60ml		
2.8x10 <sup>4</sup> /ml (Dialysate)		<1 cfu/20-30mi		
6.5x10 <sup>3</sup> /ml (Blood)			<1 cfu/60ml	
9.0x10 <sup>3</sup> /ml (Dialysate)			24 cfu/20-30ml	
1.7x10 <sup>4</sup> /ml (Blood)				<1 cfu/60ml
9.6x10 <sup>3</sup> /ml (Dialysate)				<1 cfu/20-30 ml
3.0x10 <sup>4</sup> /ml (Blood)	<1 cfu/60ml			
1.3x10 <sup>4</sup> /ml (Dialysate)	<1 cfu/20-30ml			
1.3x10 <sup>4</sup> /ml (Blood)		<1 cfu/60ml		
2.9x10 <sup>4</sup> /ml (Dialysate)		<1 cfu/20-30ml		
1.2x10 <sup>4</sup> /ml (Blood)			<1 cfu/60ml	
2.1x10 <sup>4</sup> /ml (Dialysate)			53 cfu/20-30ml	
9.5x10 <sup>3</sup> /ml (Blood)				<1 cfu/60ml
1.0x10 <sup>4</sup> /ml (Dialysate)				<1 cfu/20-30ml

Since only the blood compartment was treated with the disinfectant, this test shows that both formaldehyde and Renalin readily diffuse through the dialyzer membrane. The test results also show that .5% Renalin is more effective in destroying *Bacillus subtilis* and Mycobacterium since total kill was obtained within 2 hours, while 4% formaldehyde did not completely kill at 2 hours.

\*colony forming units

## In Vitro Aqueous Tests

To show that Renalin is effective against other microorganisms, the following *in vitro* tests were performed using a .5% solution of Renalin. All tests were performed on Aqueous Test solutions at 20°C. The longest surviving organism was *Asp. niger* requiring 120 minutes to kill. *Asp. niger* is a fungus.

In Vitro Aqueous Test at 20°C		
Renalin Concentration .5%		Time for 100% Kill
Species	Count per ML	In Minutes
Bac. subtilis	6x10 <sup>6</sup>	2.5
Bac. stearothermophilus	6x10 <sup>8</sup>	2.5
Bac. subtilis NCTC 3610	2.4x10 <sup>9</sup>	5.0
Bac. mesentericus	1.6x10 <sup>9</sup>	5.0
Clostr. perfringens	1x10 <sup>7</sup>	10.0
Clostr. tyrobutyricum	1x10 <sup>7</sup>	5.0
Sacchar. cerevisiae	6x10 <sup>7</sup>	0.5
Cand. mycoderma	1.4x10 <sup>8</sup>	0.5
Hansenula anomala	6.4x10 <sup>8</sup>	0.5
Pichia membranaefaciens	4.8x10 <sup>7</sup>	0.5
Asp. niger	2x10 <sup>7</sup>	120.0
Pen. camerunense	1.7x10 <sup>8</sup>	2.5
Mucor plumbeus	3x10 <sup>6</sup>	2.5
Geotrichum candidum	2x10 <sup>7</sup>	0.5
Byssochlamys nivea	6x10 <sup>7</sup>	10.0
Staph. aureus	2.6x10 <sup>9</sup>	0.5
Strept. faecalis	1.6x10 <sup>9</sup>	0.5
Kleb. aerogenes	2.3x10 <sup>9</sup>	0.5
Ps. fluorescens	4.6x10 <sup>9</sup>	0.5
Ps. aeruginosa	2x10 <sup>9</sup>	0.5
Salm. thyphimurium	2.8x10 <sup>9</sup>	0.5
Coryneb. rubrum	1x10 <sup>7</sup>	1.0
Leuconostoc spec.	5.3x10 <sup>8</sup>	0.5
Lactob. brevis	1.8x10 <sup>9</sup>	0.5

## AOAC Sporicidal Testing

To show that Renalin is sporicidal, the test method of the American Organization of Analytical Chemists (AOAC) was performed. This test is accepted by the F.D.A. to show that a chemical will be considered a sterilant.

Cultures of both *Bacillus subtilis* and *Clostridium sporogenes* were grown. Both silk suture loops and ceramic cylinders were contaminated with the cultures and dried for 24 hours in a vacuum oven. Tests are then run to show that after dehydration the spores are still viable and that resistance is within tolerance.

Five loops or cylinders are then placed into 10ml of the test solution, in this case diluted Renalin. The test time used for Renalin was set for 11 hours.

Following the 11 hour contact time the carriers are removed from the test solution and placed in a subculture medium. Only one carrier per test tube. The test samples are then retransferred to a fresh tube of thioglycolate and incubated for 21 days at 37°C. If no growth is observed after 21 days the tubes are heat-shocked for 20 minutes at 80°C and reincubated for 72 hours at 37°C.

The test was performed on three lots of the test chemical. Results of the test are performed as shown in the following chart. A total of 720 tests were performed on Renalin.

<b>Results of AOAC Sporicidal Test at Minimum Use concentration</b>		
Lot/Organism	Carrier Type	Positives/Total
1 Bacillus subtilis	Loop	0/60
1 Bacillus subtilis	Cylinder	0/60
1 Clostridium sporogenes	Loop	0/60
1 Clostridium sporogenes	Cylinder	0/60
2 Bacillus subtilis	Loop	0/60
2 Bacillus subtilis	Cylinder	0/60
2 Clostridium sporogenes	Loop	0/60
2 Clostridium sporogenes	Cylinder	0/60
3 Bacillus subtilis	Loop	0/60
3 Bacillus subtilis	Cylinder	0/60
3 Clostridium sporogenes	Loop	0/60
3 Clostridium sporogenes	Cylinder	0/60

Total Tests = 720

**NO POSITIVE CULTURES WERE OBTAINED THUS  
RENALIN PASSED THE AOAC SPORICIDAL TEST.**

The performance standards are as follows:

- For sporicidal claims, no more than 2 failures can be tolerated.
- For sterilizing claims, no failures can be tolerated.
- Growth must be observed in tubes with carriers exposed for 2 minutes to 2.5N HCL (as per the Dehydration/Resistance Test)
- Controls must show growth.

All of these performance standards were met by the 11 hours exposure to Renalin.

## Vapor Kill

The ability of Renalin vapor to kill *Bacillus subtilis* spores was tested *in vitro*.

An aqueous solution of Bacillus spores was made by grinding spore strips with sterile water in a sterile container. The solution was then filtered through .45 micron membrane filters. A specific amount of the solution was filtered through each membrane to give 10<sup>3</sup>, 10<sup>4</sup>, 10<sup>5</sup>, 10<sup>6</sup> spores respectively. Each of the filters was then supported approximately 1" above a 1% solution of Renalin. Controls had the filters supported 1" above sterile water. All jars were then covered tightly with plastic caps. The jars were allowed to stand for 21 hours at ambient temperature.

The filters were then removed and transferred to petri dishes containing plate count agar. The dishes were incubated for 48 hours at 35°C. The results are recorded in the chart below.

<b>Deactivation of Organisms by Renalin Vapors</b>	
Estimated Spore Concentration of Filter Suspended 1" above	B. subtilis Spores/Filter
Control: Water	
10 <sup>3</sup> spores	9.6x10 <sup>2</sup> cfu*
10 <sup>6</sup> spores	>3x10 <sup>3</sup> cfu
Renalin Solution (1%)	
10 <sup>3</sup> spores	<1 cfu*
10 <sup>4</sup> spores	<1 cfu
10 <sup>5</sup> spores	<1 cfu
10 <sup>6</sup> spores	<1 cfu

\*Colony forming units

# TOXICITY

<b>Renalin Toxicity Assessment Summary</b>			
Toxicity Summary	Specimen	Results	Reference
Oral Toxicity	Male rats Female rats	LD <sub>50</sub> =2.43 (2.04-2.88) g/kg LD <sub>50</sub> =2.10 (1.92-2.30) g/kg	Litchfield & Wilcoxon
Inhalation Toxicity	Male rats Female rats	Established lethal concentration- LC=13,439 Mg/cubic meter	
Skin Sensitivity	Mice Humans	No reaction No visible effects	Burkhardt's test
Mucous Membranes	New Zealand rabbits	Effects completely gone within 7 days	HH Draize
Dermatological Sensitivity Qualities	White Guinea pigs	No difference between control and test group	Klugman & Magnusson
Intravenous Toxicity	Male rats	LD <sub>50</sub> =212 mg/kg	Litchfield & Wilcoxon

## Test For Acute Oral Toxicity

The test for acute oral toxicity of Renalin was conducted utilizing male Wistar rats weighing 197 grams and female Wistar rats weighing 157 grams. Ten rats were used per dosage. The Renalin was given to the animals by means of a force feeding hose and diluted with water. The Renalin fed the animals was maintained at a constant 20ml/kg of body weight. The animals were observed after the treatment for a period of 8 days. As symptoms of poisoning, the following variables were observed: skin rashes, decreased mobility, difficulties in breathing, cramps, and inability to stand up. The LD<sub>50</sub> value was determined according to the method from Litchfield and Wilcoxon, it was calculated statistically and it was shown to be:

Male rats      LD<sub>50</sub> = 2.43 (2.04-2.88) g/kg

Female rats    LD<sub>50</sub> = 2.10 (1.92-2.30) g/kg

The dosage that was survived by all rats was for male rats 1.25 g/kg and for female rats 1.58 g/kg. The difference is due to the weight difference between males and females. Vivisection of the animals who died during the testing, and all animals at the end of the test were done, and an analysis of the internal organs, showed etching of internal organs, but indicated that there were no specific toxic effects.

## Inhalation Toxicity

Because the possibility could exist that persons over a short period of time may be exposed to Renalin's vapor and breathe these vapors, we have tested Renalin for acute inhalation toxicity with animals. A concentration of 5% was tested. Therefore, we tested 10 male and 10 female rats who were given a 5% solution of Renalin in the form of a fine mist in an inhalation room where the rats were present. This concentration was inhaled by the rats for a period of 4 hours continuously without any toxic symptoms. Since no toxic symptoms were observed, the test was repeated with another 20 rats. This time undiluted Renalin was sprayed into the inhalation chamber.

The quantity of Renalin that was administered in this manner over the 4 hour test period was 38.2 grams of diluted solution and 28.8 grams of undiluted solution. These products were mixed with 2123 and 2023 liters of air, respectively. The capacity of the inhalation chamber was 120 liters; therefore, 1.91 grams equivalent of undiluted Renalin from the diluted solution and 28.8 grams of undiluted Renalin were sprayed into the test chamber during the two tests. This resulted in concentration of 851 mg/M<sup>3</sup> of Renalin in the test using the 5% (diluted) concentration of Renalin and 13,439 mg/M<sup>3</sup> of Renalin in the test using undiluted Renalin.

Neither group of rats who were exposed to the Renalin solution showed any symptoms of poisoning. The rats that were sprayed with the undiluted product showed scratching their noses, inducement to sneeze, wet skin, general discomfort which was expressed by crawling together in a corner of the room, and bent backs. These symptoms stayed 1 hour after the test was ended. After that, all 40 rats survived the chamber for the observed period of 1 week.

The lethal concentration of Renalin therefore is established at greater than 13,439 milligrams per cubic meter.

We can conclude from these tests that Renalin in the vapor form, when used according to the instructions, does not create any health hazard for personnel.

### **Testing of Skin Sensitivity**

A group of 10 hairless mice was tested. Renalin was applied twice a day for 2 weeks to a surface area as large as a silver dollar. Renalin was rubbed into the skin and left there. Two mice showed a slight reddening of the skin after the fourth treatment. These were the only animals that showed any such symptoms. The other animals did not show any symptoms during or after the application. The reaction of the two mice was resolved by the eighth treatment and did not reappear.

The testing of skin sensitivity with repeated applications from human volunteers was conducted according to the W. Burkhardt-Test as described in the *Professional Dermatology Magazine* (p. 179-188, 1970). Renalin 3% solution was applied to the underarm of 5 volunteers at 30 second intervals for 30 minutes. Neither during or after this test, were there any visible effects of the product on the skin.

### **Test of the Effect of the Product on the Mucous Membranes of Rabbits**

Two groups of four white male New Zealand Rabbits were used. Both groups were given 0.1ml of a 3% solution of Renalin. The solution was applied to the tear duct of the right eye. The left eye of the rabbits was left untreated to serve as a control. Group 1 got a thorough rinse of their eye 10 seconds after the Renalin was applied, and Group 2 was left without being rinsed after the application.

The assessment of the effect on the cornea, iris, and the white of the eye followed 2, 6, 24, 48, 72, and 144 hours after the treatment, per the time schedule developed by the Draize method. (H.H. Draize, "Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics," Assn. of Food and Drug Officials of the U.S., p. 49-52, 1959). No effects on the cornea or iris were observed during these observations and no infectious symptoms were observed. A severe reddening of the white of the eye was evidenced in 57.5% of group 1 up and by 25% of group 2. This reddening of the white of the eye decreased greatly within 3 days after the treatment, and was completely gone 7 days after the treatment.

This research indicates that the contact with mucous membranes in the eye with the undiluted or slightly diluted product must be avoided. Based on these results, if Renalin is splashed in the eye, immediate rinsing should be applied and an ophthalmologist consulted.

## **Test of Sensitivity Qualities from a Dermatological Point of View**

This test was conducted according to the method of Kligman and Magnusson (*Journal of investigation Dermatology*, Volume 52, p. 268-276,1979). Two groups of 10 pure white Guinea pigs were used. Each Guinea pig was between 300-400 grams. Behind their shoulder blades a spot 5x6cm was carefully shaved.

The test animals in Group 1 were given three intracutaneous injections on each side of the back bone. The injections were as follows: 1) 0.1ml Freund' Schem adjuvans placebo; 2) 0.1ml solution of 1% Renalin; 3) .1ml of mixture of .05ml placebo and .05ml 1% Renalin solution.

The control Group 2 were given two intracutaneous injections on each side of the backbone. The injections were as follows: 1) 0.1ml placebo; 2) 0.1m distilled water.

Eight days later a 1% solution of Renalin and vaseline was applied to the shaved patch with a gauze and held in place with tape. This gauze was Removed 48 hours later.

After a 14-day treatment the following was performed. An area 2x2cm was shaved on the right flank, vaseline mixed with a 1% Renalin solution was applied to this area and covered with gauze. The gauze was taped in place for 24 hours. After the removal of the gauze, all animals of Group 1 and all animals of Group 2 showed that equally strong reddening of the skin, which must be given a Kligman and Magnusson value of 1. After 48 hours from test initiation only 4 animals had a reaction and after 62 hours none exhibited a reaction. Because we cannot observe a difference between the test animals and the control animals, it was concluded that Renalin does not have any sensitivity effect on the skin. In this test, only primary skin reactions were observed which were caused by the 1% Renalin solution.

## IV Toxicity Tests

To relate the effects of IV Injection of Renalin into humans, a test was performed using white male mice. This test was performed to compare the effects of formaldehyde and Renalin when injected into the test animals. Thirteen groups of 10 test animals were subjected to the test. Seven groups received injections of Renalin and six groups received injections of formaldehyde. The injection was via the tail vein. An aqueous solution was made by diluting the test chemicals with saline, the range of the injection was 158mg/kg to 398mg/kg for Renalin and 100mg/kg to 199mg/kg for formaldehyde. The volume of the injection was kept constant at 10ml/kg, the injection took place over a 1-minute period.

Observations were made following the injection at 1, 6 and 24 hours and then in 24-72 hour intervals up to 14 days.

Average Body Weight Gains					
Product	Dosage mg/kg	Before the Application	24 hrs. after the application	7 days after the application	14 days after the application
Renalin	158	27,9	28,2	31,3	31,2
	172	27,7	28,0	31,0	31,9
	186	28,6	28,5	32,8	33,7
	199	28,3	28,2	29,9	31,9
	251	27,9	28,1	31,0	31,8
	316	27,7	26,5	30,8	31,7
	398	27,5	-	-	-
Formaldehyde	100	27,4	27,3	31,7	32,9
	125	26,9	27,2	31,0	32,3
	142	25,9	25,9	29,3	30,3
	158	27,0	26,5	29,6	30,8
	179	27,7	27,7	31,5	32,2
	199	27,5	-	-	-

As the chart indicates the treatment with Renalin and formaldehyde influenced the body weight development of the animals negatively only during the first 24 hours and weight gain was observed after a week's time.

The chart below details the toxic symptoms which were observed following the application of both Renalin and formaldehyde.

<b>Renalin</b>				
Dosage mg/kg.	Decreased Activity	Spasmodic Jumping	Piloerection	Increased Breathing
158	Medium 24 hrs.	Medium 1 hr.	Medium 24 hrs.	Med/Low 6 hrs.
172	Medium 24 hrs.	Medium 1 hr.	Significant 24 hrs.	Significant 1 hr.
186	Medium 24 hrs.	Significant 1 hr.	Significant 24 hrs.	Significant 24 hrs.
199	Medium 24 hrs.	Severe 1 hr.	Significant 24 hrs.	Severe 24 hrs.
251	Severe	Severe 1 hr.	Significant 24 hrs.	Strong 24 hrs.
316	Significant	Severe 1 hr.	Significant 24 hrs.	Severe 24 hrs.
398	-	Severe	Severe	Severe

<b>Formaldehyde</b>				
Dosage mg/kg	Decreased Activity	Spasmodic Jumping	Piloerection	Increased Breathing
100	Medium decrease 24 hrs.		Slight 6 hr.	Med/High 6 hrs.
125	Significant unrest	Medium 1 hr.	Significant 24 hrs.	Significant 6 hrs.
142	Significant unrest	Severe 1 hr.	Significant 24 hrs.	Severe 24 hrs.
158	Severe unrest 6 hrs.	Severe 1 hr.	Severe 24 hrs.	Severe 24 hrs.
179	-	Severe 1 hr.	Severe 24 hrs.	Severe 24 hrs.
199	-	Severe	Severe	Severe

<b>Renalin</b>					
Dosage	Sound Expression	Ataxia	Lateral Positioning	Toxic and Clonic Spasms	Decreased Reflexes
158	-	-	-	-	-
172	-	-	-	-	-
186	Significant	Medium 1 hr.	-	-	-
199	Significant	Significant 6 hr.	Medium	-	-
251	Significant	Severe 24 hr.	Significant 1 hr.	-	Ear/slight Corneal 1 hr.
316	Severe	Severe 24 hr.	Severe 1 hr.	-	Ear/slight Corneal 1 hr.
398	Severe	-	Severe	-	-

Formaldehyde					
Dosage	Sound Expression	Ataxia	Lateral Positioning	Toxic and Clonic Spasms	Decreased Reflexes
100	-	Slight	-	-	-
125	-	Med/Staggering 6 hr.	-	Medium	-
142	-	Sig/Staggering	-	Significant	-
158	-	Severe/Staggering	-	Medium/High	-
179	-	Severe/Staggering	Severe 1 hr.	Severe	-
199	-	Severe/Staggering	Severe	Severe	-

The animals within the group which died, died within the times listed below:

Dosage mg/kg	Formaldehyde	Dosage mg/kg	Renalin
100	-	158	-
125	3-4 minutes	172	5-6 minutes
142	3-4 minutes	186	5-6 minutes
158	2-3 minutes	199	4-5 minute
179	2 minutes (8 animals)	251	3 minutes
	96 hours (1 animal)	316	2 minutes
199	10-20 seconds	398	20-30 seconds ALL

All the animals which died were dissected. No microscopically recognizable pathologic/anatomic changes could be registered on the inner organs or in the body crevices.

At the end of 14 days, the surviving mice were terminated and dissected. No pathologic/anatomic changes could be registered. This held true for both groups, Renalin and formaldehyde.

The LD<sub>50</sub> value was then statistically assessed according to procedures of Litchfield and Wilcoxon and found to be:

Renalin-LD<sub>50</sub> = 212 (190.9-235.2) mg/kg

Formaldehyde (37.6 weight % formaldehyde in water) LD<sub>50</sub>= 144 (130.9-158.4) mg/kg

The highest concentration of Renalin at which all test animals survived was 158mg/kg. From this we can speculate by calculation that 11,060mg of Renalin Concentrate can be injected into an adult human of 70kg.

Given a security factor of ten, it is felt that human beings can survive a 1100mg Injection of Renalin Concentrate.

Relating this to the solution concentration contained within a reprocessed dialyzer, an adult human of 70kg theoretically should be able to survive a 33ml injection of the solution used in a dialyzer with a security factor of ten.

## Renalin Metabolic Fate

From the literature, it is found that acetic acid will be metabolized to Acetyl CO A and at least in the citric acid cycle eliminated.

The hydrogen peroxide component of the Renalin is destroyed by the enzyme Catalase to water and oxygen.

Only indirect information is existing concerning the peracetic acid contained in Renalin. From this literature we can transfer that peracetic acid is also converted by the enzyme Catalase to acetic acid and oxygen.

## DIALYZER PERFORMANCE TESTS

Tests were then run to show the effects of Renalin on dialyzers for urea, creatinine and vitamin B<sub>12</sub> clearances, ultrafiltration coefficients, blood compartment priming volume, particle testing and pressure tests.

New regenerated cellulose and cuprophane dialyzers were tested for clearances (K) of urea (bun), creatinine (crt) and Vitamin B<sub>12</sub>, (VB<sub>12</sub>), volume and ultrafiltration coefficients (uf). These dialyzers were then filled with it solution of .5 % Renalin and allowed to stand for one week. The dialyzers were then retested for clearances, volume and ultrafiltration coefficients. The results are shown on the following tables. The results shown are felt to be within experimental error.

### In Vitro Dialyzer Performance

<b>In Vitro Performance Effects on Regenerated Cellulose Dialyzers After Exposure to Renalin</b>			
	± Standard Deviation		
	<u>Mean Pre/Post</u>	<u>Pre/Post</u>	<u>% Pre/Post</u>
K <sub>bun</sub> <sup>o</sup> ml/min	152.7/155.7	2.5/2.9	+2.0
K <sub>crt</sub> <sup>o</sup> ml/min	123.7/123.3	1.5/2.2	-3.0
K <sub>VB12</sub> <sup>o</sup> ml/min	23.0/20.7	1.0/1.5	-10.0
K <sub>uf</sub> <sup>o</sup> ml/hr/mmHg	1.8/1.7	0.1/0.1	-5.6
Volume, ml	116/115.3	0/1.2	-0.6

<b>In Vitro Performance Effects on Cuprophane Dialyzer After Exposure to Renalin</b>			
	±Standard Deviation		
	<u>Mean Pre/Post</u>	<u>Pre/Post</u>	<u>% Pre/Post</u>
K <sub>bun</sub> <sup>o</sup> ml/min	159/155.7	3.0/3.8	-2.1
K <sub>crt</sub> <sup>o</sup> ml/min	127.7/124	1.2/1.0	-2.9
K <sub>VB12</sub> <sup>o</sup> ml/min	32.7/32.3	0.6/1.5	-1.2
K <sub>uf</sub> <sup>o</sup> ml/hr/mmHg	3.1/3.2	0.1/0.1	+3.2
Volume, ml	55.7/53.3	1.5/1.5	-4.3

To test for particle shredding, dialyzers were rinsed at 200ml/min with 3 liters of filtered normal saline. This was done to remove any particles left by the manufacturing process. Following this, the dialyzers were filled with a .5% solution of Renalin and allowed to stand for 40 hours.

The dialyzer was then flushed with 1 liter of filtered normal saline. The effluent rinse was passed through a matched pair of 33MM diameter .8 micron filters (Millipore type MAAWP 037PM matched weight filter monitors). After rinsing, the filters were dried.

The upper filter will capture any particles larger than .8 micron. The weight differential between the upper filter and the lower filter is the amount of particles released from the dialyzer. This test was done on both Renalin and formaldehyde filled dialyzers. As the table below shows, *no significant shredding was found.*

<b>Weight of Particle Recovery from Dialyzer</b>		
Dialyzer Time	Formaldehyde Exposed	Renalin Exposed
Cuprophane		
Mean	-0.05	0.32
± SD	0.04	0.47
Regenerated Cellulose		
Mean	0.05	0.05
± SD	0.22	0.07
Cellulose		
Mean	-0.15	-0.08
± SD	0.08	0.12

**NOTE:** Negative value indicates control weighed more than recovery filter. The dialyzers used for the shredding test were then pressure tested. This was accomplished by pressurizing the dialyzers to 1000mmHg using an insufflator bulb and a manometer. The rate of pressure drop was determined and compared to formaldehyde-filled dialyzers.

No ruptures of the membrane occurred. Pressure drops were comparable to the formaldehyde-filled dialyzers.

**Performance Data Final Concentration**

To show that dialyzer performance will be maintained when Renalin and the Renatron are used together the following test was run.

Dialyzers were tested *in vitro* and measurements made for BUN, Creatinine and vitamin B<sub>12</sub> clearances. Ultrafiltration coefficients and blood compartment priming volumes are also measured. Dialyzers were then reprocessed and filled with Renalin via a Renatron. The dialyzers were allowed to stand for 72 hours and tested. The dialyzers were then refilled and allowed to stand for an additional 96 hours after which the ultrafiltration coefficient was determined.

In addition, dialyzers were allowed to stand filled with Renalin for 168 hours and then tested.

# Dialyzer Performance Tests

The results are shown in the following table. All pre-and post-differences in Renalin exposed dialyzers are slight and within experimental errors.

<b>Performance Data of Dialyzer Exposed to Final Use Concentration</b>			
	Time of Exposure (hours)	Value Obtained	% Change
K <sub>bun</sub> <sup>a</sup> ml/min	0	156	
	72	156	0
	168	160	+2.5%
K <sub>crt</sub> <sup>a</sup> ml/min	0	126.6	
	72	124	-2.0%
	168	127	+0.3%
K <sub>VB12</sub> <sup>a</sup> ml/min	0	31.3	
	72	30.5	-2.5%
	168	31	-0.9%
K <sub>uf</sub> <sup>a</sup> ml/hr/mmHg	0	3.0	
	72	3.1	-3.3%
	168	3.1	-3.3%
Volume, ml	0	51.6	
	168	52	+0.7%

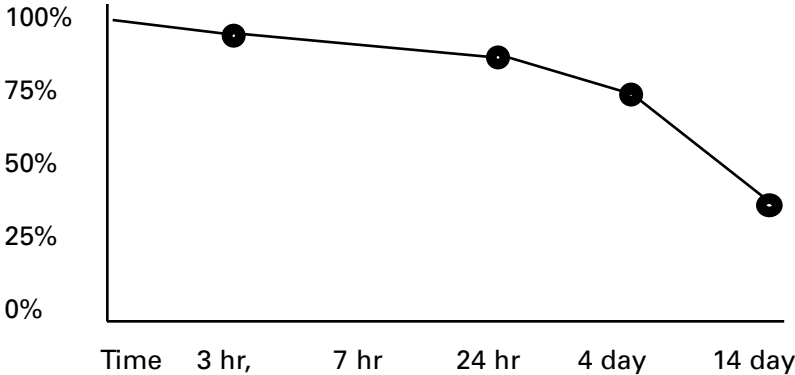
# RENALIN

## Stability

Although Renalin will remain stable in the concentrated form for over 1 year, once it is diluted a decay process begins to take place. The decay is such that when diluted with AAMI quality water, 50% of the peracetic acid will remain after 7 days. The graph that follows shows the decay curve of Renalin diluted 1:100 over a 14 day period. The ratio of dilution affects the rate of decay of peracetic acid contained in the Renalin. Thus, if the dilution ratio is less than 1:100 the rate of decay will be less.

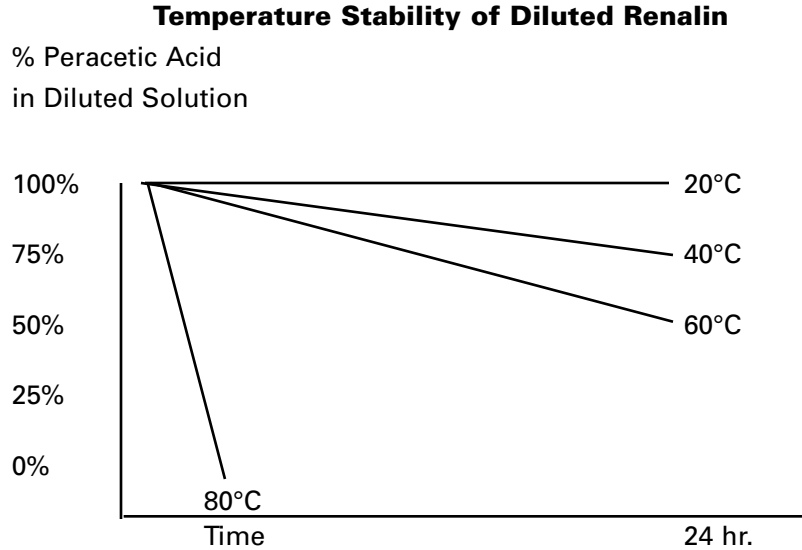
**Stability of Renalin Diluted Solution  
Dilution Ratio 1:100**

% Peracetic Acid  
in Diluted Solution



## Temperature Stability of Diluted Solution

Concentrated Renalin remains stable at temperatures up to 30°C (86°F) for a period of 1 year. However, once Renalin is diluted, the rate of decay of the peracetic acid is greatly increased as the temperature increases as is shown on the following graph:



The factors of time decay and temperature decay have been taken into account when determining the concentration of Renalin to be delivered to the dialyzer.

The level of peracetic acid as pre-diluted and further diluted by Renatron Dialyzer Reprocessing System is such that when a 50% decay is allowed, the sterilizing effects of Renalin are still retained. However, to obtain this level it is necessary to label the product as such that:

1. Once the product is diluted it must be used within 7 days, and
2. The storage and use temperature must be maintained below 30°C (86°F).

## Storage Conditions

A test was conducted on a .5% solution of Renalin. This test was conducted to show the stability of peracetic acid with various storage conditions. The storage conditions that were researched were translucent containers at room temperature, dark containers at room temperature and dark containers at 37°C. The selection of these test conditions was based on the knowledge that both temperature and light can effect the level of peracetic acid. The results of this test are shown in the following table.

<b>Stability of Peracetic Acid with Storage Conditions</b>			
Storage Condition	% of Decrease in 7 Days	% of Decrease in 14 Days	.5% Renalin Solution % of Decrease in 112 Days
Translucent Container Room Temperature	43%	58%	83%
Dark Container Room Temperature	36%	43%	80%
Dark Container 37°C	51%	78%	78%

**NOTE:** The duplication between the dark container at 37°C, 14 days and 112 days of 78% is probably an artifact of the test method used in making the concentration measurements.

The package recommendations of: “Store in Original Shipping Box”, “Do Not Store in Direct Sunlight” and “Maintain Temperature below 37°C after Dilution” are justified by these test results.

## Decrease of Peracetic Acid in the Presence of Protein

Again we look at the results of the AOAC Sporicidal test. However, this time the 4th column has been added showing the decrease in peracetic acid following exposure to the test media.

<b>Results of AOAC Sporicidal Test at Minimum Use Concentration</b>			
Lot/Organism	Carrier Type	Positives/Total	Decrease in Peracetic Acid
1 Bacillus subtilis	Loop	0/60	-44%
1 Bacillus subtilis	Cylinder	0/60	-6%
1 Clostridium sporogenes	Loop	0/60	-42%
1 Clostridium sporogenes	Cylinder	0/60	-10%
2 Bacillus subtilis	Loop	0/60	-44%
2 Bacillus subtilis	Cylinder	0/60	-8%
2 Clostridium sporogenes	Loop	0/60	-44%
2 Clostridium sporogenes	Cylinder	0/60	-12%
3 Bacillus subtilis	Loop	0/60	-40%
3 Bacillus subtilis	Cylinder	0/60	0
3 Clostridium sporogenes	Loop	0/60	-36%
3 Clostridium sporogenes	Cylinder	0/60	-8%

Total test = 72

**NO POSITIVE CULTURES WERE OBTAINED THUS  
RENALIN PASSED THE AOAC SPORICIDAL TEST**

Note that the silk suture loops had a decrease in peracetic acid from 36-44%, while the ceramic cylinders decreased only 10% at most. The suture loops due to their high porosity carried more of the culture media to the test solution and thus a greater decrease occurred.

This was also shown during the *in vitro* bacteria kill test where pseudomonas were allowed to remain in the broth and no kill was obtained.

Dialyzers with up to 60% reduction in volume were also tested at periods of up to 7 days and the concentration of peracetic acid remained at the desired sporicidal level. Because of this decrease in peracetic acid in the presence of organic loads and the resultant pressure buildup within the dialyzer as the organic material is broken down by the Renalin, Renal Systems strongly recommends that only dialyzers with greater than 80% volume be reused.

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