SUMMARY OF RESULTS

Test Substance:	Rely + On™ Virkon®, Lot 1306BA0298
Dilution:	1:100 defined as 10 g of test substance + 1 litre of 400 ppm AOAC Synthetic Hard Water
Virus:	Human Coronavirus, ATCC VR-740, Strain 229E
Exposure Time:	10 minutes
Exposure Temperature:	Room temperature (20.0°C)
Organic Soil Load:	5% fetal bovine serum
Efficacy Result:	One lot of Rely + On™ Virkon® (Lot 1306BA0298) met the performance requirements specified in the study protocol. The results indicate **complete inactivation** of Human Coronavirus under these test conditions as required by the U.S. EPA.

TEST SYSTEM

1. Virus

The 229E strain of Human Coronavirus used for this study was obtained from the American Type Culture Collection, Manassas, Virginia (ATCC VR-740). Stock virus was prepared by collecting the supernatant culture fluid from 75-100% infected culture cells. The cells were disrupted and cell debris removed by centrifugation at approximately 2000 RPM for five minutes at approximately 4°C. The supernatant was removed, aliquoted, and the high titer stock virus was stored at ≤-70°C until the day of use. On the day of use, an aliquot of stock virus (ATS Labs Lot HCV-69) was removed, thawed, and maintained at a refrigerated temperature until used in the assay. The stock virus culture was adjusted to contain 5% fetal bovine serum as the organic soil load. The stock virus tested demonstrated cytopathic effects (CPE) typical of Human Coronavirus on WI-38 cells.

2. Indicator Cell Cultures

Cultures of WI-38 (human lung) cells were originally obtained from the American Type Culture Collection, Manassas, VA (ATCC CCL-75). The cells were propagated by ATS Labs personnel. The cells were seeded into multiwell cell culture plates and maintained at 36-38°C in a humidified atmosphere of 5-7% CO_2. On the day of testing, cells were observed as having proper cell integrity and confluency and therefore, were acceptable for use in this study.

All cell culture documentation was retained for the cell cultures used in the assay with respect to source, passage number, growth characteristics, seeding densities and the general condition of the cells.

STUDY RESULTS

Results of tests with one lot of Rely + On™ Virkon® (Lot 1306BA0298), diluted 1:100 in 400 ppm AOAC Synthetic Hard Water, exposed to Human Coronavirus in the presence of a 5% fetal bovine serum organic soil load at room temperature (20.0°C) for 10 minutes are shown in Tables 1-3. All cell controls were negative for test virus infectivity. The titer of the input virus control was 6.00 $\log_{10}$. The titer of the dried virus control was 5.00 $\log_{10}$. Following exposure, test virus infectivity was not detected in the virus-test substance mixture at any dilution tested ($\leq$0.50 $\log_{10}$). Test substance toxicity was not observed at any dilution tested ($\leq$0.50 $\log_{10}$). The neutralization control (non-virucidal level of the test substance) indicates that the test substance was neutralized at $\leq$0.50 $\log_{10}$. Taking the toxicity and neutralization control results into consideration, the reduction in viral titer was $\geq$4.50 $\log_{10}$.

STUDY CONCLUSION

Under the conditions of this investigation and in the presence of a 5% fetal bovine serum organic soil load, Rely + On™ Virkon® (Lot 1306BA0298), diluted 1:100 in 400 ppm AOAC Synthetic Hard Water, demonstrated complete inactivation of Human Coronavirus following a 10 minute exposure time at room temperature (20.0°C) as required by the U.S. EPA.

In the opinion of the Study Director, there were no circumstances that may have adversely affected the quality or integrity of the data.