Kauri Dieback: Kauri Hygiene – small project

Stanley E. Bellgard, Elsa P. Paderes and Ross E. Beever

Landcare Research
231 Morrin Road
St Johns
Auckland 1142
New Zealand

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PREPARED FOR:
MAFBNZ
Pastoral House
PO Box 2526
Wellington 6140

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Reviewed by:	*Approved for release by:*
Sarah L. Dodd Scientist Landcare Research	Peter K. Buchanan Science Team Leader Biosystematics, Landcare Research

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Summary

Project and Client
MAFBNZ required research to assess the efficacy of current hygiene methods to suppress (i.e. preventing it from growing or developing) and control the plant pathogen, *Phytophthora* taxon Agathis (PTA). The current hygiene product used by Auckland Regional Council (ARC) is 2% TriGene™. MAFBNZ are also keen to identify other potential candidates for consideration as alternative hygiene methods (e.g., quaternary ammonium products, sodium hypochlorite and Citricidal®, a grapefruit seed and pulp extract).

Methods
To achieve these aims, we carried out a series of *in vitro* and soil-based bioassays to obtain specific information about:

- Expt 1. The direct biocidal efficacy of the disinfectants TriGene™ (II) Advance, Phytoclean™, Virkon® S, Janola® and Citricidal®, on PTA mycelium
- Expt 2. The direct biocidal efficacy of these disinfectants on oospores and;
- Expt 2b. The direct biocidal efficacy of these disinfectants on zoospores (i.e. the inoculum of PTA)
- Expt 3. The infective capacity of PTA inoculum
- Expt 4. The direct ability of these disinfectants to kill PTA in soil and
- Expt 5. The direct ability of these disinfectants to kill PTA in soil adhering to rubber gum-boots.

Results

Expt. 1 Sensitivity of PTA mycelium to disinfectants

TriGene, and Phytoclean completely suppressed growth of PTA mycelium at all *in vitro* concentrations tested. Only TriGene and Phytoclean resulted in complete mortality of the hyphae of the pathogen contained in the mycelial plug. Virkon (at 0.2 and 0.1% a.i.) reduced growth of PTA by at least 95%. At 0.05% a.i. it reduced growth by 77%, at 0.025% a.i. it reduced growth by 54%, and at 0.0125% a.i. it reduced growth by 27% (compared with the control). Janola (at 0.2, 0.1, 0.05% a.i.) completely suppressed PTA. At 0.025% a.i. it inhibited growth by 54% and at 0.0125 % a.i., it inhibited growth by 18% (compared with the control). Citricidal was demonstrated to be fungistatic (i.e. inhibited growth but did not kill the mycelium) at all concentrations.

Expt. 2 Sensitivity of PTA oospores to disinfectants

The majority of the oospores in the unamended control were dormant (approx. 80%). About 10% of the oospores in the control were non-viable and the remaining 10% were activated. Virkon (0.2% a.i.), and Janola (0.05% a.i.) had the most significant impact on oospore viability. Virkon killed significantly more oospores than Janola – and both Virkon and Janola were more lethal than either TriGene (0.0125% a.i.), Phytoclean (0.0125% a.i.) and/or Citricidal.

Expt. 2b Sensitivity of PTA zoospores to disinfectants

The zoospores that were placed into TriGene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%), did not survive the treatment. The zoospores that were placed in the Citricidal and Control (i.e. RO water) survived the treatment and produced a mean of 784 ±38 / ml, and 404 ± 70 / ml colonies of PTA after 2 days.

Expt. 3 Infective capacity of PTA inoculum

The soil "spiked" with 2000 oospores / g of PTA colonised 37% of leaf baits – confirming the infective capacity of PTA oospore-inoculum.

Expt. 4 Ability of disinfectants to kill PTA in soil

The spiked soil that was soaked in TriGene (2%) and Phytoclean (10%) completely suppressed PTA, and all soil fungi. Virkon (1%) and Janola (5%) suppressed PTA, but soil treated with Virkon (1%) and Janola (5%) did not suppress all soil fungi and bacteria.

Expt. 5 The ability of disinfectants to kill PTA in soil on boots

PTA was not recovered from soiled rubber gum-boots sprayed with TriGene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%). The rinsate from spraying with RO water alone did not suppress PTA. Soil adhering to boots sprayed with TriGene (2%), Phytoclean (10%), Virkon (1%) and/or Janola (5%) significantly reduced the infective capability of PTA inoculum.

Conclusions

- Disinfecting soiled rubber gum-boots with a 2% spray treatment of TriGene II Advance effectively suppresses the inoculum of PTA. TriGene achieves this in part, by being biocidal to PTA mycelium. TriGene could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA. TriGene effectively suppresses the infective capacity of PTA in soil at its recommended label rate of 2%.
- Phytoclean demonstrated a similar efficacy to TriGene, in that it completely suppressed the growth of PTA by killing the mycelium. Phytoclean could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA. Phytoclean demonstrated efficacy at its label/recommended rate (i.e. 10%) to effectively suppress the spread of PTA inoculum contained in soil.
- Virkon only suppressed growth of PTA at higher *in vitro* concentrations and at these concentrations it was lethal to mycelium. Virkon could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA at its recommended label rate. Virkon (at 1%) also demonstrated efficacy at suppressing PTA's infective capacity in soil when applied as a spray application. Further research is required to understand the difference between spray-applied efficacy versus *in vitro* efficacy.
- Janola demonstrated a similar efficacy to Virkon, in that it completely suppressed the growth of PTA by killing the mycelium at higher *in vitro* concentrations. Janola could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA at its recommended label rate. When applied as a spray-treatment, Janola (5%) demonstrated efficacy at its recommended rate to effectively suppress the spread of PTA inoculum contained in soil, but like Virkon was not as effective *in vitro.* Further research into the "mode of action" of Virkon and Janola could assist in understanding the differences between spray-treatment efficacy and *in vitro* efficacy of these disinfectants.
- Citricidal, while demonstrating successful *in vitro* fungistatic inhibition (i.e. slowed down the growth process) of PTA, did not kill mycelium like the other disinfectants. Citricidal had no effect on zoospores of PTA, and did not reduce the infective capacity of PTA inoculum contained in soil.
- Spraying with RO water alone does not reduce the infective capacity of PTA inoculum contained in soil.

1. Introduction

Phytophthora diseases worldwide

From a global perspective, more than 66% of all fine-root diseases and more than 90% of all collar-rots of woody plants are mediated by *Phytophthora* species (Erwin & Ribeiro 1996). During the recent decades a series of devastating diseases of broad-leaved tree species in Europe and the USA has focussed research on the role of *Phytophthora* in natural ecosystems Much new information has accumulated, and several new *Phytophthora* taxa have been described in Europe (Jung et al. 2002, 2003), and the western USA (Hansen et al. 2000). In California and some localities of Oregon the airborne species *P. ramorum* is responsible for the so-called "Sudden Oak Death Syndrome": a rapidly spreading epidemic of tanoak and several oak species that is characterised by multiple cankers along the stem and in the crown (Rizzo et al. 2002). In riparian and forest stands in Western, Central, and Southern Europe various alder species are suffering from a widespread and often lethal root and collar rot caused by the alder *Phytophthora* (Brasier et al 2004).

In the agricultural and horticultural context, it has been clearly demonstrated that *Phytophthora* can be prevented from spreading by a variety of integrated chemical and physical methods. In the field or glasshouse fumigation using steam heat and/or metham sodium has been shown to suppress *Phytophthora* for at least half a year (if applied before a crop is planted). Total sanitation is very expensive, however, and in the real world, it is seldom achievable, due to the resistant nature of some of the propagules of certain *Phytophthora* species, e.g., oospores and chlamydospores (Erwin & Ribeiro 1996).

Approved *Phytophthora* disease control in natural forests has a number of risk and human-health constraints that limit the widespread application of agricultural chemical-control methods. However, successful outcomes have been reported using potassium phosphonate foliar sprays (e.g., Aberton et al. 1999) and phosphonic acid injections in stopping the growth of *Phytophthora* species (Jackson et al. 2000) in natural ecosystems. Evidence from conservation areas in Eastern Australia (Tasmania) and mining operations in Western Australia have demonstrated that spread of the inoculum of the related species *P. cinnamomi* can be limited by the removal of mud/soil at "boot wash stations", which reduces the ingress of spores (Tasmanian DPI 2004; Colquhoun & Kerp 2007).

PTA defined

Phytophthora taxon Agathis (PTA) was first recovered in 1972 from unhealthy stands of kauri (*Agathis australis*) on Great Barrier Island (Gadgil 1974), where it was associated with a distinctive collar-rot. It was initially identified as *P. heveae*, the causal agent of "black stripe" of *Hevea brasiliensis* (rubber), but subsequent molecular studies indicate that while it resembles this species it is more closely related to *P. castanea* (= *P. katsurae*) from Japan and SE Asia (Beever et al. 2009). However, it lacks the highly rugose (bullate) oospore ornamentation characteristic of this species and is probably new to science, hence it has been named PTA pending further study (Plate 1 depicts the slight raised protuberances associated with PTA, while *P. heveae* is smooth in texture). Pathogenicity tests indicate that PTA is highly pathogenic to kauri, but not to other kauri ecosystem associates (Gadgil 1974; Beever et al. 2008). PTA was recovered from the Waitakere Ranges near Auckland city in 2006 and is presently also known from Pakiri Reserve and Trounson Kauri Park, Northland. However, disease symptoms are more widespread, raising concern that PTA poses a threat to both kauri ecosystems and iconic giant trees (Beever et al. 2008, 2009).

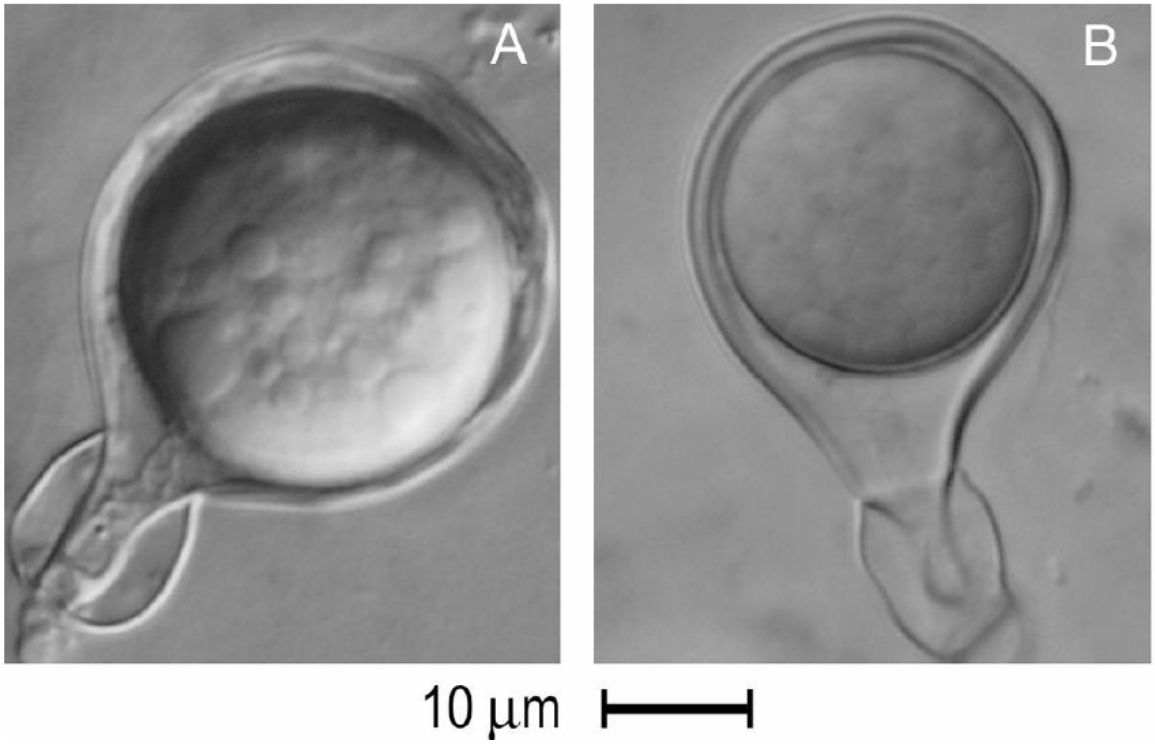

Plate 1: Oospores of PTA (A) versus *P. heveae* (B). Note oospore on left is slightly bigger and slightly rugose compared with the smaller, smoother oospore on the right hand side (from Beever et al. 2008)

Concern about this threat led the Auckland Regional Council (ARC) to initiate a management response in May 2008 (J. Craw, ARC Biosecurity, pers. comm.). In October 2008 a Joint Agency Response involving MAF Biosecurity, Department of Conservation and the 6 northern Regional Councils was initiated and PTA was declared an unwanted organism. Since that time, hygiene kits (aimed to limit the spread of soilborne PTA inoculum) have been provided at the start of walking tracks in the Waitakere Regional Park. The current recommended hygiene prescriptions for activities in kauri forest developed primarily by the ARC can be summarised as follows (http://www.arc.govt.nz/environment/biosecurity/kauri-dieback/kauri-dieback-how-you-can-help_home.cfm):

- Shoes, tyres and equipment are to be clean of dirt/soil before entering kauri forest.
- Shoes and any other equipment that comes into contact with soil need to be cleaned of adhering soil after every visit, and also if moving between bush areas.
- Keeping to defined park tracks at all times to prevent movement of soil that has the potential to spread the disease (ARC 2009).

Hygiene chemicals

TriGene™ (II) Advance (TriGene) has as its main active ingredient a group of halogenated tertiary amines. Until now, ARC has been using an earlier formulation of TriGene. We have tested the efficacy of *TriGene II Advance* on PTA on the understanding that this new formulation will likely to be adopted by ARC as the new standard. Microbial tests have demonstrated its efficacy against a range of micro-organisms including bacteria, viruses, and fungi (including the soil fungus, *Aspergillus niger*). Its recommended label rate is 2% (Table 1). It does not deteriorate if "stored correctly" (however, it is recommended that is be stored out of direct sunlight), is biodegradable (Medichem International Ltd 2008; Appendix 3), and has low mammalian toxicity.

Phytoclean™ (Phytoclean) is a disinfectant cleaner specifically designed for the control of *Phytophthora cinnamomi* in horticulture, plantation, and earth-moving industries. Phytoclean is based on the quaternary ammonium compound, benzalkonium chloride. It also contains

sodium tripolyphosphate and a surfactant. It is recommended that Phytoclean not be stored as a diluted solution. There is also a warning on the label, "do not contaminate streams, rivers or waterways with Phytoclean or used containers". For footbaths, the recommended label rate is 10% (Table 1). Noske and Shearer (1985) demonstrated that quaternary ammonium products were more effective than sodium hypochlorite at suppressing growth of *P. cinnamomi.* This work was repeated by Smith and Clements (2006), with similar suppressive results demonstrated by quaternary ammonium compounds.

Virkon® S (Virkon) is a broad spectrum disinfectant with potassium peroxymonosulphate as the main active ingredient. It is used in cleaning and disinfecting industrial, animal and agricultural facilities. It is also used for emergency disease control and is efficacious against a range of viruses, bacteria and fungi (including a range of plant pathogens, e.g., *Alternaria, Colletotrichum, Fusarium, Pythium,* and *Rhizoctonia* species). For greenhouse and horticultural applications, a 1% solution is recommended for disinfecting glasshouse structures, equipment, and tools (Table 1). There is warning on the label: "do not immerse metal objects in Virkon for long periods." The recommended maximum contact time is 10 minutes. Additionally, it is also not recommended for use on acid sensitive surfaces, e.g., copper, brass, or aluminium.

Sodium hypochlorite is available in a number of commercial formulations. Janola® (active ingredient hypochlorous acid/sodium salt solution) is a broad spectrum disinfectant at 5% concentration. Smith (1979) demonstrated that chlorine-releasing compounds (e.g., sodium hypochlorite) were fungitoxic against *P. cinnamomi.* However, sodium hypochlorite is considered hazardous in the case of skin and eye contact. It is also considered hazardous in case of inhalation in a confined space. In its diluted form, it is sensitive to light, and is extremely corrosive to brass, and moderately corrosive to bronze.

Citricidal® is synthesised from the polyphenolic compounds found in grapefruit seed and pulp. The active component of Citricidal is considered to be related to "quaternary ammonium chloride". It is recommended for the treatment of candidiasis, parasites, sinusitis, athlete's foot (in humans) and ulcers on pets and livestock. The "label rate" indicates there is 25 mg of grapefruit concentrate in each drop (i.e. approx. 0.25%).

Table 1: Percentage active ingredients of the commercial disinfectants

Disinfectant	Recommended Rates	Percentage active ingredient (% a.i.) in Label "Recommended Rates"
TriGene	2%	0.0024%
Phytoclean	10%	0.0128%
Virkon	1%	0.0020%
Janola	5%	0.0021%

NB. All MSDS information and biodegradability data (where available) is provided in Appendix 3.

In a recent comparative assessment of disinfectant products for the microbial decontamination of imported, used footwear, Cheah et al. (2009) demonstrated that sodium hypochlorite and quaternary ammonium compounds gave almost complete control of bacteria. Sodium hypochlorite and quaternary ammonium were as effective as Virkon in controlling soil fungi associated with dirty footwear.

2. Objectives

The aims of the research were to:

- Assess the efficacy of current hygiene methods (i.e. 2% TriGene) against PTA, and to
- Identify other potential candidates for consideration as alternative hygiene methods (e.g., Phytoclean (quaternary ammonium), Citricidal (grapefruit seed and pulp extract), and Janola (sodium hypochlorite)).

In order to achieve these objectives, a series of experiments were run both sequentially and concurrently. The five experiments were designed to provide specific information about:

Expt 1. The direct biocidal efficacy of the disinfectants TriGene, Phytoclean, Virkon, Janola, and Citricidal on PTA mycelium

Expt 2. The direct biocidal efficacy of these disinfectants on inoculum of PTA (i.e. oospores and zoospores)

Expt 3. The infective capacity of PTA inoculum (i.e. oospores)

Expt 4. The direct ability of these disinfectants to kill PTA in soil and;

Expt 5. The direct ability of these disinfectants to kill PTA in soil adhering to boots.

3. Methods

Experiment 1: Sensitivity of PTA mycelium to disinfectants

All PTA isolates examined to date have the same ITS sequence and are morphologically similar. Strain REB316-1 (= ICMP17021) (Beever et al. 2009), an isolate from an active tree lesion at Piha (Waitakere Ranges), was chosen for testing. Isolate REB 316-1 was grown on potato dextrose agar (PDA; Appendix 1) in petri dishes at 20ºC. From the growing edge of cultures, 6.5 mm diameter plugs of agar were placed on PDA amended with the five disinfectant treatments; TriGene, Phytoclean, Virkon, Janola and Citricidal at 0.2 (C1), 0.1 (C2), 0.05 (C3), 0.025 (C4), and 0.0125% (C5) active ingredient (a.i.) and a control containing water (5 plates per treatment).

The plates were incubated at 20ºC (under fluorescent light) and colony growth marked the same time each day at 4, 5 and 8 days after inoculation. Survival of the culture plugs was assessed by transferring them onto fresh, unamended PDA after 10 days, and growth responses assessed after a further 4 days.

Experiment 2: Sensitivity of PTA oospores and zoospores to disinfectants

PTA (isolate REB 326-1, = ICMP 18244, confirmed to be PTA by ITS obtained from the symptomatic kauri tree from Pakiri Scenic Reserve) was grown on PDA in petri dishes at 20ºC. From the growing edge of cultures, 6.5-mm-diameter plugs of agar were placed into clarified V8 juice broth (Appendix 1) and incubated at 20ºC for 56 days. PTA was harvested from the V8 juice broth and macerated in a Waring Blender for 20 seconds. Oospore numbers were estimated by haemocytometry at approx. 200 000 oospores/ml.

Four replicate oospore suspensions of 25 µl were added to plates containing 0.6% water agar amended with each of the five disinfectant treatments at their lethal concentrations as determined in Experiment 1; TriGene Advance (0.0125% a.i.), Phytoclean (0.0125% a.i.), Virkon S (0.2% a.i.), Janola (0.05% a.i.) and Citricidal (6 drops/100 ml) and a control that was the unamended water agar. The plates were incubated at 20ºC and after 10 days the viability and/or dormancy of 50 oospores from each replicate (giving a total of 200 oospores in total) were assessed by light microscopy using tetrazolium salt (BDH) as a vital stain (Jiang & Erwin 1990). Data were analysed using a χ-squared contingency table (comparing the response of the oospores in the unamended control with each of the disinfectant treatments in a pair-wise manner).

A second experiment (comprising five replicates) assessed the efficacy of the hygiene agents on zoospores of PTA. Sterile zoospore suspensions were made by incubating blocks of colonised V8 juice agar in sterilised soil extract (Appendix 1) overnight (under white and blue fluorescent light at 18ºC). The next day, the blocks were transferred to Eppendorf tubes (1.5 ml) with 50 µl of sterile soil extract and incubated in the refrigerator for 1 hour (to induce sporangial release). A 50-µl aliquot of each disinfectant at recommended label rates (i.e. TriGene 2%; Phytoclean 10%; Virkon 1%, Janola 5% and Citricidal (6 drops/100 ml)) with a sterile RO water control was added to the zoospore suspension. The tubes were vortexed and incubated for 1 minute at room temperature and the contents plated to P_5ARP Selective Medium for *Phytophthora* species (Appendix 1) selective agar and incubated in the dark for 2 days. Colony forming units (CFU's) per ml were estimated after 3 days by counting the number of fungal colonies, and representative isolates were plated to PDA and V8 juice agar to confirm their identity. This experiment was repeated 5 times.

Experiment 3: Soil spiking and quantification of PTA infection potential

Field soil from the infested Huia site was collected in sterile, 20 l plastic pails. Soil was collected around kauri (of ricker-age) exhibiting gummosis, crown decline and/or crown chlorosis and transported back to Landcare Research, Tamaki and stored at 10ºC in the dark.

Soils were passed through a 2-cm screen to remove coarse woody debris. At least 15 kg of soil was kept as the "control". The remaining soil was split into 5 lots, each having one of the five oospore suspensions added to it, before being thoroughly mixed. This resulted in five "spiked" soils with final oospore concentrations of 2000, 1000, 500, 250 or 125 oospores/g of soil.

Three 20-g sub-samples of the "control" and each of the "spiked" soils were assayed for the presence of PTA, using the extended leaf-bait soil bioassay methods (modified from Stack & Millar 1985). The soil was air-dried on the Dingley laboratory bench for two days, moist incubated for four days (see Stack & Millar 1985) and then flooded with 200 ml RO water in 400 ml beakers. The beakers were baited with 10, trimmed, Himalayan cedar (*Cedrus deodara*) needles and incubated at 20ºC for 4 days at 60%RH under blue and cool white fluorescent light (Light intensity of 180 µE). All leaf-baits were surface-sterilised for 30 seconds in 50% ethanol, rinsed three times in sterile RO water and plated onto P_5ARP selective media. Three replicate aliquots (100 µl) of the leaf-bait soil bioassay water were taken from each bioassay and were plated onto P_5ARP selective agar, and the number of CFU's/ml resembling PTA in colony morphology on the selective agar plate assessed after 3 days. Representative isolates were plated to V8 juice agar to confirm their identity. The proportion of leaf baits colonised by PTA were transformed using the angular transformation (i.e. by taking the arcsine of the square root of each proportion). Data were then compared using a t-test.

Experiment 4: Ability of disinfectants to kill PTA in soil

The ability of the disinfectants to kill/inhibit PTA in soil was assessed by soaking replicates of the spiked soil (produced for Experiment 3) in the disinfectants. Replicate spiked soil (20-g samples containing 1500 oospores/g) were placed in mesh bags and soaked in the disinfectants at their label rates (for Citricidal, the equivalent of 6 drops/100 ml was used). The control involved soaking the soil in RO water (Plates 2a, 2b). The soil was then washed three times in sterile RO water following the initial treatment and allowed to drain.

The treated soil was bioassayed using the extended bioassay methods described in Experiment 3. Three 100-µl aliquots of the soil bioassay water from each bioassay were plated directly on P_5ARP to determine the number of CFUs/ml. Representative isolates were plated on V8 juice agar to confirm their identity. The experiment was replicated three times.

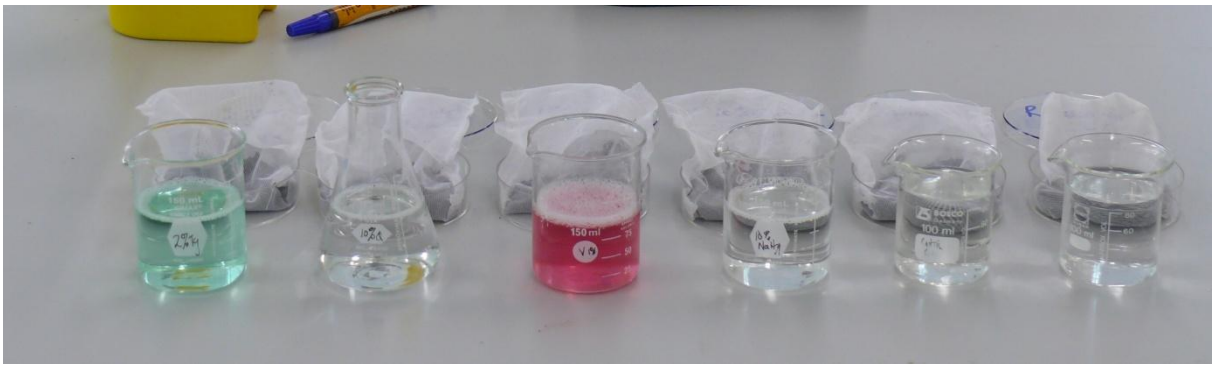

Plate 2a: Disinfectant solutions

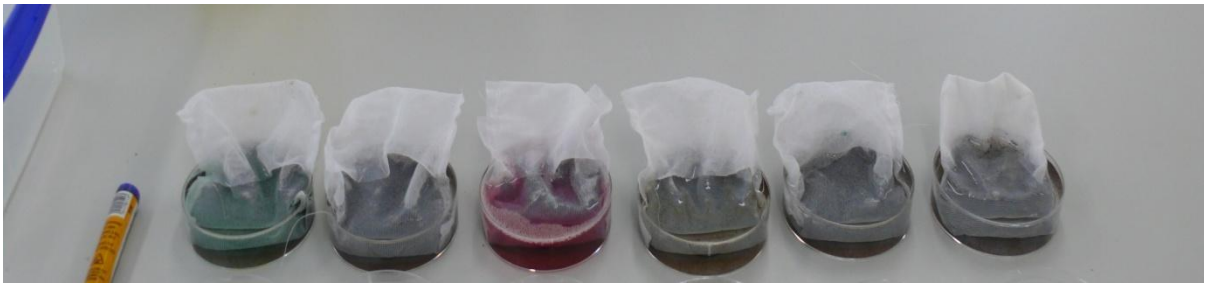

Plate 2b: Spiked soil in bags soaked in disinfectant solutions.

Experiment 5: The ability of disinfectants to kill PTA in soil on boots

The ability of the disinfectants to kill PTA in soil adhering to rubber-soled gum-boots utilised the following approach. The boot was surface sterilised by scrubbing with 95% ethanol, then rinsing three times with sterile RO water (the same boot was used throughout the experiment). A sterile cotton swab sample from the sole surface was taken before the boot was treated. The boot was then pressed into spiked soil (Plate 3a). The boots were then cleaned by spraying the boot to run-off using hand-held, commercial pump-packs sprayer containing the disinfectants at label rates (Plate 3b). The "rinsate" from each of the chemical treatments was collected (Plate 3c) and plated (one or two plates depending upon volume of rinsate collected) to P_5ARP selective agar (15 ml per plate).

Plate 3a: Boot pressed into soil

Plate 3b: Boot sprayed

Plate 3c: Rinsate collected

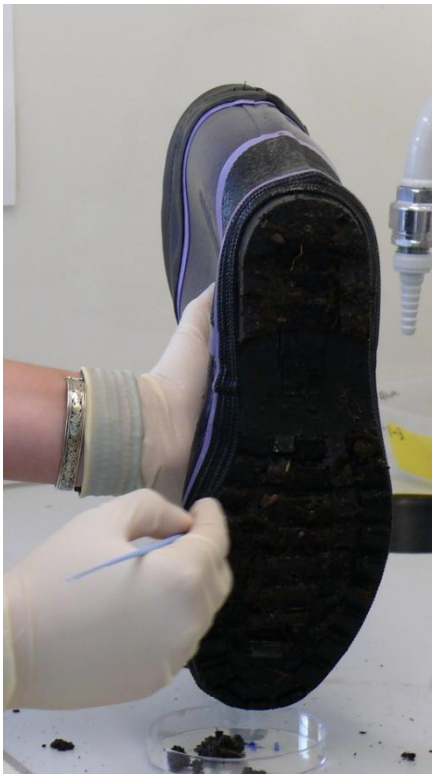

Plate 3d: Treated soil collected

The treated soil left adhering to the boot was scraped off after the spray treatment (Plate 3d) and bioassayed for PTA using the extended leaf-bait soil bioassay technique described in Experiment 3 (Plate 4).

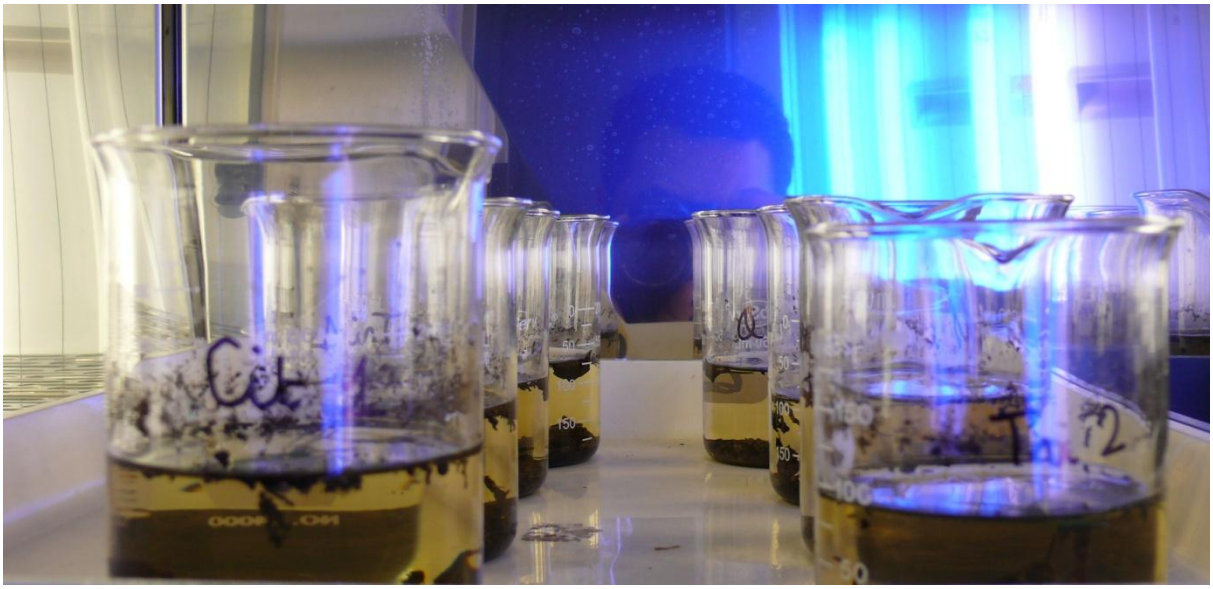

Plate 4: Leaf-bait soil bioassays after 4 days incubation in blue and cool white fluorescent light

Percentage data of leaf baits colonised by PTA before and after spray treatment with disinfectants, were transformed using the angular transformation (i.e. arcsine of the square root of each proportion). Data were then compared using a t-test. The experiment was repeated twice.

4. Results

Experiment 1: Sensitivity of PTA mycelium to disinfectants

Table 2 provides a summary of the sensitivity of PTA growth rates when grown on agar amended with the five hygiene products at five concentrations. TriGene and Phytoclean completely suppressed growth of PTA mycelium at all concentrations incorporated into agar (Table 2). Virkon (at 0.2 and 0.1% a.i.) completely suppressed growth of PTA: at 0.05% a.i. it reduced growth by 77%; at 0.025% a.i. it reduced growth by 54%; and at 0.0125% a.i. it reduced growth by 27% (compared with the control). Janola (at 0.2, 0.1, 0.05% a.i.) completely suppressed PTA: at 0.025% a.i. it inhibited growth by 54%; and at 0.0125 % a.i. it inhibited growth by 18% (compared with the control). Citricidal inhibited PTA growth at all concentrations, but did not kill the mycelium in the plug.

Table 2: Growth rates (mm/day) of PTA grown on agar amended with 5 disinfectants at 5 concentrations (mean of five replicates) after 4 days

	C1	C2	C3	C4	C5
	0.2% a.i.	**0.1% a.i.**	**0.05% a.i.**	**0.025% a.i.**	**0.0125% a.i.**
TriGene	0	0	0	0	0
Phytoclean	0	0	0	0	0
Virkon	0	0	0.6	1.45	2.10
Janola	0	0	0	1.25	2.30
Citricidal	0	0	0	0	0
Control			2.75		

Table 3 provides a summary of the ability of the PTA agar plugs exposed to the disinfectants to re-commence growth on fresh, unamended PDA agar. TriGene and Phytoclean resulted in complete mortality of the PTA in the plug at all concentrations (Table 3). Virkon was lethal at 0.2 and 0.1% a.i. Janola was lethal at a.i. concentrations of between 0.2 and 0.05%. Citricidal demonstrated *fungistasis* at all concentrations i.e. inhibited growth, but did not result in mortality at any of the concentrations assessed.

Table 3: Ability of PTA to re-grow from plugs exposed to the 5 disinfectants at 5 concentrations after 10 days. Results display re-growth after 4 days on fresh, unamended PDA (data are the number of plugs out of five that commenced growth).

	C1	C2	C3	C4	C5
	0.2% a.i.	**0.1% a.i.**	**0.05% a.i.**	**0.025% a.i.**	**0.0125% a.i.**
TriGene	0/5	0/5	0/5	0/5	0/5
Phytoclean	0/5	0/5	0/5	0/5	0/5
Virkon	0/5	0/5	5/5	5/5	5/5
Janola	0/5	0/5	0/5	5/5	5/5

	C1 0.2% a.i.	C2 0.1% a.i.	C3 0.05% a.i.	C4 0.025% a.i.	C5 0.0125% a.i.
Citricidal	5/5	5/5	5/5	5/5	5/5
Control	5/5	5/5	5/5	5/5	5/5

Experiment 2: Sensitivity of PTA oospores to disinfectants

No activated oospores (i.e. staining red in BDH) were observed in any of the hygiene treatments (Figure 1).

The majority of the oospores (approx. 80%) in the unamended control were dormant (pink bars; Fig. 1). About 10% of the oospores were activated (i.e. red oospores) and the remainder (about 10%) were non-viable (i.e. stained black).

In comparison, Virkon and Janola significantly reduced on oospore viability (Fig. 1). Virkon killed significantly more oospores than Janola – and both Virkon and Janola were more lethal than TriGene, Phytoclean or Citricidal (6 drops/100 ml). There was no difference in response between the oospores exposed to either TriGene or Citricidal, with Phytoclean having a greater negative impact than either of these two treatments (see Appendix 2 for Chi-squared results).

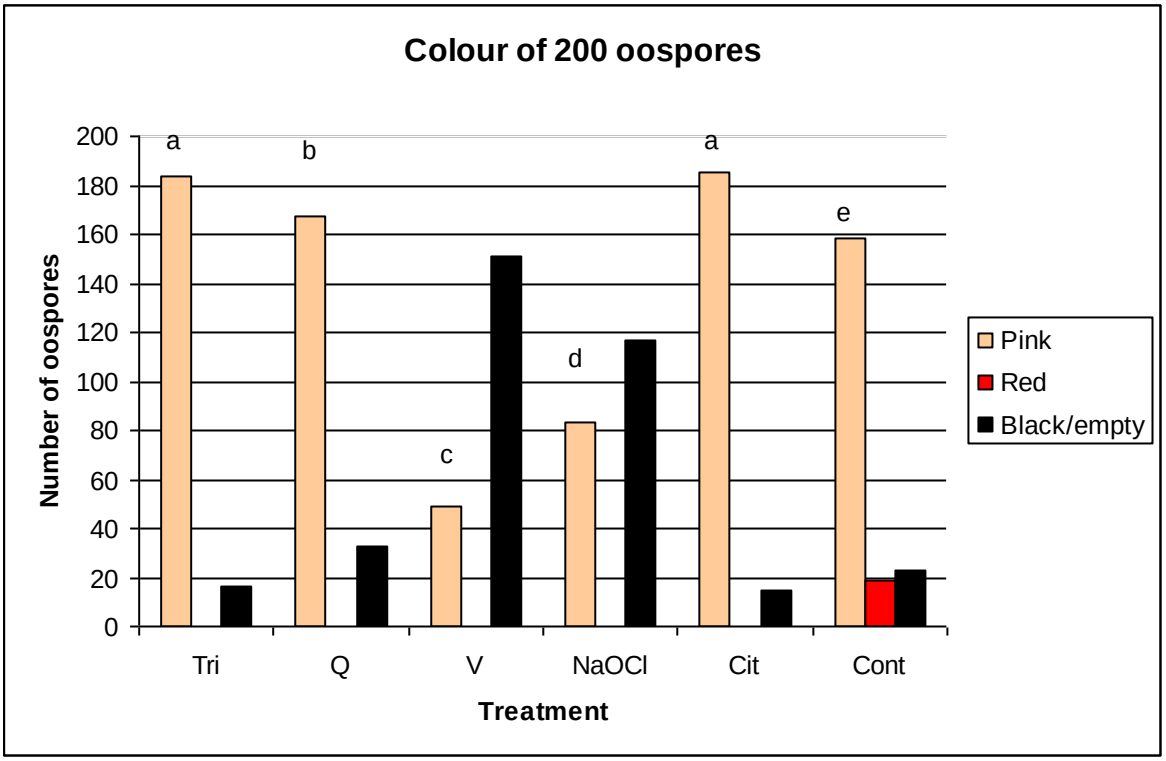

Figure 1: Oospore viability counts after 10-days being incubated in the 5 disinfectants (Tri = TriGene; Q = Phytoclean; V = Virkon; NaOCl = Janola; Cit = Citricidal; Cont = unamended control). Bars with the same letter are not significantly different (*P* = 0.05). Bar colour: Pink = dormant oospores, red = active spores, black = non-viable spores.

Experiment 2b: Sensitivity of PTA zoospores to disinfectants

Trigene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%) all proved lethal to zoospores (Table 4). The zoospores that were placed in the Citricidal and Control (i.e. RO water) survived the treatment and produced a mean of 784 ±38, and 404 ± 70 colonies of PTA/ml respectively, after 3 days (see Appendix 2 for raw data).

Table 4: Survival of PTA zoospores after being treated with disinfectants at label/recommended rates. Data represent mean number of PTA colonies/ml after 3 days growth on P_5ARP (data are means ± s.e.m., n = 5).

Disinfectant treatment	Mean CFUs of PTA/ml
TriGene (2%)	0
Phytoclean (10%)	0
Virkon (1%)	0
Janola (5%)	0
Citricidal	784 ± 38
Control	404 ± 70

Experiment 3: Soil spiking and quantification of PTA infection potential

The spiked soil containing 2000 oospores/g of soil colonised approximately 37% of the leaf baits (Table 5). In comparison, the PTA recovery from the unamended Huia Composite field soil was about 3% (1 out of 30 baits from three repeats of the experiment). However, due to the high degree of variability *within* each of the oospore concentration treatments, there was no significant difference *between* treatments (*t*-value of 2.776, α=0.05; see Appendix 2 for *t*-table). From the soil bioassay water, PTA was only recovered from the soil containing 2000 oospores/g (Table 5). No CFU's of PTA were obtained from the Huia Composite soil.

Table 5: Results of spiked soil bioassay trial to enumerate PTA inoculum. Data represent number of leaf baits colonised out of 30 and mean number CFUs/ml on P_5ARP (n = 3). Leaf bait proportions with same subscripted letter are not significantly different at *P* = 0.05.

Oospore concentration	Leaf Baits	Mean CFU's/ml from soil bioassay	
		PTA	Other fungi
2000 oospores/g	$11/30_a$	23±33	233 ± 23
1000 oospores/g	$7/30_a$	0	87 ± 40
500 oospores/g	$4/30_a$	0	30 ± 19
250 oospores/g	$1/30_a$	0	0
125 oospores/g	$0/30_a$	0	150 ± 85
Huia Composite	$1/30_a$	0	76 ± 11

Experiment 4: Ability of disinfectants to kill/suppress PTA in soil

Spiked soil containing 1500 PTA oospores/g, which was soaked in TriGene (2%) and Phytoclean (10%), completely suppressed PTA and all soil fungi/bacteria (Table 6). Virkon (1%) and Janola (5%) also completely suppressed PTA (Table 6).

However, Virkon- and Janola-treatment soil did not suppress all soil fungi and bacteria (Table 6). 17.5±24.8 and 45.0±26.1 CFUs/ml of a commonly recovered zygomycete were found in the soils treated with Virkon and Janola respectively. Approximately 12.5 CFUs/ml of bacteria were also associated with the Virkon treated soil.

In comparison to the above four disinfectant treatments, Citricidal and RO water (i.e. control) did not suppress PTA. PTA was recovered from 10% of leaf baits from spiked soil soaked in Citricidal and RO water. PTA CFUs were only recovered from the soil bioassay water from the soils treated in Citricidal and RO water (Table 6).

Table 6: Ability of disinfectants to kill PTA in soil. Data represent total number of leaf baits colonised out of 30 and mean number of colonies formed on P_5ARP after 3 days (n = 3).

Soil treatment	Leaf Baits	Mean CFUs/ml from soil bioassay water		
		PTA	Zygomycetes	Bacteria
TriGene (2%)	0	0	0	0
Phytoclean (10%)	0	0	0	0
Virkon (1%)	0	0	17.5 ± 24.8	12.5 ± 17.7
Janola (5%)	8 (zygomycetes only)	0	45.0 ± 26.1	0
Citricidal	3 PTA 1 *Phytophthora cinnamomi* 4 zygomycetes	5.0 ± 5.8	47.5 ± 41.0	13.3 ± 14.1
RO water Control	3 PTA 1 *Pythium* sp. 8 zygomycetes	10.0 ± 5.7	44.7 ± 12.0	67.0 ± 23.0

Experiment 5: The ability of disinfectants to kill PTA in soil on boots

Rinsates collected from spray-treated boots

PTA was not recovered from the rinsate of boots sprayed with TriGene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%) (Table 7). In a similar trend to that observed in Experiment 4, TriGene and Phytoclean completely suppressed all soil fungi (compare Table 7 with Table 6).

Table 7: Soil fungi and PTA recoveries from spray rinsate collected from boots being treated with hygiene treatments. Data represent mean number of CFUs/ml (n=2).

Soil treatment	Mean CFUs/ml formed on P_5ARP
TriGene (2%)	0

Soil treatment	Mean CFUs/ml formed on P_5ARP
Phytoclean (10%)	0
Virkon (1%)	15.0 ± 5.0
Janola (5%)	20.0 ± 10.0
Citricidal	115.0 ± 65.0*****
Control	260.0 ± 30.0*****

* **indicates PTA confirmed.**

Colonies of common soil zygomycetes were recovered from the rinsates produced from treatments with Virkon, Janola, Citricidal and the RO water treated control. Significantly, PTA was only recovered from the rinsates resulting from spray treatments with Citricidal and RO water (Table 7).

Before and after treatment swabs

The swabs taken from the boots before and between treatments returned negative results, i.e. no PTA (Table 8). There was, however, some carry-over between treatments, with a maximum of 20.0 CFUs/ml recovered between treatments (Table 8).

The swabs taken after the soil was removed from the spray-treated boot returned a number of soil fungi. PTA was only recovered from the boot soil treated with Citricidal and RO water (control). PTA was not recovered from boots sprayed with TriGene, Phytoclean, Virkon and Janola.

Table 8: Soil fungi and PTA recovered from cotton swabs taken before and after spray treatment of soil on boots. Data represent the mean number of CFUs/ml (n = 3).

Soil treatment	Number of colonies from swabs before spray treatment	Number of colonies from swabs after spray treatment
TriGene (2%)	2.5 ± 3.5	295.0 ± 3.0 **(no PTA)**
Phytoclean (10%)	2.5 ± 3.5	442.0 ± 3.0 **(no PTA)**
Virkon (1%)	20.0 ± 16.0	190.0 ± 160 **(no PTA)**
Janola (5%)	20.0 ± 19.8	165.0 ± 20.0 **(no PTA)**
Citricidal	0	470.0 ± 142*** (PTA)**
Control	5.0 ± 4.1	315.0 ± 4.0*** (PTA)**

* **indicates PTA confirmed.**

Soil bioassay of soil before and after spray treatment

Before spray treatment, all spiked soils produced PTA on leaf baits (Table 9), which confirmed the infective potential of the artificially spiked soil.

Spray treatment of spiked soil on boots with TriGene (2%), Phytoclean (10%) and Virkon (1%) significantly decreased the number of leaf baits colonised by soil fungi and completely suppressed PTA (Table 9).

Janola did not significantly decrease the amount of soil fungi in total, but did suppress PTA after spray application (Table 9).

Post-spray treatment, PTA was only recovered from boots sprayed with Citricidal and/or RO water (Table 9).

Table 9: PTA recoveries from leaf-bait soil bioassay before and after treatment with spray disinfectants. Data represents the proportion of leaf baits colonised by PTA (n = 2; total of 20 leaf baits). Data with same subscripted letter are not significantly different at *P* = 0.05.

Soil treatment	Colonised leaf baits (before spray treatment)	Colonised leaf baits (after spray treatment)
TriGene (2%)	20/20*$_a$	3/20$_c$
Phytoclean (10%)	20/20*$_a$	0/20$_c$
Virkon (1%)	17/20*$_a$	0/20$_c$
Janola (5%)	17/20*$_a$	7/20$_{a,b}$
Citricidal	16/20*$_b$	7/20*$_b$
Control	11/20*$_b$	12/20*$_b$

*** indicates PTA confirmed.**

5. Conclusions

Expt 1. Sensitivity of PTA mycelium to disinfectants

TriGene and Phytoclean completely suppressed growth (i.e. preventing it from growing or developing) of PTA mycelium at all *in vitro* concentrations tested. Only TriGene and Phytoclean resulted in complete mortality of the hyphae of the pathogen contained in the mycelial plug. Both Virkon (at 0.2 and 0.1% a.i.) and Janola (at 0.2, 0.1, 0.05% a.i.) completely suppressed PTA, but only at higher *in vitro* concentrations. Citricidal was demonstrated to be fungistatic (i.e. inhibited growth but did not kill the mycelium) at all concentrations.

Expt 2. Sensitivity of PTA oospores to disinfectants

The majority of the oospores in the unamended control were dormant (approx. 80%). The unamended control was the only treatment where activated oospores were observed (about 10%), and the remainder were non-viable (approx. 10%). Virkon (0.2% a.i.) and Janola (0.05% a.i.) had the most significant impact on oospore viability. Virkon killed significantly more oospores than Janola – and both Virkon and Janola were more lethal than TriGene (0.0125% a.i.), Phytoclean (0.0125% a.i.), or Citricidal – but again, only at higher *in vitro* concentrations than either TriGene or Phytoclean.

Expt 2b. Sensitivity of PTA zoospores to disinfectants

The zoospores that were placed into Trigene (2%), Phytoclean (10%), Virkon (1%) and Janola (5%), did not survive the treatment. The zoospores that were placed in the Citricidal and RO water Control survived the treatment and produced colonies of PTA after 2 days.

Expt 3. Infective capacity of PTA inoculum

The soil "spiked" with 2000 oospores / g of PTA colonised 37% of leaf baits – confirming the infective capacity of PTA oospore-inoculum.

Expt 4. Ability of disinfectants to kill PTA in soil

Soaking the spiked soil in TriGene (2%) and Phytoclean (10%) completely suppressed PTA and all soil fungi. Virkon (1%) and Janola (5%) suppressed PTA, but soil treated with Virkon (1%) and Janola (5%) did not suppress all soil fungi and bacteria.

Expt 5. The ability of disinfectants to kill PTA in soil on boots

PTA was not recovered from the rinsate collected from the sole of rubber gum-boots sprayed with TriGene (2%), Phytoclean (10%), Virkon (1%) and/or Janola (5%). The rinsate collected after spraying with Citricidal and RO water did not suppress PTA. Spray treatment of spiked soil on boots with TriGene, Phytoclean and Virkon significantly decreased the number of leaf baits colonised by soil fungi and completely suppressed PTA. Janola did not significantly decrease the amount of soil fungi in total, but did suppress PTA after spray application.

6. Comparative summary of hygiene performance

- Disinfecting soiled rubber gum-boots with a 2% spray treatment of TriGene will effectively suppress the inoculum of PTA. TriGene achieves this in part, by being biocidal to PTA mycelium. TriGene could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA. TriGene effectively suppresses the infective capacity of PTA in soil at its recommended label rate of 2%.

- Phytoclean demonstrated a similar efficacy to TriGene in that it completely suppressed the growth of PTA by killing the mycelium. Phytoclean could also limit the spread of propagules of PTA, by its ability to kill zoospores of PTA at its label/recommended rate (i.e. 10%). Phytoclean demonstrated efficacy at its label/recommended rate to effectively suppress the infective capacity of PTA inoculum contained in soil.

- Virkon only suppressed growth of PTA at higher *in vitro* concentrations, and at these concentrations it was lethal to mycelium. Virkon could also limit the spread of propagules of PTA through its ability to kill zoospores of PTA at its label/recommended rate (i.e. 1%). Virkon (at 1%) also demonstrated efficacy at suppressing PTA's infective capacity in soil when applied as a spray-treatment.

- Janola demonstrated a similar efficacy to Virkon in that it completely suppressed the growth of PTA by killing the mycelium at higher *in vitro* concentrations. Janola could also limit the spread of propagules of PTA, through its ability to kill zoospores of PTA at its label/recommended rate (i.e. 5%). Janola (5%) demonstrated efficacy at its recommended rate to effectively suppress the spread of PTA inoculum contained in soil when applied as a spray-treatment, but did not suppress all soil fungi.

- Citricidal, while demonstrating good *in vitro* fungistatic inhibition of PTA, did not kill mycelium like the other disinfectants. Citricidal had no effect on zoospores of PTA, and did not reduce the infective capacity of PTA contained in soil.

- Spraying with RO water alone does not reduce the infective capacity of PTA contained in soil.

7. Recommendations

TriGene II Advance (2%) is a suitable hygiene prescription for controlling PTA, effectively killing propagules of PTA, and reducing the infective capacity of soil containing PTA.

Quaternary ammonium compounds registered for phytosanitary applications in New Zealand (e.g., Sterbac™, Trimove®, Flurosan®) should be considered as alternative hygiene options for controlling PTA should TriGene become unavailable. We consider it likely they will behave similarly to Phytoclean. An alternative could be to encourage registration of Phytoclean in NZ, as we have shown this to be efficacious against PTA.

Further research is necessary to understand the difference between *in vitro* concentrations and spray-efficacy of Virkon and Janola at recommended/label rates. The "mode of action" of these two disinfectants may explain, in part, why spray-treatments of Virkon and Janola effectively suppress the spread of PTA inoculum contained in soil.

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Appendix 1 Media Recipes

Potato Dextrose Agar (PDA)

Difco™ PDA	39 g
RO water	1 litre

Autoclave at 121ºC for 15 minutes at 15 p.s.i. (15 ml per plate)

V8 juice agar

V8 Juice	200 ml
$CaCO_3$	3.0 g
RO water	800 ml
Agar	15.0 g

Autoclave at 121ºC for 15 minutes at 15 p.s.i. (15 ml per plate)

Clarified V8 juice broth

Clarified V8 juice	100 ml
$CaCO_3$	2% (in 100 ml)
RO water	800 ml

Clarify V8 juice by centrifugation at 4000 rpm for 15 minutes. Vacuum filter three times through one layer of Whatman No. 42 filter paper and twice through two layers.

Autoclave at 121º C for 15 minutes at 15 p.s.i.

Sterile soil extract:

Garden soil	200 grams of soil (collcted from landscaped area in Tamaki carpark, 231 Morrin Road, St Johns)
RO water	1 litre

Stirred vigorously for 2 minutes, then stirred vigorously again 30 minutes later and allowed to stand overnight.

The solution was filtered through paper hand towel, bottled and autoclaved at 121º C for 15 minutes at 15 p.s.i. Stored in refrigerator.

PARP-CMA Selective Medium for *Phytophthora* species

Difco corn meal agar	17 g
RO water	1 litre
Pimaricin	5 mg/l
Sodium Ampicillin	250 mg/l
Rifamycin-SV (sodium salt)	10 mg/l
PCNB (75%)	66.7 mg/l

Autoclave at 121ºC for 15 minutes at 15 p.s.i. (15 ml / plate).

Appendix 2 Raw data and statistical analysis

EXPERIMENT 1 RAW DATA

Growth of the PTA colonies

		Growth in mm			
		2 days	4 days	5 days	8 days
TriC1R1	*REB 316-1, TriGene Advance 0.2% a.i, rep 1*				
TriC1R2	*REB 316-1, TriGene Advance 0.2% a.i, rep 2*				
TriC1R3	*REB 316-1, TriGene Advance 0.2% a.i, rep 3*				
TriC1R4	*REB 316-1, TriGene Advance 0.2% a.i, rep 4*				
TriC1R5	*REB 316-1, TriGene Advance 0.2% a.i, rep 5*				
TriC2R1	*REB 316-1, TriGene Advance 0.1% a.i, rep 1*				
TriC2R2	*REB 316-1, TriGene Advance 0.1% a.i, rep 2*				
TriC2R3	*REB 316-1, TriGene Advance 0.1% a.i, rep 3*				
TriC2R4	*REB 316-1, TriGene Advance 0.1% a.i, rep 4*				
TriC2R5	*REB 316-1, TriGene Advance 0.1% a.i, rep 5*				
TriC3R1	*REB 316-1, TriGene Advance 0.05% a.i, rep 1*				
TriC3R2	*REB 316-1, TriGene Advance 0.05% a.i, rep 2*				
TriC3R3	*REB 316-1, TriGene Advance 0.05% a.i, rep 3*				
TriC3R4	*REB 316-1, TriGene Advance 0.05% a.i, rep 4*				
TriC3R5	*REB 316-1, TriGene Advance 0.05% a.i, rep 5*				
TriC4R1	*REB 316-1, TriGene Advance 0.025% a.i, rep 1*				
TriC4R2	*REB 316-1, TriGene Advance 0.025% a.i, rep 2*				
TriC4R3	*REB 316-1, TriGene Advance 0.025% a.i, rep 3*				
TriC4R4	*REB 316-1, TriGene Advance 0.025% a.i, rep 4*				
TriC4R5	*REB 316-1, TriGene Advance 0.025% a.i, rep 5*				
TriC5R1	*REB 316-1, TriGene Advance 0.0125% a.i, rep 1*				
TriC5R2	*REB 316-1, TriGene Advance 0.0125% a.i, rep 2*				
TriC5R3	*REB 316-1, TriGene Advance 0.0125% a.i, rep 3*				
TriC5R4	*REB 316-1, TriGene Advance 0.0125% a.i, rep 4*				
TriC5R5	*REB 316-1, TriGene Advance 0.0125% a.i, rep 5*				
VirC1R1	*REB 316-1, Virkon S 0.2% a.i, rep 1*				
VirC1R2	*REB 316-1, Virkon S 0.2% a.i, rep 2*				
VirC1R3	*REB 316-1, Virkon S 0.2% a.i, rep 3*				
VirC1R4	*REB 316-1, Virkon S 0.2% a.i, rep 4*				
VirC1R5	*REB 316-1, Virkon S 0.2% a.i, rep 5*				
VirC2R1	*REB 316-1, Virkon S 0.1% a.i, rep 1*			<1	~1
VirC2R2	*REB 316-1, Virkon S 0.1% a.i, rep 2*			<1	~1
VirC2R3	*REB 316-1, Virkon S 0.1% a.i, rep 3*			<1	~1
VirC2R4	*REB 316-1, Virkon S 0.1% a.i, rep 4*			<1	~1
VirC2R5	*REB 316-1, Virkon S 0.1% a.i, rep 5*			<1	~1
VirC3R1	*REB 316-1, Virkon S 0.05% a.i, rep 1*	3	3	2	5
VirC3R2	*REB 316-1, Virkon S 0.05% a.i, rep 2*	2	2	1.5	5
VirC3R3	*REB 316-1, Virkon S 0.05% a.i, rep 3*	2	2	1.5	5
VirC3R4	*REB 316-1, Virkon S 0.05% a.i, rep 4*	2	3	1.5	5
VirC3R5	*REB 316-1, Virkon S 0.05% a.i, rep 5*	2	2	1.5	6
VirC4R1	*REB 316-1, Virkon S 0.025% a.i, rep 1*	5	5	3	9
VirC4R2	*REB 316-1, Virkon S 0.025% a.i, rep 2*	5	6	2.5	10
VirC4R3	*REB 316-1, Virkon S 0.025% a.i, rep 3*	5	6	2.5	10
VirC4R4	*REB 316-1, Virkon S 0.025% a.i, rep 4*	4	6	2.5	10
VirC4R5	*REB 316-1, Virkon S 0.025% a.i, rep 5*	4	6	2.5	9
VirC5R1	*REB 316-1, Virkon S 0.0125% a.i, rep 1*	8	8	4	12
VirC5R2	*REB 316-1, Virkon S 0.0125% a.i, rep 2*	7	8	2.5	11

VirC5R3	*REB 316-1, Virkon S 0.0125% a.i, rep 3*	7	9	3	12
VirC5R4	*REB 316-1, Virkon S 0.0125% a.i, rep 4*	7	9	3	12
VirC5R5	*REB 316-1, Virkon S 0.0125% a.i, rep 5*	8	8	3	11
NaOCl1R1	*REB 316-1, NaHypochlorite 0.2% a.i, rep 1*				
NaOCl1R2	*REB 316-1, NaHypochlorite 0.2% a.i, rep 2*				
NaOCl1R3	*REB 316-1, NaHypochlorite 0.2% a.i, rep 3*				
NaOCl1R4	*REB 316-1, NaHypochlorite 0.2% a.i, rep 4*				
NaOCl1R5	*REB 316-1, NaHypochlorite 0.2% a.i, rep 5*				
NaOCl2R1	*REB 316-1, NaHypochlorite 0.1% a.i, rep 1*				
NaOCl2R2	*REB 316-1, NaHypochlorite 0.1% a.i, rep 2*				
NaOCl2R3	*REB 316-1, NaHypochlorite 0.1% a.i, rep 3*				
NaOCl2R4	*REB 316-1, NaHypochlorite 0.1% a.i, rep 4*				
NaOCl2R5	*REB 316-1, NaHypochlorite 0.1% a.i, rep 5*				
NaOCl3R1	*REB 316-1, NaHypochlorite 0.05% a.i, rep 1*				
NaOCl3R2	*REB 316-1, NaHypochlorite 0.05% a.i, rep 2*				
NaOCl3R3	*REB 316-1, NaHypochlorite 0.05% a.i, rep 3*				
NaOCl3R4	*REB 316-1, NaHypochlorite 0.05% a.i, rep 4*				
NaOCl3R5	*REB 316-1, NaHypochlorite 0.05% a.i, rep 5*				
NaOCl4R1	*REB 316-1, NaHypochlorite 0.025% a.i, rep 1*		5	2.5	12
NaOCl4R2	*REB 316-1, NaHypochlorite 0.025% a.i, rep 2*		5	2.5	12
NaOCl4R3	*REB 316-1, NaHypochlorite 0.025% a.i, rep 3*		5	3	11
NaOCl4R4	*REB 316-1, NaHypochlorite 0.025% a.i, rep 4*		6	2.5	11
NaOCl4R5	*REB 316-1, NaHypochlorite 0.025% a.i, rep 5*		4	2.5	10
NaOCl5R1	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 1*	4	9	4	14
NaOCl5R2	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 2*	5	9	4	13
NaOCl5R3	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 3*	5	10	4	13
NaOCl5R4	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 4*	4	9	4	14
NaOCl5R5	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 5*	4	9	4	13
CitC1R1	*REB 316-1, Citricidal 20 drops rep 1*				
CitC1R2	*REB 316-1, Citricidal 20 drops rep 2*				
CitC1R3	*REB 316-1, Citricidal 20 drops rep 3*				
CitC1R4	*REB 316-1, Citricidal 20 drops rep 4*				
CitC1R5	*REB 316-1, Citricidal 20 drops rep 5*				
CitC2R1	*REB 316-1, Citricidal 12 drops rep 1*				
CitC2R2	*REB 316-1, Citricidal 12 drops rep 2*				
CitC2R3	*REB 316-1, Citricidal 12 drops rep 3*				
CitC2R4	*REB 316-1, Citricidal 12 drops rep 4*				
CitC2R5	*REB 316-1, Citricidal 12 drops rep 5*				
CitC3R1	*REB 316-1, Citricidal 6 drops rep 1*				
CitC3R2	*REB 316-1, Citricidal 6 drops rep 2*				
CitC3R3	*REB 316-1, Citricidal 6 drops rep 3*				
CitC3R4	*REB 316-1, Citricidal 6 drops rep 4*				
CitC3R5	*REB 316-1, Citricidal 6 drops rep 5*				
CitC4R1	*REB 316-1, Citricidal 3 drops rep 1*				
CitC4R2	*REB 316-1, Citricidal 3 drops rep 2*				
CitC4R3	*REB 316-1, Citricidal 3 drops rep 3*				
CitC4R4	*REB 316-1, Citricidal 3 drops rep 4*				
CitC4R5	*REB 316-1, Citricidal 3 drops rep 5*				
CitC5R1	*REB 316-1, Citricidal 1 drop rep 1*				
CitC5R2	*REB 316-1, Citricidal 1 drop rep 2*				
CitC5R3	*REB 316-1, Citricidal 1 drop rep 3*				
CitC5R4	*REB 316-1, Citricidal 1 drop rep 4*				
CitC5R5	*REB 316-1, Citricidal 1 drop rep 5*				
ConC1R1	*REB 316-1, Control ,water, rep 1*	10	11	4	13
ConC1R2	*REB 316-1, Control ,water, rep 2*	10	11	4.5	11

ConC1R3	*REB 316-1, Control ,water, rep 3*	10	11	5	11
ConC2R1	*REB 316-1, Control ,water, rep 1*	9	11	4	14
ConC2R2	*REB 316-1, Control ,water, rep 2*	9	11	5	16
ConC2R3	*REB 316-1, Control ,water, rep 3*	10	11	4.5	13
ConC3R1	*REB 316-1, Control ,water, rep 1*	10	10	4	13
ConC3R2	*REB 316-1, Control ,water, rep 2*	10	11	4	12
ConC3R3	*REB 316-1, Control ,water, rep 3*	10	12	5	12
ConC4R1	*REB 316-1, Control ,water, rep 1*	11	11	5	11
ConC4R2	*REB 316-1, Control ,water, rep 2*	10	11	4	13
ConC4R3	*REB 316-1, Control ,water, rep 3*	9	11	4.5	13
ConC5R1	*REB 316-1, Control ,water, rep 1*	10	11	4.5	14
ConC5R2	*REB 316-1, Control ,water, rep 2*	9	11	4.5	14
ConC5R3	*REB 316-1, Control ,water, rep 3*	9	11	4	15

Indicates Colony has hit edge of plate

	Observations			
	2 days	4 days	5 days	8 days
TriC1R1				
TriC1R2				
TriC1R3				
TriC1R4				
TriC1R5				
TriC2R1				
TriC2R2				
TriC2R3				
TriC2R4				
TriC2R5				
TriC3R1				
TriC3R2				
TriC3R3				
TriC3R4				
TriC3R5				
TriC4R1				
TriC4R2				
TriC4R3				
TriC4R4				
TriC4R5				
TriC5R1				
TriC5R2				
TriC5R3				
TriC5R4				
TriC5R5				
VirC1R1				
VirC1R2				
VirC1R3				Mycelia growing in plug but not radial growth
VirC1R4				
VirC1R5				
VirC2R1				
VirC2R2				
VirC2R3				Mycelia growing all over plug and just starting to grow radially
VirC2R4		Mycelia still alive on plug		
VirC2R5				
VirC3R1				
VirC3R2				
VirC3R3				
VirC3R4				
VirC3R5	Mycelia growing upwards into the air rather than outwards into the media	Mycelia still growing upwards into the air rather than outwards into the media		Mycelia also growing vigorously upwards of the plug
VirC4R1				
VirC4R2				
VirC4R3				Mycelia also growing vigorously upwards of the plug
VirC4R4				
VirC4R5				
VirC5R1				
VirC5R2				Mycelia growing on top

	Observations			
	2 days	4 days	5 days	8 days
VirC5R3				of plug but not as vigorous as VirC3 & 4
VirC5R4				
VirC5R5				
NaOCl1R1				
NaOCl1R2				
NaOCl1R3				
NaOCl1R4				
NaOCl1R5				
NaOCl2R1				
NaOCl2R2				
NaOCl2R3			A few mycelial threads	A few tiny mycelial threads growing radially into agar
NaOCl2R4				
NaOCl2R5				
NaOCl3R1				
NaOCl3R2				
NaOCl3R3				
NaOCl3R4				Surprisingly no action?
NaOCl3R5				
NaOCl4R1				
NaOCl4R2				
NaOCl4R3				
NaOCl4R4				
NaOCl4R5				
NaOCl5R1				
NaOCl5R2				
NaOCl5R3				
NaOCl5R4				
NaOCl5R5				
CitC1R1				
CitC1R2				
CitC1R3				
CitC1R4			Mycelia just emerging from top of plug	Very tiny mycelia emerging from top of plug
CitC1R5				
CitC2R1				
CitC2R2				
CitC2R3				
CitC2R4			Mycelia just emerging from top of plug	Very tiny mycelia emerging from top of plug
CitC2R5				
CitC3R1				
CitC3R2				
CitC3R3			Mycelia just emerging from top of plug	Very tiny mycelia emerging from top of plug
CitC3R4				
CitC3R5				
CitC4R1				
CitC4R2				
CitC4R3			Mycelia just emerging from top of plug	Some mycelia starting to grow well on top of the plug
CitC4R4				
CitC4R5				
CitC5R1	Mycelia	Mycelia still	All have	All replicas

	Observations			
	2 days	**4 days**	**5 days**	**8 days**
CitC5R2	growing upwards on agar plug, not into the surrounding agar	growing upwards on agar plug, not into the surrounding agar	mycelia emerging on the top of plug. No radial growth	have mycelia starting to grow well on top of plug
CitC5R3				
CitC5R4				
CitC5R5				
ConC1R1				
ConC1R2				
ConC1R3				
ConC2R1				
ConC2R2				
ConC2R3				
ConC3R1				
ConC3R2				
ConC3R3				
ConC4R1				
ConC4R2				
ConC4R3				
ConC5R1				
ConC5R2				
ConC5R3				

EXPERIMENT 1: AVERAGED DATA

Average growth of the PTA colonies

Virkon

		Growth in mm			
		2 days	4 days	5 days	8 days
VirC3R1	*REB 316-1, Virkon S 0.05% a.i, rep 1*	3	3	2	5
VirC3R2	*REB 316-1, Virkon S 0.05% a.i, rep 2*	2	2	1.5	5
VirC3R3	*REB 316-1, Virkon S 0.05% a.i, rep 3*	2	2	1.5	5
VirC3R4	*REB 316-1, Virkon S 0.05% a.i, rep 4*	2	3	1.5	5
VirC3R5	*REB 316-1, Virkon S 0.05% a.i, rep 5*	2	2	1.5	6
VirC4R1	*REB 316-1, Virkon S 0.025% a.i, rep 1*	5	5	3	9
VirC4R2	*REB 316-1, Virkon S 0.025% a.i, rep 2*	5	6	2.5	10
VirC4R3	*REB 316-1, Virkon S 0.025% a.i, rep 3*	5	6	2.5	10
VirC4R4	*REB 316-1, Virkon S 0.025% a.i, rep 4*	4	6	2.5	10
VirC4R5	*REB 316-1, Virkon S 0.025% a.i, rep 5*	4	6	2.5	9
VirC5R1	*REB 316-1, Virkon S 0.0125% a.i, rep 1*	8	8	4	12
VirC5R2	*REB 316-1, Virkon S 0.0125% a.i, rep 2*	7	8	2.5	11
VirC5R3	*REB 316-1, Virkon S 0.0125% a.i, rep 3*	7	9	3	12
VirC5R4	*REB 316-1, Virkon S 0.0125% a.i, rep 4*	7	9	3	12
VirC5R5	*REB 316-1, Virkon S 0.0125% a.i, rep 5*	8	8	3	11

Virkon				
	Average growth in mm			
Concentration	**2 days**	**4 days**	**5 days**	**8 days**
0.050%	2.2	2.4	1.6	5.2
0.025%	4.6	5.8	2.6	9.6
0.0125%	7.4	8.4	3.1	11.6

n = 5

Sodium hypochlorite

		Growth in mm			
		2 days	4 days	5 days	8 days
NaOCl4R1	*REB 316-1, NaHypochlorite 0.025% a.i, rep 1*	0	5	2.5	12
NaOCl4R2	*REB 316-1, NaHypochlorite 0.025% a.i, rep 2*	0	5	2.5	12
NaOCl4R3	*REB 316-1, NaHypochlorite 0.025% a.i, rep 3*	0	5	3	11
NaOCl4R4	*REB 316-1, NaHypochlorite 0.025% a.i, rep 4*	0	6	2.5	11
NaOCl4R5	*REB 316-1, NaHypochlorite 0.025% a.i, rep 5*	0	4	2.5	10
NaOCl5R1	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 1*	4	9	4	14
NaOCl5R2	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 2*	5	9	4	13
NaOCl5R3	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 3*	5	10	4	13
NaOCl5R4	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 4*	4	9	4	14
NaOCl5R5	*REB 316-1, NaHypochlorite 0.0125% a.i, rep 5*	4	9	4	13

Sodium Hypochlorite (NaOCl)				
	Average growth in mm			
Concentration	**2 days**	**4 days**	**5 days**	**8 days**
0.025%	0	5	2.6	11.2
0.0125%	4.4	9.2	4	13.4

n = 5

Control

		Growth in mm			
		2 days	4 days	5 days	8 days
ConC1R1	*REB 316-1, Control ,water, rep 1*	10	11	4	13
ConC1R2	*REB 316-1, Control ,water, rep 2*	10	11	4.5	11
ConC1R3	*REB 316-1, Control ,water, rep 3*	10	11	5	11
ConC2R1	*REB 316-1, Control ,water, rep 1*	9	11	4	14
ConC2R2	*REB 316-1, Control ,water, rep 2*	9	11	5	16
ConC2R3	*REB 316-1, Control ,water, rep 3*	10	11	4.5	13
ConC3R1	*REB 316-1, Control ,water, rep 1*	10	10	4	13
ConC3R2	*REB 316-1, Control ,water, rep 2*	10	11	4	12
ConC3R3	*REB 316-1, Control ,water, rep 3*	10	12	5	12
ConC4R1	*REB 316-1, Control ,water, rep 1*	11	11	5	11
ConC4R2	*REB 316-1, Control ,water, rep 2*	10	11	4	13
ConC4R3	*REB 316-1, Control ,water, rep 3*	9	11	4.5	13
ConC5R1	*REB 316-1, Control ,water, rep 1*	10	11	4.5	14
ConC5R2	*REB 316-1, Control ,water, rep 2*	9	11	4.5	14
ConC5R3	*REB 316-1, Control ,water, rep 3*	9	11	4	15

Indicates that Colony has hit the edge of the plate

Control				
	Average growth in mm			
	2 days	**4 days**	**5 days**	**8 days**
Controls	9.733333	11	4.433333	13

n=15

EXPERIMENT 1: DATA AND RESULTS

Net growth

Net growth in mm

Virkon				
	Average net growth in mm			
Concentration	2 days	4 days	5 days	8 days
0.0500%	2.2	2.4	1.6	5.2
0.0250%	4.6	5.8	2.6	9.6
0.0125%	7.4	8.4	3.1	11.6

n = 5

Sodium Hypochlorite (NaOCl)				
	Average net growth in mm			
Concentration	2 days	4 days	5 days	8 days
0.0250%	0	5	2.6	11.2
0.0125%	4.4	9.2	4	13.4

n = 5

Control				
	Average net growth in mm			
	2 days	4 days	5 days	8 days
Controls	9.733333	11	4.433333	13

n = 15

Total growth

Total growth in mm (measured from start of plug each time)

Virkon				
	Average total growth in mm			
Concentration	2 days	4 days	5 days	8 days
0.0500%	2.2	4.6	6.2	11.4
0.0250%	4.6	10.4	13	22.6
0.0125%	7.4	15.8	18.9	30.5

n = 5

Sodium Hypochlorite (NaOCl)				
	Average total growth in mm			
Concentration	2 days	4 days	5 days	8 days
0.0250%	0	5	7.6	18.8
0.0125%	4.4	13.6	17.6	31

n = 5

Control				
	Average total growth in mm			
	2 days	4 days	5 days	8 days
Controls	9.733333	20.733333	25.16667	38.16667

n = 15

	Conc.	Rate of growth (mm/day)	R^2
Virkon	0.05%	1.33	0.98
	0.025%	2.72	0.993
	0.0125%	3.82	0.999

	0.025%	3.16	0.987
Sodium hypochlorite	0.0125%	3.66	0.978
Control	0%	5.07	0.999

Virkon – Average total growth in mm			
Days	0.05%	0.025	0.0125
0	0	0	0
2	2.2	4.6	7.4
4	4.6	10.4	15.8
5	6.2	13	18.9
8	11.4	22.6	30.5

Sodium hypochlorite – Average total growth in mm		
Days	0.03%	0.01%
0	0	0
2	0	4.4
4	5	13.6
5	7.6	17.6
8	18.8	31

Control – Average total growth in mm	
Days	
0	0
2	9.733333333
4	20.73333333
5	25.16666667
8	38.16666667

Note : Day 8 not included – Cultures grown to edge of plate

EXPERIMENT 2: DATA COMBINED (200 OOSPORES)

Colour of 200 oospores

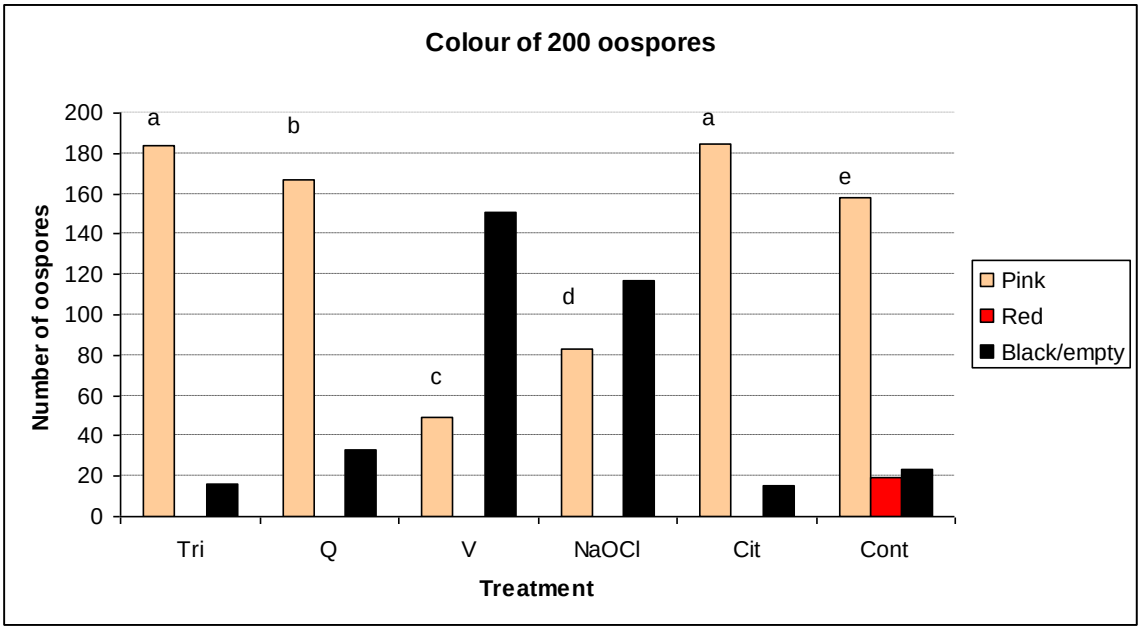
Colour of 200 oospores

	Pink	Red	Black/empty
Tri	184	0	16
Q	167	0	33
V	49	0	151
NaOCl	83	0	117
Cit	185	0	15
Cont	158	19	23

	Pink	Red	Black/empty
Tri	0.92	0	0.08
Q	0.835	0	0.165
V	0.245	0	0.755
NaOCl	0.415	0	0.585
Cit	0.925	0	0.075
Cont	0.79	0.095	0.115

Chi2 test (alpha = 0.05, critical value = 5.99)

	Distances			*Sum squares*	Chi2 (= sum squares × 200)	
	Pink	**Red**	**Black/empty**			
Tri/Cont	0.021392405	0.095	0.010652174	0.127044579	25.4089158	difference
Q/Cont	0.002563291	0.095	0.02173913	0.119302422	23.86048431	difference
V/Cont	0.375981013	0.095	3.56173913	4.032720143	806.5440286	difference
NaOCl/Cont	0.178006329	0.095	1.920869565	2.193875894	438.7751789	difference
Cit/Cont	0.02306962	0.095	0.013913043	0.131982664	26.39653275	difference
Tri/Q	0.008652695	0	0.043787879	0.052440573	10.48811468	difference
Tri/V	1.859693878	0	0.603476821	2.463170699	492.6341397	difference
Tri/NaOCl	0.614518072	0	0.435940171	1.050458243	210.0916486	difference
Tri/Cit	2.7027E-05	0	0.000333333	0.00036036	0.072072072	NO DIFFERENCE
Q/V	1.420816327	0	0.461059603	1.881875929	376.3751858	difference
Q/NaOCl	0.425060241	0	0.301538462	0.726598703	145.3197405	difference
Q/Cit	0.008756757	0	0.108	0.116756757	23.35135135	difference
V/NaOCl	0.069638554	0	0.049401709	0.119040264	23.80805272	difference
V/Cit	0.499891892	0	6.165333333	6.665225225	1333.045045	difference
NaOCl/Cit	0.281189189	0	3.468	3.749189189	749.8378378	difference



EXPERIMENT 2: Individual Replicates

Colour of 100 oospores (replicate 1)

Colour of 100 oospores

	Pink	Red	Black/empty
TriR1	100	0	0
QR3	70	0	30
VR2	28	0	72
NaOClR1	40	0	60
CitR1	94	0	6
ContR1	93	11	6

Frequency of each colour

	Pink	Red	Black/empty
TriR1	1	0	0
QR3	0.7	0	0.3
VR2	0.28	0	0.72
NaOClR1	0.4	0	0.6
CitR1	0.94	0	0.06
ContR1	0.93	0.11	0.06

Colour of 100 oospores (replicate 2)

Colour of 100 oospores

	Pink	Red	Black/empty
TriR4	84	0	16
QR4	97	0	3
VR3	21	0	79
NaOClR5	43	0	57
CitR2	91	0	9
ContR4	75	8	17

Frequency of each colour

	Pink	Red	Black/empty
TriR4	0.84	0	0.16
QR4	0.97	0	0.03
VR3	0.21	0	0.79
NaOClR5	0.43	0	0.57
CitR2	0.91	0	0.09
ContR4	0.75	0.08	0.17

Chi2 test (alpha = 0.05, critical value = 5.99)

	Distances			*Sum Squares*	*Chi2 (= sum squares × 100)*	
	Pink	**Red**	**Black/empty**			
ContR1/TriR1	0.005268817	0.11	0.06	0.175269	17.52688	difference
ContR1/QR3	0.05688172	0.11	0.96	1.126882	112.6882	difference
ContR1/VR2	0.454301075	0.11	7.26	7.824301	782.4301	difference
ContR1/NaOClR1	0.302043011	0.11	4.86	5.272043	527.2043	difference
ContR1/CitR1	0.000107527	0.11	0	0.110108	11.01075	difference
ContR4/TriR1	0.083333333	0.08	0.17	0.333333	33.33333	difference
ContR4/QR3	0.003333333	0.08	0.099411765	0.182745	18.27451	difference
ContR4/VR2	0.294533333	0.08	1.779411765	2.153945	215.3945	difference
ContR4/NaOClR1	0.163333333	0.08	1.087647059	1.33098	133.098	difference
ContR4/CitR1	0.048133333	0.08	0.071176471	0.19931	19.93098	difference
TriR1/TriR4	0.03047619	0	0.16	0.190476	19.04762	difference
QR3/QR4	0.075154639	0	2.43	2.505155	250.5155	difference
VR2/VR3	0.023333333	0	0.006202532	0.029536	2.953586	no difference
NaOClR1/NaOClR5	0.002093023	0	0.001578947	0.003672	0.367197	no difference
CitR1/CitR2	0.000989011	0.01125	0.01	0.022239	2.223901	no difference
ConTR1/ContR4	0.03483871	0.008182	0.201666667	0.244687	24.46872	difference
TriR1/QR3	0.128571429	0	0.3	0.428571	42.85714	difference
TriR1/VR2	1.851428571	0	0.72	2.571429	257.1429	difference
TriR1/NaOClR1	0.9	0	0.6	1.5	150	difference
TriR1/CitR1	0.003829787	0	0.06	0.06383	6.382979	difference

QR3/VR2	0.63	0	0.245	0.875	87.5	difference
QR3/NaOClR1	0.225	0	0.15	0.375	37.5	difference
QR3/CitR1	0.061276596	0	0.96	1.021277	102.1277	difference
VR2/NaOClR1	0.036	0	0.024	0.06	6	difference
VR2/CitR1	0.463404255	0	7.26	7.723404	772.3404	difference
NaOClR1/CitR1	0.310212766	0	4.86	5.170213	517.0213	difference

	Distances			*Sum Squares*	*Chi2 (= sum squares × 100)*	
	Pink	**Red**	**Black/empty**			
ContR1/TriR4	0.00871	0.11	0.166667	0.285376	28.53763	difference
ContR1/QR4	0.00172	0.11	0.015	0.12672	12.67204	difference
ContR1/VR3	0.557419	0.11	8.881667	9.549086	954.9086	difference
ContR1/NaOClR5	0.268817	0.11	4.335	4.713817	471.3817	difference
ContR1/CitR2	0.00043	0.11	0.015	0.12543	12.54301	difference

ContR4/TriR4	0.0108	0.08	0.000588	0.091388	9.138824	difference
ContR4/QR4	0.064533	0.08	0.115294	0.259827	25.98275	difference
ContR4/VR3	0.3888	0.08	2.261176	2.729976	272.9976	difference
ContR4/NaOClR5	0.136533	0.08	0.941176	1.15771	115.771	difference
ContR4/CitR2	0.034133	0.08	0.037647	0.15178	15.17804	difference

TriR4/QR4	0.017423	0	0.563333	0.580756	58.0756	difference
TriR4/VR3	1.89	0	0.502405	2.392405	239.2405	difference
TriR4/NaOClR5	0.39093	0	0.294912	0.685843	68.58425	difference
TriR4/CitR2	0.005385	0	0.054444	0.059829	5.982906	difference
QR4/VR3	2.750476	0	0.731139	3.481615	348.1615	difference
QR4/NaOClR5	0.67814	0	0.511579	1.189718	118.9718	difference
QR4/CitR2	0.003956	0	0.04	0.043956	4.395604	NO DIFFERENCE
VR3/NaOClR5	0.112558	0	0.084912	0.19747	19.74704	difference
VR3/CitR2	0.538462	0	5.444444	5.982906	598.2906	difference
NaOClR5/CitR2	0.253187	0	2.56	2.813187	281.3187	difference

EXPERIMENT 2b: Zoospore sensitivity data 3 Replicates 1-5

Rep 1: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	840
Cont	440

Rep 2: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	800
Cont	500

Rep 3: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	780
Cont	320

Rep 4: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	760
Cont	360

Rep 5: Zoospore CFUs/ml

	Pink
Tri	0
Q	0
V	0
NaOCl	0
Cit	740
Cont	400

	Average number of CFUs/ml	Standard deviation
Tri	0	0
Q	0	0
V	0	0
NaOCl	0	0
Cit	784	38.47076812
Cont	404	69.85699679

Experiment 3 ***Raw data and statistical analysis***

		Number of leaf baits (10 leaves/replicate)			*Total of leaf baits (out of 30)*
	Oospores / g of soil	**Replicate 1** **Leaf baits**	**Replicate 2** **Leaf baits**	**Replicate 3** **Leaf baits**	
1	2 × 103	0	8	3	11
2	1 × 103	0	5	2	7
3	500	0	4	0	4
4	250	0	0	1	1
5	125	0	0	0	0
Control	Huia Composite	0	0	0	1

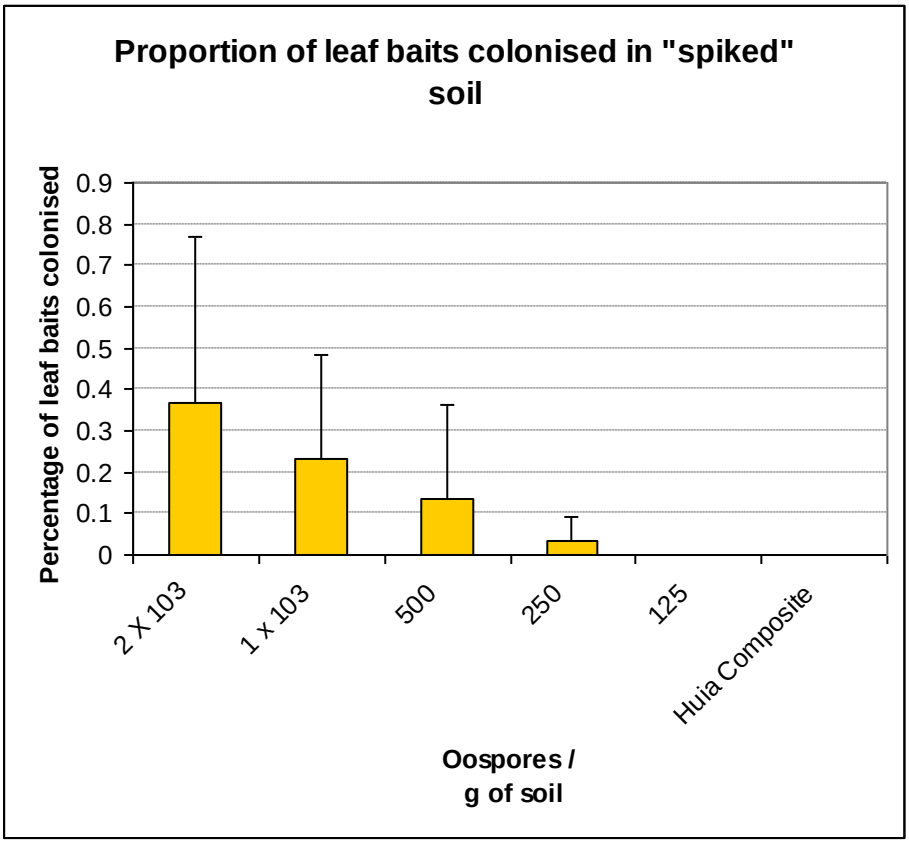
		Number of CFUs ./ ml				
Oospores/g of soil	**PTA recoveries**	**Replicate 1** **CFUs**	**Replicate 2** **CFUs**	**Replicate 3** **CFUs**	*Mean*	*Standard deviation*
2 × 103	7	220	270	210	233.3333	32.14550254
1 × 103	0	70	40	150	86.66667	56.86240703
500	0	60	10	20	30	26.45751311
250	0	0	0	0	0	0
125	0	220	220	10	150	121.2435565
Huia Composite	0	60	80	90	76.66667	15.27525232

Frequency of leaf baits

	Oospores/g of soil	**Replicate 1** **Leaf baits**	**Replicate 2** **Leaf baits**	**Replicate 3** **Leaf baits**	*Mean*	*Variance*	*SS*
1	2 × 103	0	0.8	0.3	0.366666667	0.108888889	0.326666667
2	1 × 103	0	0.5	0.2	0.233333333	0.042222222	0.126666667
3	500	0	0.4	0	0.133333333	0.035555556	0.106666667
4	250	0	0	0.1	0.033333333	0.002222222	0.006666667
5	125	0	0	0	0	0	0
Control	Huia Composite	0	0	0	0	0	0

Student test (alpha = 0.05, critical value = 2.776)

	sp2	*sX1-X2*	*t*	
1 v. Control	0.081666667	0.233333333	1.571428571	No difference
2 v. Control	0.031666667	0.145296631	1.605910137	No difference
3 v. Control	0.026666667	0.133333333	1	No difference
4 v. Control	0.001666667	0.033333333	1	No difference
5 v. Control				No difference



Angular transformation and Student test

	Oospores/g of soil	Replicate 1 Leaf baits	Replicate 2 Leaf baits	Replicate 3 Leaf baits	*Mean*	*Varpa*	*SS*
1	2 × 103	0	1.107148718	0.57963974	0.562262819	0.204447359	0.613342078
2	1 × 103	0	0.785398163	0.463647609	0.416348591	0.103926978	0.311780933
3	500	0	0.684719203	0	0.228239734	0.104186753	0.312560258
4	250	0	0	0.321750554	0.107250185	0.023005204	0.069015613
5	125	0	0	0	0	0	0
Control	Huia Composite	0	0	0	0	0	0

STUDENT TEST alpha = 0.05 limit value = 2.776

	sp2	*sX1-X2*	*t*	
1/Control	0.153335519	0.319724381	1.758585999	No difference
2/Control	0.077945233	0.227955015	1.826450674	No difference
3/Control	0.078140064	0.228239734	1	No difference
4/Control	0.017253903	0.107250185	1	No difference
5/Control				No difference

EXPERIMENT 4: After 3-days

Raw data

	Leaf baits	Soil extract	Soil extract	Soil extract
2% TriGene	0	0	0	0
2% TriGene	0	0	0	0
10% Phytoclean	0	0	0	0
10% Phytoclean	0	0	0	0
1% Virkon	0	0	0	0
1% Virkon	0	5 bact	7 zygos	0
5% NaOCl	12 zygos	0	0	0
5% NaOCl	8 zygos	3 zygos	7 zygos	0
Citricidal 6 drops/100 ml	1 pta, 1 pc, 4 zygos	7 zygos	3 zygos, 1 pta	4 zygos, 4 bact
Citricidal 6 drops/100 ml	2 pta	20 zygos	2 zygos	5 bact
Control (RO water)	1 pta, 1 py	14 zygos, 1 pta	16 bact, 16 zygos	20 bact, 10 zygos
Control (RO water)	8 zygos, 2 pta	16 zygos, 1 pta	17 bact, 10 zygos	16 bact, 5 zygos

CFUs / ml

	Mean bact	*s.d. bact*	*Mean zygo*	*s.d. zygo*	*Mean PTA*	*s.d. PTA*
2% TriGene	0	0	0	0	0	0
2% TriGene	0	0	0	0	0	0
10% Phytoclean	0	0	0	0	0	0
10% Phytoclean	0	0	0	0	0	0
1% Virkon	0	0	0	0	0	0
1% Virkon	12.5	25	17.5	35	0	0
5% NaOCl	0	0	30	60	0	0
5% NaOCl	0	0	45	36.96845502	0	0
Citricidal 6 drops/100 ml	13.33333333	20	40	17.32050808	5	5.773502692
Citricidal 6 drops/100 ml	12.5	25	55	107.0825227		
Control (RO water)	40	80	100	86.986589	10	5.773502692
Control (RO water)	40	80	97.5	46.45786622		

EXPERIMENT 5: RINSATES

CFUs / ml

	Rinsates PTA	Rinsates Zygos
2% TriGene	0	0
2% TriGene	0	0
10% Phytoclean	0	0
10% Phytoclean	0	0
1% Virkon	0	10
1% Virkon	0	20
5% NaOCl	0	10
5% NaOCl	0	30
Citricidal 6 drops/100 ml	10	180
Citricidal 6 drops/100 ml	10	50
Control (RO water)	10	290
Control (RO water)	10	230

	Mean		*Standard deviation*	
	Rinsates PTA	Rinsates Zygos	Rinsates PTA	Rinsates Zygos
2% TriGene	0	0	0	0
10% Phytoclean	0	0	0	0
1% Virkon	0	15	0	7.071068
5% NaOCl	0	20	0	14.14214
Citricidal 6 drops/100 ml	10	115	0	91.92388
Control (RO water)	10	260	0	42.42641

EXPERIMENT 5: SWABS CFUs / ml

	Swabs before Fungi	Swabs before Bacteri	Swabs after Fungi	Swabs after Bacteria
2% TriGene	0	0	230	340
2% TriGene	10	0	190	10
2% TriGene	0	0	480	630
2% TriGene	0	0	280	1000
10% Phytoclean	0	0	560	0
10% Phytoclean	1	0	440	0
10% Phytoclean	0	0	350	0
10% Phytoclean	0	0	420	0
1% Virkon	0	0	450	0
1% Virkon	0	0	290	0
1% Virkon	40	0	20	0
1% Virkon	40	0	0	0
5% NaOCl	0	0	0	0
5% NaOCl	0	1000	60	0
5% NaOCl	2	0	540	0
5% NaOCl	6	0	70	0
Citricidal 6 drops/100 ml	0	0	530	0
Citricidal 6 drops/100 ml	0	0	720	0

Citricidal 6 drops/100 ml	0	0	240	0
Citricidal 6 drops/100 ml	0	0	390	0
Control (RO water)	0	0	550	500
Control (RO water)	0	0	430	420
Control (RO water)	10	0	150	330
Control (RO water)	10	0	130	120

Mean *Standard deviation*

	Before		After		Before		After	
	Fungi	**Bacteria**	**Fungi**	**Bacteria**	**Fungi**	**Bacteria**	**Fungi**	**Bacteria**
2% TriGene	2.5	0	295	495	5	0	128.7116	421.3075
10% Phytoclean	0.25	0	442.5	0	0.5	0	87.32125	0
1% Virkon	20	0	190	0	23.09401	0	218.0214	0
5% NaOCl	2	250	167.5	0	2.828427	500	250.2499	0
Citricidal 6 drops/100 ml	0	0	470	0	0	0	204.4505	0
Control (RO water)	5	0	315	342.5	5.773503	0	208.0865	163.7834

EXPERIMENT 5: LEAF BAITS

Raw data

Number of leaf baits

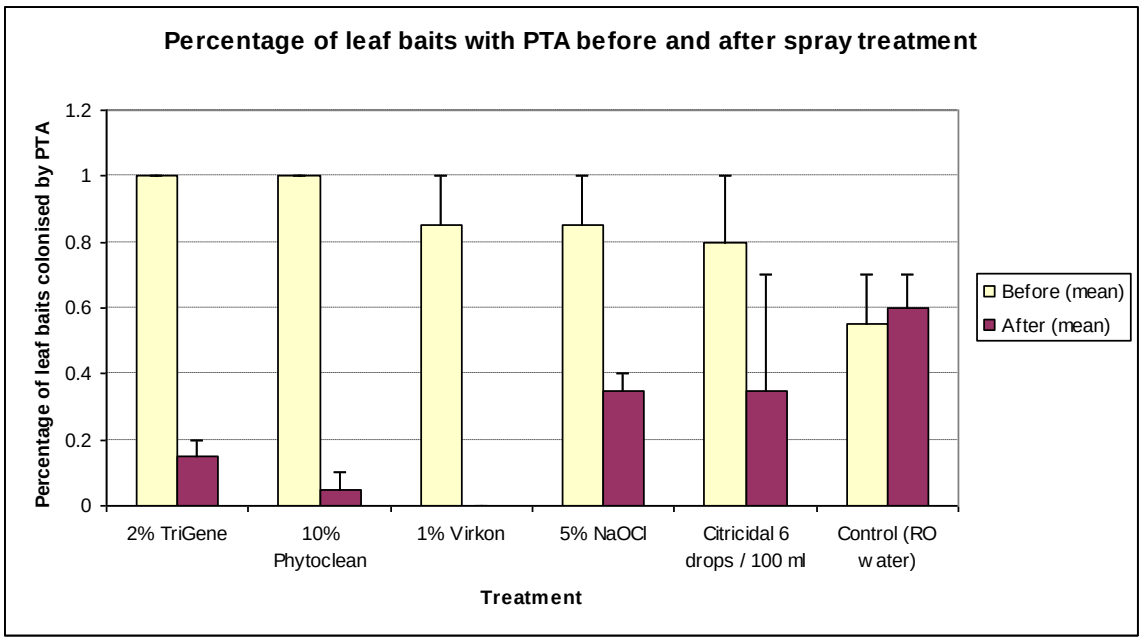
	Before	After	Total
2% TriGene	20	3	**23**
10% Phytoclean	20	1	**20**
1% Virkon	17	0	**17**
5% NaOCl	17	7	**24**
Citricidal 6 drops/100 ml	16	7	**17**
Control (RO water)	11	12	**23**
	101	**23**	**124**

Treatment	Before (Rep 1)	Before (Rep 2)	After (Rep 1)	After (Rep 2)
2% TriGene	1	1	1/5	1/10
10% Phytoclean	1	1	0	1/10
1% Virkon	1	7/10	0	0
5% NaOCl	1	7/10	2/5	3/10
Citricidal 6 drops/100 ml	3/5	1	7/10	0
Control (RO water)	7/10	2/5	7/10	1/2

	Before		After	
Treatment	*Before (mean)*	*Variance*	*After (mean)*	*Variance*
2% TriGene	1	0	0.15	0.0025
10% Phytoclean	1	0	0.05	0.0025
1% Virkon	0.85	0.0225	0	0
5% NaOCl	0.85	0.0225	0.35	0.0025
Citricidal 6 drops/100 ml	0.8	0.04	0.35	0.1225
Control (RO water)	0.55	0.0225	0.6	0.01

Student test (alpha = 0.05, critical value = 4.403)

sp2	*sX1-X*	*t*	
0.0025	0.05	17	effect of the treatment
0.0025	0.05	19	effect of the treatment
0.0225	0.15	5.666667	effect of the treatment
0.025	0.158113883	3.162278	no effect of the treatment
0.1625	0.403112887	1.116313	no effect of the treatment
0.0325	0.180277564	–0.27735	no effect of the treatment



Reduction of number of baits with lesions

Reduction of number of baits with lesions

	Rep1	**Rep2**	*Mean*	*Var*
2% TriGene	8	9	8.5	0.5
10% Phytoclean	10	9	9.5	0.5
1% Virkon	10	7	8.5	4.5
5% NaOCl	6	4	5	2
Citricidal 6 drops/100 ml	1	10	5.5	40.5
Control (RO water)	0	1	0.5	0.5

Student test (alpha=0.05, critical value=4.403)

	sp2	*sqrt(sp2)*	*t*	
Trigene/Phytoclean	0.5	0.707106781	-1.41421	no difference
Trigene/Control	0.5	0.707106781	11.3137	difference
Phytoclean/Control	0.5	0.707106781	12.7279	difference
Virkon/Control	2.5	1.58113883	5.05964	difference
Citricidal/Control	20.5	4.527692569	1.10432	no difference
NaOCl/Control	1.25	1.118033989	4.02492	difference
Trigene/Virkon	2.5	1.58113883	0	no difference
Phytoclean/Virkon	2.5	1.58113883	0.63246	no difference

Angular transformation and Student test (raw data)

Frequence of leaf baits

Before (Rep 1)	**Before (Rep 2)**	**After (Rep 1)**	**After (Rep 2)**

2% TriGene	1	1	1/5	1/10
10% Phytoclean	1	1	0	1/10
1% Virkon	1	7/10	0	0
5% NaOCl	1	7/10	2/5	3/10
Citricidal 6 drops/100 ml	3/5	1	7/10	0
Control (RO water)	7/10	2/5	7/10	1/2

Sqrt(frequence)

	Before (Rep 1)	Before (Rep 2)	After (Rep 1)	After (Rep 2)
2% TriGene	1	1	17/38	6/19
10% Phytoclean	1	1	0	6/19
1% Virkon	1	41/49	0	0
5% NaOCl	1	41/49	43/68	23/42
Citricidal 6 drops/100 ml	55/71	1	41/49	0
Control (RO water)	41/49	43/68	41/49	70/99

Arcsin(sqrt(frequence))

	Before (Rep 1)	Before (Rep 2)	After (Rep 1)	After (Rep 2)
2% TriGene	1 4/7	1 4/7	32/69	28/87
10% Phytoclean	1 4/7	1 4/7	0	28/87
1% Virkon	1 4/7	1	0	0
5% NaOCl	1 4/7	1	63/92	40/69
Citricidal 6 drops/100 ml	70/79	1 4/7	1	0
Control (RO water)	1	63/92	1	11/14

STUDENT TEST

alpha = 0.05 limit value = 4.403

	Before			After		
	Mean	*Variance*	*SS*	*Mean*	*Variance*	*SS*
2% TriGene	1 4/7	0	0	11/28	0.005033694	0.010067387
10% Phytoclean	1 4/7	0	0	14/87	0.025880855	0.05176171
1% Virkon	1 25/89	0.083995557	0.167991114	0	0	0
5% NaOCl	1 25/89	0.083995557	0.167991114	55/87	0.002760423	0.005520847
Citricidal 6 drops/100 ml	1 8/35	0.117210097	0.234420193	1/2	0.245597845	0.491195689
Control (RO water)	31/37	0.023475967	0.046951935	8/9	0.010584132	0.021168264

sp2	*sqrt(sp2)*	*t*	
0.00503	0.070948527	16.604957	effect of the treatment
0.02588	0.160875277	8.764062907	effect of the treatment
0.084	0.28981987	4.419905563	effect of the treatment
0.08676	0.294543682	2.20271907	no effect
0.36281	0.602335406	1.216694926	no effect
0.03406	0.184553785	-0.272763195	no effect

Angular transformation and Student test (reduction of number of baits)

Reduction of number of baits with lesions

	Rep1	Rep2	*Mean*	*Var*	*SS*
2% TriGene	0.4	0.45	0.425	0.000625	0.00125
10% Phytoclean	0.5	0.45	0.475	0.000625	0.00125
1% Virkon	0.5	0.35	0.425	0.005625	0.01125
5% NaOCl	0.3	0.2	0.25	0.0025	0.005
Citricidal 6 drops/100 ml	0.05	0.5	0.275	0.050625	0.10125

Control (RO water)	0	0.05	0.025	0.000625	0.00125

Reduction of number of baits with lesions (angular transformation)

	Rep1	**Rep2**	*Mean*	*Var*	*SS*
2% TriGene	0.6847192	0.735314453	0.710016828	0.00064	0.00127994
10% Phytoclean	0.78539816	0.735314453	0.760356308	0.0006271	0.001254189
1% Virkon	0.78539816	0.633051836	0.709225	0.0058024	0.011604702
5% NaOCl	0.57963974	0.463647609	0.521643675	0.0033635	0.006727087
Citricidal 6 drops/100 ml	0.22551341	0.785398163	0.505455785	0.0783677	0.156735471
Control (RO water)	0	0.225513406	0.112756703	0.0127141	0.025428148

STUDENT TEST alpha = 0.05 limit value = 4.403

	sp2	*sqrt(sp2)*	*t*	
Trigene/Phytoclean	0.00126706	0.035595847	-1.41419531	no difference
Trigene/Control	0.01335404	0.115559698	5.168411941	difference
Phytoclean/Control	0.01334117	0.115503976	5.606729959	difference
Virkon/Control	0.01851642	0.136075071	4.383376715	difference
Citricidal/Control	0.09108181	0.30179763	1.301200018	no difference
NaOCl/Control	0.01607762	0.126797546	3.224723067	no difference
Trigene/Virkon	0.00644232	0.080264068	0.009865286	no difference
Phytoclean/Virkon	0.00642945	0.080183822	0.637676114	no difference

EXPERIMENT 5: SOIL EXTRACTS

CFUs / nl

	Soil extract 1	**Soil extract 1(b)**	**Soil extract 2**	**Soil extract 2(b)**
2% TriGene (fungi)	80	0	30	90
2% TriGene (bacteria)	0	0	0	0
10% Phytoclean (fungi)	120	20	0	0
10% Phytoclean (bacteria)	0	0	0	0
1% Virkon (fungi)	50	30	0	30
1% Virkon (bacteria)	0	0	0	0
5% NaOCl (fungi)	20	70	0	30
5% NaOCl (bacteria)	60	0	0	0
Citricidal 6 drops/100 ml (fungi)	40	40	0	90
Citricidal 6 drops/100 ml (bacteria)	0	0	80	0
Control (RO water) (fungi)	50	40	60	80
Control (RO water) (bacteria)	0	0	0	0

	Mean	**Standard deviation**
2% TriGene (fungi)	50	42.42640687
2% TriGene (bacteria)	0	0
10% Phytoclean (fungi)	35	57.44562647
10% Phytoclean (bacteria)	0	0
1% Virkon (fungi)	27.5	20.61552813
1% Virkon (bacteria)	0	0
5% NaOCl (fungi)	30	29.43920289

5% NaOCl (bacteria)	15	30
Citricidal 6 drops/100 ml (fungi)	42.5	36.85557398
Citricidal 6 drops/100 ml (bacteria)	20	40
Control (RO water) (fungi)	57.5	17.07825128
Control (RO water) (bacteria)	0	0

Appendix 3 MSDS of Disinfectants Assessed in this Study

NB: MSDS Sheets Commence on Next Page

MATERIAL SAFETY DATA SHEET

1. IDENTIFICATION OF THE PRODUCT AND COMPANY

1.1	Trade Name:	TRIGENE ADVANCE LABORATORY PRE-DILUTED
1.2	Type of Product:	Disinfectant cleaner.
1.3	Manufacturer/Supplier:	MediChem International Ltd
1.4	Marketing Address:	PO Box 237, Sevenoaks, Kent TN15 0ZJ TEL: 01732 763555, FAX: 01732 763530 E-mail: info@medichem.co.uk www.medi-chem.com
1.5	Manufacturing Address:	Stalham Business Park, Rushenden Road, Queenborough, Kent ME11 5HE TEL: 01795 581151, FAX: 01795 581256

2. COMPOSITION/INFORMATION ON INGREDIENTS

2.1	Chemical type:	Halogenated Tertiary Amine	
2.2	Major ingredients:	Polymeric Biguanide Hydrochloride	<1%
		Alkyl Dimethyl Benzyl Ammonium Chloride)	<1%
		Didecyl Dimethyl Ammonium Chloride)	

3 HAZARDS IDENTIFICATION

No specific hazards

4 FIRST-AID MEASURES

(Must be taken immediately)

4.1	Inhalation:	Non-toxic: Remove to fresh air. Avoid using fine mist sprays, avoid inhalation of fine mist.
4.2	Eye contact:	Rinse eyes with water copiously for 10 minutes. Seek medical advice as necessary.
4.3	Skin contact:	Wash affected area with soap and water. Avoid prolonged contact.
4.4	Ingestion:	Do not induce vomiting. Give milk or water to drink. Seek medical advice where necessary.

5 FIRE-FIGHTING MEASURES

(Not flammable solution)

5.1	Extinguishing media:	Any available means.
5.2	Explosive quality:	Nil.
5.3	Specific hazard:	Burning of residue produces irritating fumes.
5.4	Specific protective measures for fire fighters:	Breathing apparatus should be worn

6 ACCIDENTAL RELEASE MEASURES

6.1	Environmental precautions:	Product is biodegradable under OECD conditions operational 6/1995.
6.2	Clean up method:	Flush to drain with copious water or soak up onto inert material and dispose of with clinical waste.
6.3	Clothing for disposal:	Wear gloves and apron, avoid prolonged skin contact.

7 HANDLING AND STORAGE

7.1	Handling guidelines:	Safe handling by trained professional staff in accordance with label instructions only. Not to be mixed with other chemicals. Keep from children.
7.2	Storage guidelines:	Store in dry place not below 0°C or above 30°C and out of direct sunlight. Keep lidded. Keep from foodstuffs and drinks.

8 EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1	Personal protection:	Use with care, avoid eye contact and prolonged skin contact. Gloves and safety glasses recommended if available. External use only. Not for ingestion.
8.2	Skin contact:	Low risk: may degrease skin leading to dryness if excessive contact.
8.3	Eye contact:	Low risk: may cause temporary discomfort.
8.4	Inhalation:	Low risk: avoid inhalation of fine mist spray.
8.5	Inhalation (long term):	Low risk: avoid inhalation of fine mist spray.
8.6	Ingestion:	Low risk: substantial ingestion will cause discomfort to mouth and digestive tissues.

9 PHYSICAL AND CHEMICAL PROPERTIES

9.1	Physical State:	Liquid.
9.2	Appearance and Odour:	Clear with low odour, blue with Eucalyptus fragrance
9.3	Evaporation Rate:	As water.
9.4	Boiling Point:	110°C.
9.5	Freezing Point:	-20°C.
9.6	% Volatile (by weight):	>95%.
9.7	Solubility in Water (20°C):	Soluble.
9.8	pH:	5.5 approximately.
9.9	Specific Gravity:	0.990 @ 20°C.

10 STABILITY AND REACTIVITY

10.1 No decomposition if stored and used as directed.
10.2 Hazardous decomposition products:None under normal use.
10.3 If mixed with strong alkalis, may neutralise or reduce disinfectant qualities.

11 TOXICOLOGICAL INFORMATION

11.1 Human Studies: 4 hour and 20 hours patch tests have shown minor skin reddening but no harmful effects.

12 ECOLOGICAL INFORMATION

12.1 No known adverse effects from normal use.

13 DISPOSAL CONSIDERATIONS

13.1	Packaging:	Can be disposed of as normal waste in accordance with local authority regulations.
13.2	Contaminated Packaging:	May be disposed of safely under normal conditions in accordance with local authority regulations.
13.3	Product:	Solution to be disposed of in accordance with spillage instructions as detailed in Section 6.

14 TRANSPORT INFORMATION

14.1 No special conditions apply. Not dangerous.

15 REGULATORY INFORMATION

SAFETY PHRASES

(2) Keep out of reach of children
(24/25) Avoid contact with the skin and eyes
(50) Do not mix with other chemicals

16 OTHER INFORMATION

- Not a licensed medicine

MEDICHEM
INTERNATIONAL

TRIGENE ADVANCE MICROBIOLOGICAL TESTS

ANIMAL AND ENVIRONMENTAL

ORGANISM	DILUTION	METHOD	REDUCTION
SPORICIDAL ACTIVITY			
Bacillus subtilis	1:100	EN13704	>Log 6
Clostridium difficile	1:100	EN13704	>Log 5
Clostridium sporogenes	1:100	EN13704	>Log 6
Clostridium perfringens	1:100	EN13704	>Log 6
MYCOBACTERICIDAL ACTIVITY			
Mycobacterium avium	1:100	EN14204	>Log 6
Mycobacterium bovis	1:100	EN14348	>Log 5
Mycobacterium fortuitum	1:100	EN14348	>Log 6
Mycobacterium terrae	1:100	EN14348	>Log 6
VIRUCIDAL ACTIVITY			
Canine parvovirus	1:100	EPA Protocol	complete deactivation
Feline calicivirus	1:100	EPA Protocol	complete deactivation
Feline infectious peritonitis	1:100	EPA Protocol	complete deactivation
Feline rhinotracheitis	1:100	EPA Protocol	complete deactivation
Equine herpes	1:100	EPA Protocol	complete deactivation
Infectious Bursal Disease Virus	1:100	EPA Protocol	complete deactivation
Adenovirus type 5	1:100	EPA Protocol	complete deactivation
H5N1	1:200	Harbin Veterinary Research Institute	Total kill
FUNGICIDAL ACTIVITY			
Aspergillus niger	1:200	EN13624	>Log 4
Candida albicans	1:200	EN13624	>Log 4
Cladosporium fulvum	1:200	EN1657	>Log 4
Microsporum canis	1:200	EN1657	>Log 4
Penicillium verrucosum	1:200	EN1657	>Log 4
Saccharomyces cerevisiae	1:200	EN1657	>Log 4
Trichophyton rubrum	1:200	EN13624	>Log 4
Trichophyton mentagrophytes	1:200	EN1657	>Log 4
BACTERICIDAL ACTIVITY			
Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Enterococcus hirae	1:200	EN1276 by HIRL	>Log 5
Acinetobacter calcoaceticus	1:200	EN13727	>Log 5
Bordetella bronchiseptica	1:200	EN13727	>Log 5
Campylobacter jejuni	1:200	EN1656	>Log 5
Enterococcus hirae	1:200	EN1276	>Log 6
Enterococcus faecium	1:200	EN13727	>Log 6
Escherichia coli	1:200	EN1276	>Log 6
Klebsiella pneumoniae	1:200	EN1276	>Log 6
Legionella pneumophila	1:200	EN13623	>Log 6
Listeria monocytogenes	1:200	EN13727	>Log 6
Methicillin Resistant *staphylococcus aureus*	1:200	EN13727	>Log 6
Proteus vulgaris	1:200	EN1276	>Log 6
Pseudomonas aeruginosa	1:200	EN1276	>Log 6
Rhodococcus equi	1:200	EN1276	>Log 5
Salmonella choleraesuis	1:200	EN13727	>Log 6
Salmonella dublin	1:200	EN13727	>Log 6
Salmonella enteritidis	1:200	EN13727	>Log 6
Salmonella typhimurium	1:200	EN13727	>Log 6
Serratia marcescens	1:200	EN13727	>Log 6
Staphylococcus aureus	1:200	EN1276	>Log 6

keep for file.

MEDICHEM
INTERNATIONAL

TECHNICAL BULLETIN NO. 270

TRIGENE ADVANCE – BIODEGRADABILITY TEST

We recently commissioned a biodegradability test on TriGene ADVANCE using OECD Guideline No. 301B (1992) Ready Biodegradability; CO_2 Evolution Test. A copy of this test is attached.

The study concluded that TriGene ADVANCE attained 116% degradation after 28 days.

Technical Advisory Service
April 2008

MEDICHEM International (Marketing) Limited
P. O. Box 237, Sevenoaks, Kent, TN15 0ZJ
Tel: - 01732 763555 Fax: - 01732 763530
Email: - info@medichem.co.uk

SafePharm Laboratories

SafePharm Laboratories Ltd
Shardlow Business Park
London Road, Shardlow
Derby DE72 2GD United Kingdom
Tel: 01332 792896 Fax: 01332 799018
Website: www.safepharm.co.uk

SPL PROJECT NUMBER: 2460/0002

ASSESSMENT OF READY BIODEGRADABILITY; CO_2 EVOLUTION TEST

1. INTRODUCTION

At the request of the Sponsor, Medichem International, the following study was undertaken to assess the ready biodegradability of TriGene Advance.

At the request of the Sponsor the study was conducted as a screening test using a limited number of test vessels and a reduced number of sampling occasions.

2. METHODS AND MATERIALS

2.1 Test Method

The test was based upon the following Test Guidelines - OECD Guideline No. 301B (1992) "Ready Biodegradability; CO_2 Evolution Test" referenced as Method C.4-C of Commission Directive 92/69/EEC (which constitutes Annex V of Council Directive 67/548/EEC) and US EPA Fate, Transport and Transformation Test Guidelines OPPTS 835.3110 (Paragraph (m)) but adapted to provide a screening test at the request of the Sponsor.

2.2 Test Material Description, Identification and Storage Conditions

Sponsor's identification	:	TriGene Advance
Description	:	clear colourless liquid
Date received	:	25 October 2007
Storage conditions	:	room temperature in the dark

2.3 Method of Preparation

The test material is a disinfectant, therefore following the recommendations of the Test Guidelines, in the definitive test, the test material was reduced to a concentration of 5 mg C/l to minimize possible inhibitory effects.

An amount of test material (1000 mg) was dissolved in culture medium and the volume adjusted to 1 litre to give a 1000 mg/l stock solution. An aliquot (171 ml) of this stock solution was dispersed in inoculated culture medium and the volume adjusted to 3 litres to give a final concentration of 57 mg/l, equivalent to 5 mg carbon/l. The volumetric flask containing the test material was inverted several times to ensure homogeneity of the solution.

Data from the control vessels was shared with similar concurrent studies.

2.4 Standard Material

Sodium benzoate (C_6H_5COONa), at a concentration of 17.1 mg/l, equivalent to 10 mg carbon/l.

Data from the standard material vessels was shared with similar concurrent studies.

2.5 Toxicity Control

57 mg test material/l plus 17.1 mg sodium benzoate/l, equivalent to a total of 15 mg carbon/l.

2.6 Source of Inoculum

A mixed population of activated sewage sludge micro-organisms was obtained on 4 February 2008 from the aeration stage of the Severn Trent Water PLC sewage treatment plant at Loughborough, Leicestershire, which treats predominantly domestic sewage.

2.7 Preparation of Inoculum

A sample of activated sewage sludge was washed 3 times by settlement and resuspension in culture medium to remove any excessive amounts of Dissolved Organic Carbon (DOC) that may have been present. A sub-sample of the washed sewage sludge was then removed and the suspended solids concentration determined.

2.8 Loading Rate

30 mg suspended solids (ss)/l.

2.9 Dilution Water

Standard culture medium (see Appendix 1).

2.10 Duration

28 days

2.11 Test Concentrations

a) A control consisting of inoculated culture medium.

b) 17.1 mg/l sodium benzoate in inoculated culture medium to give a final concentration of 10 mg C/l.

c) 57 mg/l TriGene Advance in inoculated culture medium to give a final concentration of 5 mg C/l.

d) 57 mg/l TriGene Advance plus 17.1 mg/l sodium benzoate in inoculated culture medium to give a final concentration of 15 mg carbon/l to act as a toxicity control.

At the request of the Sponsor a single control, standard and test vessel were prepared as opposed to duplicate vessels as stated in the test guidelines.

2.12 Study Dates

Between 16 January 2008 and 5 March 2008

2.13 Sampling and Analysis

2.13.1 CO_2 analysis

Samples (2 ml) were taken from the first CO_2 absorber vessel on Days 0, 6, 14, 22, 28 and 29. The second absorber vessel was sampled on Days 0 and 29. All samples were analysed for CO_2 immediately.

On Day 28, 1 ml of concentrated hydrochloric acid was added to each vessel to drive off any inorganic carbonates formed. The vessels were resealed, aerated overnight and the final samples taken from both absorber vessels on Day 29.

The samples were analysed for CO_2 using a Tekmar-Dohrmann Apollo 9000 TOC analyser. Samples (300 µl) were injected into the IC (Inorganic Carbon) channel of the TOC analyser. Inorganic carbon analysis occurs by means of the conversion of an aqueous sample to CO_2 by orthophosphoric acid using zero grade air as the carrier gas. Calibration was by standard solutions of sodium carbonate (Na_2CO_3). Each analysis was carried out in triplicate.

2.13.2 Dissolved organic carbon (DOC) analysis

On Days 0 and 28 samples (20 ml) were removed from all culture vessels and filtered through Gelman 0.45 µm Acrocap filters (approximately 5 ml discarded) prior to DOC analysis.

The samples were analysed for DOC using a Shimadzu TOC-5050A TOC analyser. Samples (27 or 13 µl) were injected into the Total Carbon (TC) and Inorganic Carbon (IC) channels of the TOC analyser. Total carbon analysis is carried out at 680°C using a platinum based catalyst and zero grade air as the carrier gas. Inorganic carbon analysis involves conversion by orthophosphoric acid at ambient temperature. Calibration was performed using standard solutions of potassium hydrogen phthalate ($C_8H_5KO_4$) and sodium carbonate (Na_2CO_3) in deionised water. Each analysis was carried out in triplicate.

3. DATA EVALUATION

3.1 Determination of carbon content

The test material contains 8.78% carbon (data supplied by the Sponsor) and so for a concentration of 5 mg C/l (a total of 171 mg) the total organic carbon present was 15 mg C.

The theoretical amount of carbon present in the standard material, sodium benzoate (C_6H_5COONa) was calculated as follows:

$$\frac{\text{No of C atoms x mol wt of C}}{\text{mol wt of standard material}} \times 100\%$$

$$= \frac{7 \times 12.011}{144.11} \times 100 = 58.34\%$$

Thus for a 10 mg C/l test concentration (a total of 51.4 mg) the total organic carbon present for sodium benzoate was 30 mg C.

3.2 Percentage degradation

The percentage degradation or percentage Theoretical Amount of Carbon Dioxide ($ThCO_2$) produced is calculated by substituting the inorganic carbon values given in Table 1 in the following equation:

$$\%ThCO_2 (= \%\ \text{degradation}) = \frac{\text{mg IC in test flask} - \text{mg IC in control}}{\text{mg TOC as test material}} \times 100\%$$

The percentage degradation from the results of the DOC analysis, see Table 4, is calculated from the equation below. Values are corrected for the control value prior to the calculation of percentage degradation.

$$\text{Percentage degradation} = \left[1 - \frac{\text{mg DOC in test flask on day 28}}{\text{mg DOC in test flask on day 0}}\right] \times 100\%$$

The total CO_2 evolution in the control vessel at the end of the test is calculated from the equation below.

$$\text{Total } CO_2 \text{ evolution} = \text{mg IC in control} \times \frac{100}{\%C \text{ of } CO_2} \times \frac{1}{\text{test volume}}$$

$$= \text{mg IC in control} \times \frac{100}{27.29} \times \frac{1}{3}$$

4. RESULTS

Inorganic carbon values for the test material, standard material, toxicity control and control vessels at each analysis occasion are given in Table 1. Percentage biodegradation of the test and standard materials and the toxicity control is given in Table 2 and the biodegradation curves are presented in Figure 1. Total and Inorganic Carbon values in the culture vessels on Day 0 are given in Table 3, and the results of the Dissolved Organic Carbon analyses performed on Days 0 and 28 are given in Table 4.

The total CO_2 evolution in the control vessel on Day 28 was 34.01 mg/l and therefore satisfied the validation criterion given in the OECD Test Guidelines.

The IC/TC ratio of the test material suspension in the mineral medium at the start of the test (see Table 3) was below 5% and hence satisfied the validation criterion given in the OECD Test Guidelines.

Acidification of the test vessels on Day 28 followed by the final analyses on Day 29 was conducted according to the methods specified in the Test Guidelines. This acidification effectively kills the micro-organisms present and drives off any dissolved CO_2 present in the test vessels. Therefore any additional CO_2 detected in the Day 29 samples originated from dissolved CO_2 that was present in the test vessels on

Day 28 and hence the biodegradation value calculated from the Day 29 analyses is taken as being the final biodegradation value for the test material.

The results of the inorganic carbon analysis of samples from the first absorber vessels on Day 29 showed an increase in all vessels. Inorganic carbon analysis of the samples from the second absorber vessels on Day 29 confirmed that no significant carry-over of CO_2 into the second absorber vessels occurred.

The test material attained 116% degradation after 28 days. Degradation values in excess of 100% were considered to be due to an increase in the numbers of viable micro-organisms in the test material vessel as a result of the readily biodegradable nature of the test material. This effect occurs due to the micro-organisms utilizing the test material as a carbon source for cellular growth resulting in a greater number of viable micro-organisms in this vessel when compared to the control vessel. This increased number of micro-organisms in this vessel gave rise to increased respiration rates and hence background CO_2 evolution was greater than in the control vessel. This increase in background CO_2 evolution resulted in biodegradation rates in excess of 100%.

The toxicity control attained 16% degradation after 14 days and 2% degradation after 28 days. This result implies that the test material was toxic to the activated sewage micro-organisms used in the test as at least 25% degradation should be attained in the toxicity control vessel by Day 14 for the test material to be considered to have caused no inhibitory effects. However, as over 100% degradation was attained from inorganic carbon analysis in the test material vessel, and Dissolved Organic Carbon (DOC) analysis from both the test material and toxicity control vessels showed complete degradation, the percentage degradation obtained from inorganic carbon analysis from the toxicity control vessel was considered to be low due to a leak in the system and not because the test material was causing any inhibitory effects.

Sodium benzoate attained 62% degradation after 14 days and 88% degradation after 28 days thereby confirming the suitability of the inoculum and test conditions.

Analysis of the test media from the test material culture vessels on Days 0 and 28 for Dissolved Organic Carbon (DOC), see Table 4, gave percentage degradation values of 100% for both the test material and toxicity control. Sodium benzoate attained 98% degradation calculated from the results of the DOC analyses. The degradation rates calculated from the results of the DOC analyses were similar to those calculated from inorganic carbon analysis except for the toxicity control vessel. The considered reason for this is explained above.

5. CONCLUSION

The test material attained 116% degradation after 28 days.

This study was conducted in a facility operating to Good Laboratory Practice within the UK national GLP monitoring programme, but the study report has not been audited by the QA Unit. No formal claim of GLP compliance is made for this study.

..... DATE: .....

C Mead BSc

STUDY DIRECTOR

..... DATE: .....

A Hurt BSc

HEAD OF ECOTOXICOLOGY AND ENVIRONMENTAL FATE

TriGene Advance : ASSESSMENT OF READY BIODEGRADABILITY; CO_2 EVOLUTION TEST

Table 1 Inorganic Carbon Values on Each Sampling Occasion

DAY	Control (mg IC)		Sodium Benzoate (mg IC)		Test Material (mg IC)		Test Material plus Sodium Benzoate Toxicity Control (mg IC)	
	Abs1	Abs 2	Abs 1	Abs 2	Abs 1	Abs 2	Abs 1	Abs 2
0	1.17	1.98	1.63	1.75	1.52	2.92	1.75	2.10
6	17.10	-	24.05	-	25.88	-	30.21	-
14	25.49	-	43.98	-	36.41	-	32.62	-
22	31.08	-	57.38	-	46.18	-	32.71	-
28	27.84	-	57.71	-	42.77	-	30.19	-
29	31.16	2.32	57.56	2.44	48.12	2.78	31.27	3.02

Table 2 Percentage Biodegradation Values

Day	% Degradation Sodium Benzoate	% Degradation Test Material	% Degradation Test Material plus Sodium Benzoate Toxicity Control
0	0	0	0
6	23	59	29
14	62	73	16
22	88	101	4
28	100	100	5
29*	88	116	2

Abs = CO_2 absorber vessel

* Day 29 values corrected to include any carry-over of CO_2 detected in Absorber 2

TriGene Advance : ASSESSMENT OF READY BIODEGRADABILITY; CO_2 EVOLUTION TEST

Table 3 **Total and Inorganic Carbon Values in the Culture Vessels on Day 0**

Test vessel	Total Carbon* (mg/l)	Inorganic Carbon* (mg/l)	IC/TC Ratio (%)
Sodium Benzoate 10 mg C/l	9.83	-0.11	0
Test Material 5 mg C/l	4.07	-0.87	0
Test Material plus Sodium Benzoate Toxicity Control 15 mg C/l	13.63	0.26	2

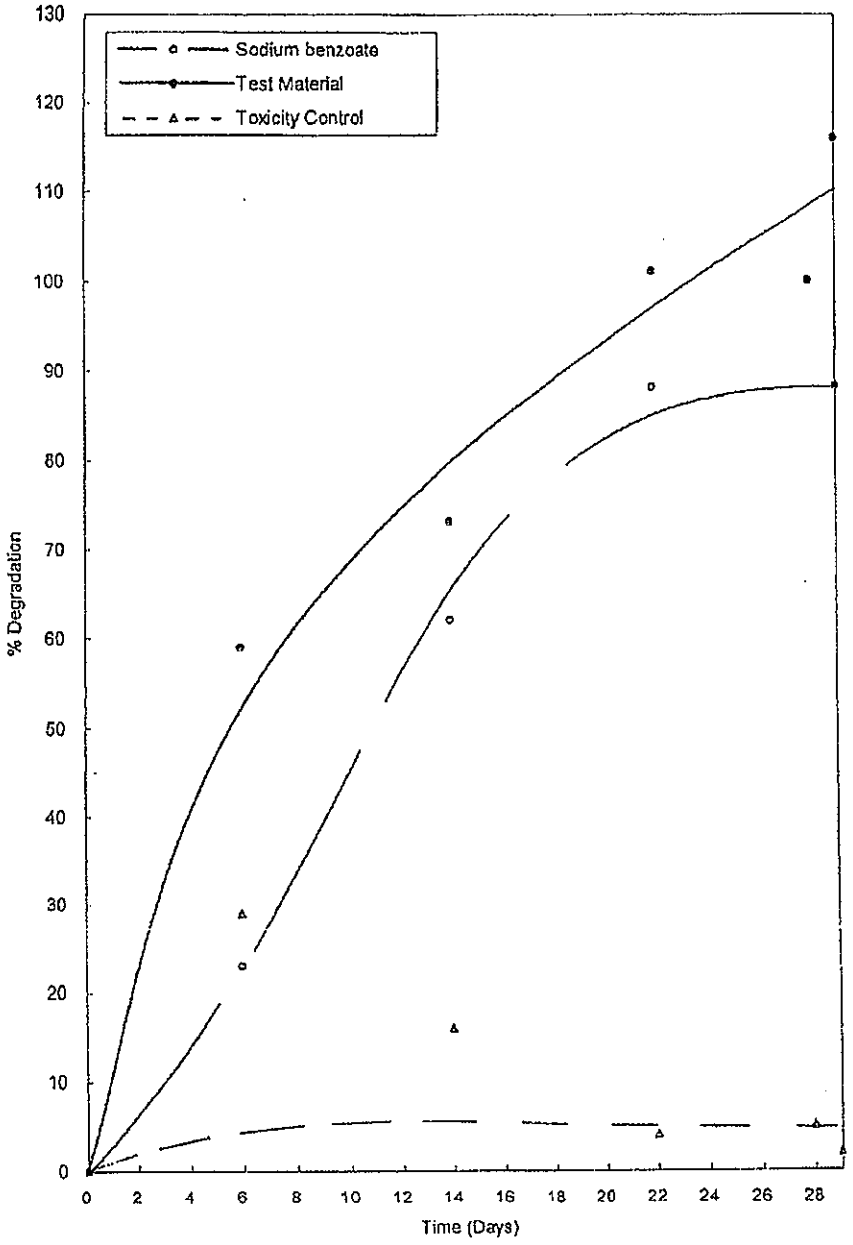
Table 4 **Dissolved Organic Carbon (DOC) Values in the Culture Vessels on Days 0 and 28**

Test Vessel	DOC* Concentration				
	Day 0		Day 28		
	mg C/l	% Nominal Carbon Content	mg C/l	% Initial Carbon Concentration	% Degradation
Sodium Benzoate 10 mg C/l	9.94	99	0.18	2	98
Test Material 5 mg C/l	4.94	99	<LOQ	0	100
Test Material plus Sodium Benzoate Toxicity Control 15 mg C/l	13.38	89	<LOQ	0	100

* Corrected for control values. Negative values are due to measured concentration values being less than control values

TriGene Advance : ASSESSMENT OF READY BIODEGRADABILITY; CO_2 EVOLUTION TEST

Figure 1 Biodegradation Curves



TriGene Advance : ASSESSMENT OF READY BIODEGRADABILITY; CO_2 EVOLUTION TEST

Appendix I Culture Medium

Solution a:	KH_2PO_4		8.50 g/l
	K_2HPO_4		21.75 g/l
	$Na_2HPO_4.2H_2O$		33.40 g/l
	NH_4Cl		0.50 g/l
		pH =	7.4
Solution b:	$CaCl_2$		27.50 g/l
Solution c:	$MgSO_4.7H_2O$		22.50 g/l
Solution d:	$FeCl_3.6H_2O$		0.25 g/l

To 1 litre (final volume) of purified water* are added the following volumes of solutions a to d.

10 ml of Solution a
1 ml of Solution b
1 ml of Solution c
1 ml of Solution d

* Reverse osmosis purified and deionised water (Elga Optima 15+)

Personal protection: Use with care, avoid eye contact and prolonged skin contact. Gloves recommended if available. External use only. Not for ingestion.

INGREDIENTS:
Chemical type: Halogenated Tertiary Amine
Major ingredients: Polymeric Biguanide Hydrochloride <1%
Alkyl Dimethyl Benzyl Ammonium Chloride <1%
Didecyl Dimethyl Ammonium Chloride

Working together to protect kauri:
MAF Biosecurity New Zealand
Department of Conservation
Auckland Regional Council,
Northland Regional Council
Environment Waikato
Environment Bay of Plenty

0800 NZ KAURI www.kauridieback.co.nz

STOP KAURI DIEBACK

TRIGENE disinfectant

(2% solution)

To disinfect footwear and equipment to restrict the transfer of PTA (Phytophthora taxon Agathis) via soil.

Instructions: Remove all soil or mud prior to disinfection. Spray entire surface of dirty footwear/ equipment. Ensure complete coverage. Leave to dry for one minute before moving to another area of kauri.

Date _____

TriGene effective for up to six months from the date above

SAFETY:
(1) Keep out of reach of children (2) Avoid contact with the skin and eyes (3) Do not mix with other chemicals

Store in dry place below 30°C and away from direct sunlight. Keep lidded. Keep away from foodstuffs and drinks.

DISPOSAL:
This product is biodegradable. Flush to drain with copious water.

FIRST-AID MEASURES (Must be followed immediately)

4.1 Inhalation: Non-toxic: Remove to fresh air. Avoid using fine mist sprays, avoid inhalation of fine mist.
4.2 Eye contact: Rinse eyes with water copiously for 10 minutes. Seek medical advice as necessary.
4.3 Skin contact: Wash affected area with soap and water. Avoid prolonged contact.
4.4 Ingestion: Do not induce vomiting. Give milk or water to drink. Seek medical advice where necessary.

Personal protection: Use with care, avoid eye contact and prolonged skin contact. Gloves recommended if available. External use only. Not for ingestion.

INGREDIENTS:
Chemical type: Halogenated Tertiary Amine
Major ingredients: Polymeric Biguanide Hydrochloride <1%
Alkyl Dimethyl Benzyl Ammonium Chloride <1%
Didecyl Dimethyl Ammonium Chloride

Working together to protect kauri:
MAF Biosecurity New Zealand
Department of Conservation
Auckland Regional Council,
Northland Regional Council
Environment Waikato
Environment Bay of Plenty

0800 NZ KAURI www.kauridieback.co.nz

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4.4 Ingestion: Do not induce vomiting. Give milk or water to drink. Seek medical advice where necessary.

GENERAL INSTRUCTIONS

PRECAUTION
PHYTOCLEAN® should not be stored as a diluted solution.
PYTOCLEAN® should not be mixed with other chemicals.
PHYTOCLEAN® should be used within 12 months of purchase.
PHYTOCLEAN® should be stored below 30°c.

PROTECTION OF WILDLIFE, FISH, CRUSTACEANS AND THE ENVIRONENT.
DO NOT contaminate streams, rivers or waterways with PHYTOCLEAN® or used containers.

STORAGE AND DISPOSAL

Store in closed, original container in a cool, well ventilated area. Do not store for prolonged periods in direct sunlight. Triple or preferably pressure rinse containers before disposal. Add rinsings to spray tank. Do not dispose of undiluted chemical on site. If recycling, replace cap and return clean containers to recycler or designated collection point. If not recycling, break, crush, or puncture and bury empty container below 500mm in a disposal pit specifically marked and set up for this purpose clear of waterways, desirable vegetation and tree roots. Empty containers and product should not be burnt.

FIRST AID

If poisoning occurs, contact a doctor or Poisons Information Centre on **1311265.**

A Material Safety Data Sheet is available on request.

Date of manufacture.

Batch code.

PHYTOCLEAN™

CAUTION: KEEP OUT OF REACH OF CHILDREN

ACTIVE CONSTITUENT: 100g/L Benzalkonium chloride

PHYTOCLEAN® IS A DISINFECTANT CLEANER, SPECIALLY DESIGNED FOR THE CONTROL OF Phytophthora cinnamomi IN HORTICULTURAL, PLANTATION, EARTH MOVING AND QUARRYING INDUSTRIES. PHYTOCLEAN IS AN EFFECTIVE GENERAL PURPOSE MICROBIOCIDE AND ALGICIDE.

Contents 20L / 200L

PHYTOCLEAN P/L
ABN 84 135 445 966
PO BOX 499
BELGRAVE VICTORIA 3160
FAX: 03 9754 1849
www.phytoclean.com.au

Covered by Australian Patent.

Distributed By:

APVMA Approval No. 49873

DIRECTIONS FOR USE:

SITUATION	
Footbaths	1L/10L of water
Washdown	2L/100L of water
Hard surface and tools	200ml/10L of water

CRITICAL COMMENTS

Footbaths: To reduce the spread of *Phytophthora* cinnamomi and other pathogens from infected soil adhering to footwear it is recommended that the PHYTOCLEAN® solution should be topped up when required to maintain an adequate level.
Footwear should be as free of soil as possible. Footbaths should be cleaned and replenished at least weekly.

Washdown: For high pressure or automatic washdown of machinery and equipment, particular attention must be paid to the underneath and out of the way areas of machines to remove all soil matter. Allow solution to remain in surface contact for at least 30 seconds before rinsing with fresh water.
Where possible, use PHYTOCLEAN® in conjunction with hot water.

Hard surface and tools: PHYTOCLEAN® can be used for spraying and wiping of benches, shelves, walls, floors etc, also for dipping and scrubbing of hand tools, make sure to remove all soil. Allow solution to remain in contact with the surface for at least 30 seconds before hosing or wiping off.

NOT TO BE USED FOR ANY PURPOSE OR IN ANY MANNER, CONTRARY TO THIS LABEL UNLESS AUTHORISED UNDER APPROPRIATE LEGISLATION.

Product: **Phytoclean**

HEALTH HAZARD INFORMATION	
HEALTH EFFECTS - ACUTE	
SWALLOWED:	Irritation of the mouth and throat will occur and nausea is likely.
EYE:	Irritant, reddening will occur and pain may be experienced.
SKIN:	Irritant, reddening will occur, prolonged contact may lead to dermatitis.
INHALED:	Inhalation of mist or spray can lead to respiratory irritation.
HEALTH EFFECTS - CHRONIC	
FIRST AID	
SWALLOWED:	DO NOT induce vomiting. Give water or milk to drink, followed by raw egg (if available). Seek immediate medical attention.
EYE:	Hold eyes open and flood with water for at least 15 minutes. Seek immediate medical attention.
SKIN:	Remove any contaminated clothing. Wash affected area with soap and water. Seek medical attention if irritation develops.
INHALED:	Remove from exposure to a well ventilated area. Seek medical attention if any persistent irritation or discomfort is experienced.
If poisoning occurs, contact a doctor or Poisons Information Centre.	
ADVICE TO DOCTOR	
Treat symptomatically.	

PRECAUTIONS FOR USE	
EXPOSURE STANDARDS:	No value assigned for this material by the National Health and Medical Research Council.
ENGINEERING CONTROLS:	General ventilation is adequate.
PERSONAL PROTECTION Wear rubber gloves and eye protection.	
FLAMMABILITY Non-flammable	

SAFE HANDLING INFORMATION	
STORAGE AND DISPOSAL:	Classified as Non-Hazardous Goods for storage and transport. Store away from oxidisers and foodstuffs. Store between 0 and 40° Celsius
SPILLS AND DISPOSAL:	Contain spill with absorbent material. Shovel it into labelled drums and dispose of in accordance with local government regulations. Wash area down with large quantities of water.
FIRE/EXPLOSION HAZARD:	N/A

Product: **Phytoclean**

OTHER INFORMATION

Oral LD50 (rat): 366 mg/kg (80% active)
Dermal LD50 (rabbit): 421 mg/kg (80% active)
Skin irritation (rabbit): 0.5ml applied to the intact and abraded skin produces severe skin irritation that was not reversed by 72 hours, post dose (primary irritation score = 7.0).
Eye irritation (rabbit): 0.1 ml applied to the ye without washing produced severe eye irritation that was not reversed by day 7, post dose.
A 0.2% active solution was not a skin sensitiser in guinea pigs.

CONTACT POINT

Phytoclean Pty Ltd
P.O. Box 499, Belgrave Victoria 3160
Contact: Wendy Edwards
Phone: 03 97525301
Facsimile: (03) 9754 1849

This information relates to the specific material designated and may not be valid for such material used in combination with other chemicals or in any process. Such information is to the best of Phytoclean's knowledge and believed accurate and reliable as of the date indicated. However, no representation, warranty or guarantee is made as to its accuracy, reliability or completeness. It is the user's responsibility to satisfy himself/herself as to the suitability and compactness of such information for his/her own particular use.

Master Label

Virkon® S

BROAD SPECTRUM DISINFECTANT

For Use in Cleaning and Disinfecting Industrial, Animal and Agricultural Facilities (OPT.)

Effective against Viruses
(including CANINE PARVOVIRUS) ! Bacteria ! Fungi

For Use in Emergency Disease Control (OPT.)

For use in Cleaning and Disinfecting Institutional and Service Facilities including stores, factories, schools, hotels, offices, ships, planes, transportation terminals, supermarkets and food warehouses. (OPT.)

For Use in Emergency Response and On-site Cleanup (emergency response calls, crime scenes, traffic accidents, fires, flood, natural and other disasters) e.g. cars, trucks, ambulances, and similar emergency apparatus, tires, wheels, floors, walls, ceilings, paved surfaces; and equipment such as SCBA, coats, boots, hats, masks, gloves, axes, Jaws of Life and similar emergency equipment.(OPT.)

For Use in Greenhouses, Horticulture, and Aquaculture (OPT.)

ACTIVE INGREDIENTS:	
Potassium peroxymonosulfate.....	20.4%
Sodium Chloride.....	1.5%
OTHER INGREDIENTS.....	78.1%
TOTAL.....	100.00%

Equivalent to 9.75% Available Chlorine

KEEP OUT OF REACH OF CHILDREN
DANGER
See [Back] [Side] Panel[s] [Inside Booklet] for Additional Precautions

Front Panel Continued

FIRST AID

Have the product container or label with you when calling a poison control center or doctor, or going for treatment.

If Swallowed:

- Call Poison Control Center or doctor immediately for treatment advice.
- Have person sip a glass of water if able to swallow.
- Do not induce vomiting unless told to do so by the poison control center or doctor
- Do not give anything by mouth to an unconscious person

If Inhaled:

- Move person to fresh air.
- If person is not breathing, call 911 or an ambulance, then give artificial respiration, preferably mouth-to-mouth, if possible.
- Call a Poison Control Center or doctor for further treatment advice.

If in Eyes:

- Hold eye open and rinse slowly and gently with water for 15-20 minutes.
- Remove contact lenses, if present after 5 minutes, then continue rinsing eye.
- Call a Poison Control Center or doctor for further treatment advice.

If on Skin:

- Take off contaminated clothing.
- Rinse skin immediately with plenty of water for 15-20 minutes.
- Call a Poison Control Center or doctor for further treatment advice.

Note to Physician: Probable mucosal damage may contraindicate the use of gastric lavage.

___ lbs. (__) Net Weight

EPA Reg. No. 62432-1

EPA Est. No. 62432-EN-001

Antec
International
LEADERS IN BIOSECURITY

Manufactured By:
ANTEC INTERNATIONAL LTD.
Windham Road, Chilton Industrial Estates
Sudbury Suffolk C010 2XD, England

Virkon® S is a registered trademark of and manufactured by Antec International Limited
US Patent No. 4822512

[Comment: The list of claims (sites) under "EFFECTIVE AGAINST" may be placed in any order as long as each subheading and its contents remains intact.]

EFFECTIVE AGAINST THE FOLLOWING PATHOGENS:

ANIMAL AND ZOONOTIC PATHOGENS

BACTERIA

Actinobacillus pleuropneumonia
Bordetella avium
Bordetella bronchiseptica
Campylobacter pyloridis
Clostridium perfringens
Dermatophilus congolensis
Escherichia coli
Fistulous withers (Poll Evil)
Haemophilus somnus
Klebsiella pneumoniae
Moraxella bovis (Pink Eye)
Mycobacterium bovis
Mycoplasma gallisepticum
Mycoplasma mycoides
Pasteurella multocida
Pseudomonas aeruginosa
Pseudomonas mallei (Glanders)
Pseudomonas vulgaris
Salmonella choleraesuis
Salmonella typhimurium
Staphylococcus aureus
Staphylococcus epidermidis
Streptococcus equi (Strangles)
Streptococcus pyogenes
Streptococcus suis
Taylorella equigenitalis
Treponema hyodysenteriae

VIRUSES

Adenovirus Pneumonia
African Horse Sickness Virus
African Swine Fever Virus
Avian Influenza Virus
Avian Laryngotracheitis Virus
Bovine Adenovirus Type 4
Bovine Polyoma Virus
Bovine Pseudocowpox Virus
Bovine Viral Diarrhea Virus
Calf Rotavirus
Canine Adenovirus
Canine Coronavirus
Canine Parainfluenza Virus
Canine Parvovirus
Chicken Anemia Virus
Coital Exantherma Virus
Distemper Virus
Duck Adenovirus
Duck Enteritis Virus
Egg Drop Syndrome Adenovirus
Equine Infectious Anemia Virus (Swamp Fever)
Equine Arteritis Virus
Equine Herpes Virus (Type 1)
Herpes Virus Equine (Type 3)
Hog Cholera Virus
Equine Contagious Abortion Virus
Equine Papillomatosis Virus
Equine Influenza Virus (Type A)
Equine Influenza Virus (The Cough)
Feline Calicivirus
Feline Herpes Virus
Feline Infectious Peritonitis Virus
Feline Panleukopenia Virus
Feline Parvovirus
Feline Rhinotracheitis Virus
Foot and Mouth Disease Virus
Infectious Bronchitis Virus
Infectious Bursal Disease Virus

Infectious Canine Hepatitis Virus
Infectious Pancreatic Necrosis Virus
Infectious Salmon Anaemia Virus
Infective Bovine Rhinotracheitis Virus
Leptospira Canicola Virus
Maedi- Visna Virus
Marek's Disease Virus
Newcastle Disease Virus
PCV2 Virus (PMWS)
Porcine Parvovirus
Porcine Reproductive and Respiratory Syndrome Virus (PRRS)
Pseudorabies Virus (Aujesky's Disease)
Rotaviral Diarrhea Virus
Snakehead rhabdovirus
SV40 Virus
Swine Influenza Virus
Transmissible Gastroenteritis Virus (TGE)
Turkey Herpes Virus
Turkey Rhinotracheitis Virus
Vesicular Stomatitis Virus

PLANT PATHOGENS

Alternaria solani
Botrytis cinera
Colletotrichum coccodes
Didymella bryoniae
Fusarium oxysporum
Fusarium solani
Penicillium oxalicum
Phomopsis sclerotioides
Pyrenochaeta lycoopersici
Pythium aphanidermatium
Rhizoctonia solani
Sclerotinia sclerotiorum
Thielaviopsis basicola
Verticillium dahliae

FUNGI

Aspergillus fumigatus
Candida albicans
Fusarium moniliforme
Microsporum canis
Trichophyton spp. (Ringworm)
Trichophyton spp. (Mud Fever)

EFFECTIVE AGAINST THE FOLLOWING HUMAN HEALTH PATHOGENS

Human Immuno-Deficiency Virus (HIV) Type 1 (on hard, non-porous surfaces), Streptococcus pyogenes, Campylobacter pyloridis, klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium, Salmonella choleraesuis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, and Candida albicans.

PRECAUTIONARY STATEMENTS
HAZARDS TO HUMANS AND DOMESTIC ANIMALS

DANGER. Powder is corrosive. Causes skin burns and irreversible eye damage. Harmful if swallowed, absorbed through skin, or inhaled. Do not get in eyes, on skin, or on clothing. Wear protective clothing and rubber gloves. Avoid breathing dust. Wear goggles, face shield, or safety glasses. Wash thoroughly with soap and water after handling. Remove contaminated clothing and wash before reuse. **Corrosive statement refers to powder only not in use solution.**

[Comment: The instructions under "DIRECTIONS FOR USE" may be placed in any order as long as they remain a continuous section on the label.]

BROAD SPECTRUM DISINFECTANT

Virkon® S is effective against numerous microorganisms affecting animals: viruses, gram positive and gram negative bacteria, fungi (molds and yeasts), and mycoplasma. Efficacy of the 1% solution was determined in the presence of 400 ppm AOAC hard water and 5% organic material. Virkon® S passes the AOAC germicidal and detergent sanitizer test at a concentration of 0.5% (1:200) in the presence of 200 ppm hard water. Apply a 0.5% (1:200) solution for routine sanitation.

DIRECTIONS FOR USE

It is a violation of Federal law to use this product in a manner inconsistent with its labeling

GENERAL INSTRUCTIONS—POULTRY AND FARM PREMISES

1. Remove all poultry or other animals and feeds from premises, trucks or other vehicles, coops, crates or other enclosures.

2. Remove all litter droppings and manure from floors, walls and surfaces of barns pens, stalls, chutes and other facilities and fixtures occupied or traversed by poultry or other animals.

3. Empty all troughs, racks, and other feeding and watering appliances.

4. Thoroughly clean all surfaces with soap or detergent and rinse with water.

5. Saturate surfaces with the recommended disinfecting solution for a period of 10 minutes.

6. Immerse all halters, ropes, and other types of equipment used in handling and restraining animals, as well as forks, shovels, and scrapers used for removing litter and manure.

7. Ventilate buildings, cars, boats, coops, and other closed spaces. Do not house poultry or livestock or employ equipment until treatment has been absorbed, set, or dried.

8. Thoroughly scrub treated feed racks, mangers, troughs, automatic feeders, fountains, and waterers with soap or detergent, and rinse with potable water before reuse.

This powder formula is easily diluted for use in manual or machine operations.

Virkon® S DILUTION CHART
Fill container with desired amount of water and add Virkon® S powder to achieve recommended solution concentration.

Quantity of Water	*0.5% Solution*	*1% Solution*	*2% Solution*
1 Quart	*0.15 ounces*	*0.3 ounces*	*0.7 ounces*
1 Gallon	*0.65 ounces*	*1.3 ounces*	*2.7 ounces*
10 Gallons	*6.7 ounces*	*13.4 ounces*	*26.7 ounces*
50 Gallons	*33.4 ounces*	*66.8 ounces*	*133.5 ounces*

Measuring cup provided.

Solutions are stable for 7 days. Do not soak metal objects in Virkon® S for long periods - 10 minutes is maximum necessary contact time. One gallon of solution is sufficient to treat 135 sq. ft.

POULTRY [PRODUCTION] [AND RATITE PRODUCTION]

[CONTROLS: Viruses of Newcastle Disease, Infectious Bronchitis, Infectious Bursal Disease, Avian Laryngotracheitis, Marek's Disease, Egg Drop Syndrome, Avian Influenza, Turkey Herpes Virus and Duck Viral Enteritis. Fungi (molds and yeasts) - Aspergillus flavus, Aspergillus fumigatus and Candida albicans. Bacteria - Streptococcus pyogenes, Campylobacter pyloridis, Klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium, Salmonella chloeraesuis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, Bordetella avium and Mycoplasma gallisepticum.] (OPT.)

HATCHERIES: Virkon® S at 1% solution can be used for cleaning and disinfecting hatchers, setters, evaporative coolers, humidifying systems, ceiling fans, chicken houses, transfer trucks, trays, and plastic chick boxes.

Virkon® S at 1-2% solution is recommended for use in fogging (wet misting) operations as a supplemental measure, either before or after regular cleaning and disinfecting procedures. Fog (wet mist) until the area is moist using automatic foggers according to manufacturer's use directions.

BROILER/BREEDER HOUSES: Follow General Instructions to remove poultry and preclean area to be treated. Spray floors and walls with Virkon® S at 1% solution. Thoroughly wash waterers and feeders with a 1% solution of Virkon® S. After contact for 10 minutes, rinse with water. Do not house poultry or use equipment until treatment has dried.

FOR AIR SANITIZING: Use Virkon® S at 0.5-1% solution, and fog until surfaces are moist. Allow at least 2 hours before entering treated area. Rinse foggers and sprayers with water following use.
PROCESSING PLANTS: Spray Virkon® S at 1% solution to disinfect and clean walls, ceilings and floors.

SWINE PRODUCTION

[CONTROLS: Viruses of Hog Cholera, Swine influenza, Porcine Parvovirus, Pseudorabies, Porcine Reproductive and Respiratory Syndrome (PRRS), Rotoviral Diarrhea, African Swine Fever and Foot and Mouth Disease. Bacteria of Pleuropneumonia, Treponema hyodysenteriae, and Clostridium perfringens. Fungi: Fusarium moniliforme.] (OPT.)

Follow General Instructions to remove swine and preclean area to be treated. Virkon® S at 1% solution is recommended for cleaning and disinfecting farrowing units, nurseries, finisher houses, processing plants, and agricultural production equipment such as trucks, waterproof footwear (such as rubber boots), and associated livestock equipment and instruments.

Virkon® S at 0.5-1% solution is recommended for use in fogging (wet misting) operations or as a supplemental measure either before or after regular cleaning and disinfecting procedures. Fog (wet mist) until the area is moist using automatic foggers according to manufacturer's use directions. Rinse foggers and sprayers with water following use.

EQUINE PRODUCTION

BROAD SPECTRUM EQUINE DISINFECTANT/DETERGENT/WASH FOR CLEANING AND DISINFECTING STABLES, EQUIPMENT, AND AERIAL DISINFECTION

[CONTROLS: Viruses of African Horse Sickness, Equine Viral Arteritis (Pink Eye), Coital Exantherma, Myeloencephalopathy, Rhinopneumonitis, Equine Contagious Abortion, Equine Papillomatosis, Equine Infectious anemia (Swamp Fever), Adenovirus Pneumonia, Equine Influenza (The Cough) and Rhinitis. Bacterial: Clostridial Diarrhea, Fistulous Withers (Poll Evil), Taylorella equigenitalis, Bordetella bronchiseptica, Streptococcus equi (Strangles) and Pseudomonas mallei (Glanders). Fungi: Dermatophytosis (Ringworm), Dermatophylosis (Mud Fever), and Fusarium moniliforme.] (OPT.)

APPLICATIONS: For cleaning and disinfecting all surfaces, equipment, utensils and instruments in Veterinary practices, kennels, stables, catteries, etc.

USES:

Stables, Horse Boxes, Box Stalls, Tack, Equipment, and Feed Rooms: Thoroughly clean and dry [dry clean] surfaces, then wash the area manually or with pressure washer with a 1% Virkon® S solution. Rinse with clean water.

Blankets, Saddle Pads and Rugs: Shampoo by hand or spray lightly with a hand-sprayer and leave to dry. Shake or vacuum to remove residue.

Aerial Spraying to control airborne diseases: Use a hand or knapsack sprayer with fine setting, or an automatic spraying system. Spray a 1% Virkon® S solution for 2-3 minutes twice daily , first thing in the morning and last thing at night. Rinse sprayers with water after use.

BOVINE PRODUCTION

[CONTROLS: Viruses of Calf rotavirus, Infectious Bovine Rhinotracheitis, Bovine Adenovirus Type 4 and Pseudorabies and Foot and Mouth Disease; Bacteria of Maraxella bovis, Haemophilus somnus and Mycobacterium bovis; Fungi of Fusarium moniliforme.] (OPT.)

Follow General Instructions to remove livestock and preclean area to be treated. A 1% solution of Virkon® S is recommended to clean and disinfect areas associated with bovine housing stabling, hospital quarantine pens, feedlot facilities, and agricultural production equipment such as trucks, water-proof footwear (such as rubber boots), and associated livestock equipment and instruments.

COMPANION ANIMALS

[CONTROLS: Viruses of Canine Parvovirus, Distemper, Leptospira canicola. Feline parvovirus, Feline herpes and Feline calicivirus. Bacteria of Staphylococcus aureus, Streptococcus pyogenes, Klebsiella pneumoniae, and Pseudomonas aeruginosa; Fungi of Microsporum canis.] (OPT.)

[APPLICATIONS] A 1% solution of Virkon® S is recommended as a "one step" cleaning and disinfecting procedure for all surfaces, equipment, instruments, utensils and cages [caging systems]within [associated with]Veterinary Medical Hospitals, infections disease wards, quarantine areas, Humane Society facilities, laboratory animal quarters, grooming and boarding facilities, kennels, catteries and animal transportation vehicles.

Do not immerse metal objects in Virkon® S for long periods - 10 minutes is maximum contact time.

GREENHOUSES AND HORTICULTURE

Virkon® S is intended to disinfect inanimate environmental surfaces, glasshouse structures, equipment, utensils, trays, containers, and vehicles in greenhouses and other horticultural settings prior to introduction or reintroduction of plants, seeds, or soil. It is not intended to directly affect agricultural production and must not be applied to plants, seeds, or soil. If necessary, remove or cover these items prior to use of the product.

Remove all crop debris, strings and other deposits from structures, empty trays and pots. Power wash all the dust off the covering and superstructure and let dry. Using a sprayer or fogger saturate all surfaces with a 1% solution of Virkon® S. Let air dry.

Virkon® S may also be used to disinfect irrigation tanks and lines. Run a 1% solution through the system or soak equipment in a 1% solution. Let stand for ten minutes and flush system with clean water after treatment.

Virkon® S at 0.5-1%% solution is recommended for use in fogging (wet misting) operations or as a supplemental measure either before or after regular cleaning and disinfecting procedures. Fog (wet

mist) until the area is moist using automatic foggers according to manufacturer's use directions. Rinse foggers and sprayers with water following use.

AQUACULTURE

Virkon® S is intended to disinfect inanimate environmental surfaces associated with aquaculture including vehicles, nets, boots, waders, dive suits, hoses, brushes and other similar equipment. Virkon® S may also be used in foot dips. Virkon® S must not be applied directly to water.

Equipment used in separate sites, tanks, ponds in aquacultural settings should be disinfected before each new use by soaking for 20-30 minutes in a 1% Virkon® S solution followed by a water rinse.

Virkon® S at 0.5-1% solution is recommended for use in fogging (wet misting) operations or as a supplemental measure either before or after regular cleaning and disinfecting procedures. Fog (wet mist) until the area is moist using automatic foggers according to manufacturer's use directions. Rinse foggers and sprayers with water following use.

EMERGENCY DISEASE CONTROL (ANIMAL HEALTH)

CONTROLS: OIE List A Disease organisms including Foot and Mouse Disease Virus, African Horse Sickness Virus, Vesicular Stomatitis Virus, Classical Swine Fever Virus (Hog Cholera Virus), African Swine Fever Virus, Newcastle Disease Virus, and Highly Pathogenic Avian Influenza Virus. (OPT.)

A 1% solution of Virkon® S is recommended to clean and disinfect agricultural facilities and equipment, military facilities and equipment; airport facilities and equipment, port facilities and equipment, rail facilities and equipment, quarantine facilities and equipment, slaughter facilities and equipment, and other shipping facilities and equipment where animals or soils suspected of harboring foot and mouth disease virus might have been previously present.

Within these facilities, treated objects include but are not limited to vehicles, farm equipment (including tractors, ploughing shares, cars and trucks, farm engines, harvesters, loaders, mowers, tillers and slaughter machinery), military equipment (including tanks and troop carriers), and shipping equipment (pallets, bins, and containers).

Spray Virkon® S at 1% solution to disinfect and clean walls, ceilings, floors, decks, container surfaces, vehicles, wheels, water proof footwear (such as rubber boots), livestock equipment, utensils and instruments.

Do not immerse metal objects in Virkon® S for long periods - 10 minutes is maximum contact time.

INSTITUTIONAL AND SERVICE FACILITIES (HUMAN HEALTH)

CONTROLS: Human Immuno-Deficiency Virus (HIV) Type 1 (on hard, non-porous surfaces), Streptococcus pyogenes, Campylobacter pyloridis, klebsiella pneumoniae, Escherichia coli, Salmonella typhimurium, Salmonella choleraesuis, Pseudomonas aeruginosa, Staphylococcus aureus, Staphylococcus epidermidis, and Candida albicans. (OPT.)

With Virkon® S, only one product is needed to clean and disinfect all surfaces except acid-sensitive surfaces such as copper, brass, or aluminum. Do not use Virkon S on these acid-sensitive surfaces. Avoid splashing Virkon® S solution on textiles or carpets. Virkon® S may be used on carpeting or other textiles only it area is tested for color fastness before use and treated area vacuumed when dry.

Cleaning and Disinfecting Non-Food Contact Surfaces: Remove gross dirt and use 1.0% Virkon® S solution prepared according to the Dilution Chart below. Apply to surface using a mop, sponge, brushes or spray device until the surface is visibly clean. Air dry. In cases of fungal or viral contamination of non-food contact surfaces, follow these instructions substituting a 2.0% Virkon® S solution.

Sanitizing Toilet Bowls: After flushing, sprinkle 1 oz. Virkon® S powder around the bowl, scrub with a brush, and leave for 10 minutes. Flush.

Cleaning and Disinfecting Manikins Used in CPR Training: Manikins should be cleaned as soon as possible at the end of each class to avoid drying of contaminants on surfaces. Disassemble the manikin as directed by the manufacturer's instructions. Thoroughly wash all internal and external surfaces and reusable protective face shields with a brush using a 1% Virkon® S solution. Let stand for 10 minutes and rinse with potable water.

Cleaning and Disinfecting Hard, Non-porous Surfaces Suspected of HIV Type 1 Contamination: Cover heavy spillage of body fluids with Virkon® S powder. Let stand for 10 minutes, and then scoop into plastic bag. Treat bag and its contents as infectious medical waste. Prepare 2% Virkon® S solution according to the Dilution Chart. Apply to surface to be treated using a mop, sponge, brush or spray device until the surface is visibly clean. Air dry.

EMERGENCY RESPONSE AND ON-SITE CLEANUP

Cover heavy spillage of body fluids with Virkon® S powder. Let stand for 10 minutes, and then scoop into plastic bag. Treat bag and its contents as infectious medical waste.

Prepare 2% Virkon® S solution according to the Dilution Chart. Apply to surface to be treated using a mop, sponge, brush or spray device until the surface is visibly clean. Air dry.

STORAGE AND DISPOSAL

STORAGE: Store in a cool, dry place in tightly closed container away from children. Always replace lid after use.

DISPOSAL: Wash empty container thoroughly and dispose in trash. Do not mix this product with other chemicals

MATERIAL SAFETY DATA SHEET

Pharmacal Research Laboratories Inc. • 562 Captain Neville Drive, Waterbury CT 06705
(203) 755-4908 • 800-243-5350 • FAX (203) 755-4309
www.pharmacal.com

ISSUE DATE: 03/07/2007

I. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND COMPANY

PRODUCT: VIRKON-S EPA REG # 71654-6
MSDS HSD/US41
IMPORTER: Pharmacal Research Laboratories
562 Captain Neville Drive Waterbury CT 06705
Tel: 800-243-5350
Supplier: Antec International Limited
Sudbury Suffolk CO10 2XD
Tel: 44-(0)1787-377305

All information provided in this Material Safety Data Sheet refers specifically to the Virkon S powder, as supplied, & **not** the in-use solutions, unless otherwise stated.

II.COMPOSITION/INFORMATION ON INGREDIENTS

Chemical	% Concentration	CAS	Exposure
Potassium peroxomonosulfate	40-60	70693-62-8	1mg/m^3, total dust, 8 & 12 hr. TWA – manufacturer's recommendation.
Sodium Dodecylbenzene-sulphonate	10-20	25155-30-0	None assigned.
Sulfamic Acid	1-10	5329-14-6	0.5mg/m^3, 8 & 12 hr. TWA – manufacturer's recommendation.

III.HAZARDS INFORMATION

Potential Health Effects

Danger: Powder is corrosive. Causes skin burns & irreversible eye damage. Harmful if swallowed, absorbed through skin or inhaled. Do not get into eyes, on skin, or on clothing.

None of the components present in this material at concentrations equal to or greater than 0.1% are listed by IARC, NTP, OSHA or ACCIH as a carcinogen.

HMIS
Health-3 Fire-0 Reac- 0

Pharmacal Research Laboratories Inc. • 562 Captain Neville Drive, Waterbury CT 06705
(203) 755-4908 • 800-243-5350 • FAX (203) 755-4309
www.pharmacal.com

IV. FIRST AID

INHALATION

Symptom: - Inhalation of this powder in sufficient quantities may cause irritation of the upper respiratory passages, nose & throat. Gross over exposure may cause ulceration of mucous membranes.
Treatment: - Remove to fresh air. If not breathing, give artificial respiration. If breathing is difficult, give oxygen. Call a physician.

SKIN CONTACT

Symptom: - If allowed to become moist the dry powder may cause severe irritation and in cases of prolonged contact may cause burns or ulceration. Contact with the dry powder may cause skin irritation with discomfort or rash, or allergic skin reactions in sensitive individuals.
Treatment: - Flush skin with plenty of water. Remove contaminated clothing & shoes after use. Call a physician. Wash contaminated clothing before reuse.

EYE CONTACT

Symptom: - Eye contact with the powder may cause eye corrosion or ulceration; eye irritation with discomfort, tearing or blurring of vision. Severe eye damage may result if not treated immediately.
Treatment: - In case of contact, immediately flush eyes with plenty of water for at least 15 minutes. Call a physician.

INGESTION

Symptom: - Ingestion of this product in sufficient quantities may cause gastritis, with stomach pain, nausea, vomiting, diarrhoea, headache or weakness; possibly progressing to necrosis or haemorrhage with gross overexposure.
Treatment: - If swallowed, do not induce vomiting. Give 2 glasses of water immediately. Never give anything by mouth to an unconscious person. Call a physician.

V. FIRE FIGHTING MEASURES

Flammable properties: Not applicable

Extinguishing media: Water, dry powder (sand or Met-L-X), CO_2.

Fire Fighting instructions: Evacuate personnel to a safe area. Wear self-contained breathing apparatus (SCBA) & full protective equipment. When heated above 70°C, decomposes with evolution of corrosive gas (SO_2). Virkon S itself is not flammable or oxidizing, but may assist combustion of other materials under exceptional circumstances.

VI. ACCIDENTAL RELEASE PROCEDURES

Safeguards (Personnel).
Review FIRE FIGHTING MEASURES & HANDLING sections. Use appropriate Personal Protective Equipment during clean- up.
Environmental precautions: Do not allow the powder concentrate to enter drains. Infrequent disposal of small quantities (<0.5kg) may be diluted to waste with large quantities of water, subject to local waste disposal regulations. Do not allow entry to surface waters.
Methods for clean up: Sweep up carefully, preferable with the aid of a suitable dry anti-dusting agent if available. Place in suitable containers for disposal. Prevent powder from becoming moist while awaiting disposal, if possible. Moist product awaiting disposal must be kept away from combustible material & stored in a manner that allows suitable ventilation of the waste.

VII. HANDLING AND STORING

Handling Personnel: Avoid inhalation. Do not get in eyes and avoid contact with skin. Wear Personal Protective Equipment in accordance with section 8.
Handle with sufficient care to prevent dust generation.
Storage: Keep containers tightly sealed & avoid coming into contact with moisture during storage. Keep containers tightly Keep away from combustible material. Avoid contamination of the product.
1% solution: Store in a clean, loosely capped plastic container at room temperatures, and away from direct sunlight. Do not allow solution to freeze. Discard any used or contaminated solution & dispose of any stock solutions after 7 days from date of preparation.

VIII. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Controls:
Appropriate Local Exhaust Ventilation may be necessary for handling the product where dust formation is a problem, i.e. product in bulk quantities, or operations in small and/or poorly ventilated areas. Not normally necessary for preparation of solutions from small pack sizes (10lb or less).
Personal Protection Equipment:
Respiratory: Where a Health and Safety assessment shows the dusting levels to be sufficiently high when handling the powder product, wear a NIOSH approved respiratory mask against fine particles. Respiratory protection is not normally considered necessary when handling solutions of diluted product. However, when working with spray mists of Virkon S, respiratory protection in the form of a NIOSH approved respirator unit in conjunction with an organic vapor – fine particle filter cartridge.
Protective clothing:
Eye: Chemical splash goggles.
Skin: Overalls.
Hand: Rubber gloves.

Exposure Guidelines & Applicable Exposure Limits:

Potassium peroxomonosulfate

PEL (OSHA): None Established
TLV (ACGIH): None Established
AEL* (DuPont): 1 mg/m3, total dust, 8 & 12 hr. TWA

Sulfamic Acid

PEL (OSHA): None Established
TLV (ACGIH): None Established
AEL* (DuPont): 0.5 mg/m3, 8 & 12 Hr. TWA
1.5 mg/m3, 15 minute TWA

*AEL is DuPont's Acceptable Exposure Limit. Where governmentally imposed occupational exposure limits which are lower than the AEL are in effect, such limits shall take precedence.

IX. PHYSICAL AND CHEMICAL PROPERTIES

Boiling point: Decomposes on heating
Solubility in water: Approximately 8.3oz/gal
Form: Free flowing powder
Color: Yellow
Specific gravity: ~1.07

X. STABILITY AND REACTIVITY

Chemical stability: Stable at normal temperatures & storage conditions.
Incompatibility with other materials: Incompatible with strong alkalis. In contact with halogen salts (e.g. KC1, KBr, K1, NaCl), Virkon S may react to release toxic halogen gases, such as chlorine, bromine & iodine. In exceptional cases Virkon S may support combustion; avoid contact with combustible materials.
Decomposition: Under certain extreme conditions sulphur dioxide & chlorine may be generated if the powder is allowed to become moist.
Polymerisation: Polymerisation will not occur.

XI. TOXICOLOGICAL INFORMATION (Animal Data- VIRKON-S POWDER)

Acute Dermal Toxicity: LD_{50} >2.0g/kg (rabbit).
Acute Oral Toxicity: **LD_{50} = 1.70g/kg (male rats) & 1.16g/kg (female rats)**
Acute Inhalation Toxicity: 4 hour LC_{50} > 6.147mg/1 (male & female rats).
Guinea Pig Dermal Sensitisation: Virkon S displayed no fatiguing or sensitising effects.
Primary Skin Irritation: The powder is corrosive to the skin of rabbits with an irritation index of 7.00. A dilution of 5% results in an irritation index of 0.08 in rabbits.
Primary Eye Irritation: The powder is corrosive to rabbit's eyes. A dilution of 5% produces conjunctival irritation.
Effects of Overexposure: Inhalation of dust may cause choking, coughing or wheezing. A 1% solution is normally non-irritating.

XII. ECOLOGICAL INFORMATION

Aquatic Toxicity:

Oxone Monopersulphate:

96 hour LC_{50} – rainbow trout: 53 mg/L
48 hour EC_{50} – daphnia magna: 3.5 mg/L

Sodium Dodecylbenzenesulfate:

96 hour LC_{50} – rainbow trout: 1.7 mg/L

Sulphamic Acid:

96 hour LC_{50} – fathead minnows: 7.650 mg/L

XII. WASTE DISPOSAL CONSIDERATIONS

Treatment, storage, transportation, & disposal must be in accordance with applicable Federal, State/Provincial, and Local Regulations.

XIV. TRANSPORT INFORMATION

Shipping Information:

Not Regulated as a hazardous material by DOT, IMO, or IATA.

XV. U.S. REGULATORY INFORMATION

TSCA Inventory Status: Listed
The following components are TSCA listed:

Oxone
Sodium Dodecylbenzenesulfonate
Sulphamic Acid

Those not stated are proprietory & non-hazardous. However, all components over 0.1% inclusion are TSCA listed.
This information is based upon technical information believed to be reliable. It is subject to revision as additional knowledge & experience is gained.

Material Safety Data Sheet

Sodium Hypochlorite (5% Chlorine), Reagent

ACC# 95571

Section 1 - Chemical Product and Company Identification

MSDS Name: Sodium Hypochlorite (5% Chlorine), Reagent
Catalog Numbers: AC419550000, AC419550010, AC419550250
Synonyms: Antiformin; Sodium Chloride Oxide; Sodium Oxychloride.
Company Identification:

Acros Organics N.V.
One Reagent Lane
Fair Lawn, NJ 07410

For information in North America, call: 800-ACROS-01
For emergencies in the US, call CHEMTREC: 800-424-9300

Section 2 - Composition, Information on Ingredients

CAS#	Chemical Name	Percent	EINECS/ELINCS
7681-52-9	Sodium Hypochlorite, 5% Active Chlorine, Reagent	ca. 95.0	231-668-3

Section 3 - Hazards Identification

EMERGENCY OVERVIEW

Appearance: clear colorless to pale yellow liquid.
Warning! Contact with acids liberates toxic gas. Causes eye and skin irritation. May cause respiratory tract irritation.
Target Organs: Eyes, skin.

Potential Health Effects
Eye: Causes eye irritation.
Skin: Causes skin irritation.
Ingestion: Causes gastrointestinal irritation with nausea, vomiting and diarrhea.
Inhalation: May cause severe irritation of the respiratory tract with sore throat, coughing, shortness of breath and delayed lung edema.
Chronic: No information found.

Section 4 - First Aid Measures

Eyes: Immediately flush eyes with plenty of water for at least 15 minutes, occasionally lifting the upper and lower eyelids. Get medical aid imme diately.
Skin: Get medical aid immediately. Flush skin with plenty of water for at least 15 minutes while removing contaminated clothing and shoes.
Ingestion: Do not induce vomiting. If victim is conscious and alert, give 2-4 cupfuls of milk or water. Get medical aid immediately.
Inhalation: Get medical aid immediately. Remove from exposure and move to fresh air immediately. If not breathing, give artificial respiration. If breathing is difficult, give oxygen.
Notes to Physician: Treat symptomatically and supportively.

Section 5 - Fire Fighting Measures

General Information: As in any fire, wear a self-contained breathing apparatus in pressure-demand, MSHA/NIOSH (approved or equivalent), and full protective gear. During a fire, irritating and highly toxic gases may be generated by thermal decomposition or combustion. Substance is noncombustible. Oxidizer. Greatly increases the burning rate of combustible materials.
Extinguishing Media: Use extinguishing media most appropriate for the surrounding fire.
Flash Point: Not available.
Autoignition Temperature: Not available.
Explosion Limits, Lower:N/A
Upper: N/A
NFPA Rating: (estimated) Health: 2; Flammability: 0; Instability: 1

Section 6 - Accidental Release Measures

General Information: Use proper personal protective equipment as indicated in Section 8.
Spills/Leaks: Absorb spill with inert material (e.g. vermiculite, sand or earth), then place in suitable container. Clean up spills immediately, observing precautions in the Protective Equipment section. Provide ventilation.

Section 7 - Handling and Storage

Handling: Avoid breathing dust, mist, or vapor. Keep container tightly closed. Avoid contact with clothing and other combustible materials. Avoid ingestion and inhalation. Use with adequate ventilation. Use only in a chemical fume hood. Discard contaminated shoes.
Storage: Store in a tightly closed container. Keep refrigerated. (Store below 4°C/39°F.)

Section 8 - Exposure Controls, Personal Protection

Engineering Controls: Facilities storing or utilizing this material should be equipped with an eyewash facility and a safety shower. Use adequate ventilation to keep airborne concentrations low.
Exposure Limits

Chemical Name	ACGIH	NIOSH	OSHA - Final PELs
Sodium Hypochlorite, 5% Active Chlorine, Reagent	none listed	none listed	none listed

OSHA Vacated PELs: Sodium Hypochlorite, 5% Active Chlorine, Reagent: No OSHA Vacated PELs are listed for this chemical.
Personal Protective Equipment
Eyes: Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.
Skin: Wear appropriate protective gloves to prevent skin exposure.
Clothing: Wear appropriate protective clothing to prevent skin exposure.
Respirators: Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced.

Section 9 - Physical and Chemical Properties

Physical State: Liquid
Appearance: clear colorless to pale yellow
Odor: Disagreeable, sweetish odor
pH: Not available.
Vapor Pressure: 17.5 mm Hg @ 20C
Vapor Density: 2.57
Evaporation Rate: Not available.
Viscosity: Not available.
Boiling Point: Not available.
Freezing/Melting Point: Not available.
Decomposition Temperature: Not available.
Solubility: soluble in water
Specific Gravity/Density:1.097
Molecular Formula: ClNaO
Molecular Weight:74.44

Section 10 - Stability and Reactivity

Chemical Stability: Light sensitive. Sodium hypochlorite solutions decompose slowly at normal temperatures releasing low concentrations of corrosive chlorine gas. Decomposition is influenced by temperature, concentration, pH, ionic strength, exposure to light and the presence of metals.
Conditions to Avoid: Incompatible materials, light, combustible materials.
Incompatibilities with Other Materials: Methanol, metals, oxidizing agents, reducing agents, strong acids, acids (organic, e.g. acetic acid, benzoic acid, formic acid, methanoic acid, oxalic acid), ammonium salts.
Hazardous Decomposition Products: Hydrogen chloride, chlorine, sodium oxide.
Hazardous Polymerization: Will not occur.

Section 11 - Toxicological Information

RTECS#:
CAS# 7681-52-9: NH3486300
LD50/LC50:
CAS# 7681-52-9:
Draize test, rabbit, eye: 10 mg Moderate;
Draize test, rabbit, eye: 1.31 mg Mild;
Oral, mouse: LD50 = 5800 mg/kg;

Carcinogenicity:
CAS# 7681-52-9: Not listed by ACGIH, IARC, NTP, or CA Prop 65.

Epidemiology: No information available.
Teratogenicity: No information available.
Reproductive Effects: No information available.
Mutagenicity: Mutation in microorganisms: Salmonella Bacteria = 1mg/plateDNA Repair: E. coli= 20ug/discDNA Damage: E. coli = 420 umol/LCytogenetic analysis: Human Lymphocyte = 100 ppm/24H
Neurotoxicity: No information available.

Other Studies:

Section 12 - Ecological Information

Ecotoxicity: No data available. No information available.
Environmental: No information found.
Physical: No information found.
Other: No information available.

Section 13 - Disposal Considerations

Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. US EPA guidelines for the classification determination are listed in 40 CFR Parts 261.3. Additionally, waste generators must consult state and local hazardous waste regulations to ensure complete and accurate classification.
RCRA P-Series: None listed.
RCRA U-Series: None listed.

Section 14 - Transport Information

	US DOT	Canada TDG
Shipping Name:	HYPOCHLORITE SOLUTIONS	HYPOCHLORITE SOLUTIONS
Hazard Class:	8	8
UN Number:	UN1791	UN1791
Packing Group:	III	III

Section 15 - Regulatory Information

US FEDERAL

TSCA
CAS# 7681-52-9 is listed on the TSCA inventory.
Health & Safety Reporting List
None of the chemicals are on the Health & Safety Reporting List.
Chemical Test Rules
None of the chemicals in this product are under a Chemical Test Rule.
Section 12b
None of the chemicals are listed under TSCA Section 12b.
TSCA Significant New Use Rule
None of the chemicals in this material have a SNUR under TSCA.
CERCLA Hazardous Substances and corresponding RQs
CAS# 7681-52-9: 100 lb final RQ; 45.4 kg final RQ
SARA Section 302 Extremely Hazardous Substances
None of the chemicals in this product have a TPQ.
SARA Codes
CAS # 7681-52-9: immediate.
Section 313 No chemicals are reportable under Section 313.
Clean Air Act:
This material does not contain any hazardous air pollutants.
This material does not contain any Class 1 Ozone depletors.
This material does not contain any Class 2 Ozone depletors.

Clean Water Act:
CAS# 7681-52-9 is listed as a Hazardous Substance under the CWA.
None of the chemicals in this product are listed as Priority Pollutants under the CWA.
None of the chemicals in this product are listed as Toxic Pollutants under the CWA.
OSHA:
None of the chemicals in this product are considered highly hazardous by OSHA.
STATE
CAS# 7681-52-9 can be found on the following state right to know lists: California, New Jersey, Pennsylvania, Minnesota, Massachusetts.

California Prop 65

California No Significant Risk Level: None of the chemicals in this product are listed.

European/International Regulations

European Labeling in Accordance with EC Directives
Hazard Symbols:
XI
Risk Phrases:
R 31 Contact with acids liberates toxic gas.
R 36/38 Irritating to eyes and skin.

Safety Phrases:
S 45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).
S 28A After contact with skin, wash immediately with plenty of water
S 50A Do not mix with acids.

WGK (Water Danger/Protection)
CAS# 7681-52-9: 2
Canada - DSL/NDSL
CAS# 7681-52-9 is listed on Canada's DSL List.
Canada - WHMIS
This product has a WHMIS classification of E, D2B.
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations and the MSDS contains all of the information required by those regulations.
Canadian Ingredient Disclosure List
CAS# 7681-52-9 is listed on the Canadian Ingredient Disclosure List.

Section 16 - Additional Information

MSDS Creation Date: 2/03/1999
Revision #5 Date: 11/29/2007

The information above is believed to be accurate and represents the best information currently available to us. However, we make no warranty of merchantability or any other warranty, express or implied, with respect to such information, and we assume no liability resulting from its use. Users should make their own investigations to determine the suitability of the information for their particular purposes. In no event shall Fisher be liable for any claims, losses, or damages of any third party or for lost profits or any special, indirect, incidental, consequential or exemplary damages, howsoever arising, even if Fisher has been advised of the possibility of such damages.

CITRICIDAL

Product Description

CITRICIDAL® is a broad spectrum antimicrobial compound synthesized from the seeds and pulp of grapefruit.

Application

CITRICIDAL® is an extremely potent and effective broad spectrum bactericide, fungicide, antiviral and antiparasitic compound. CITRICIDAL® is environmentally safe with a low toxicity to man and animals.

Mode of Activity

Studies indicate that the antimicrobial activity of CITRICIDAL® is in the cytoplasmic membrane where the uptake of amino acids is prevented and disorganization of the cytoplasmic membrane and leakage of low molecular weight cellular contents.

Biodegradability

CITRICIDAL® is biodegradable according to the "Standard Test Methods for Determining the Anaerobic Biodegradation Potential of Organic Chemicals", ASTM Standards, Section 11, Water and Environmental Technology, Procedure E 1196-2, pp. 879-901,1993.

Uses

Agriculture: Bactericide and fungicide in both pre-harvest and post-harvest treatment - *range: 50 ppm to 250 ppm**
Fish & Poultry: Disinfectant for fresh fish and poultry, preservative for processed fish and poultry - *range: 100 ppm to 1000 ppm**
Animal Feed: Mold inhibitor and antiparasitic - *range: 50 ppm to 250 ppm**
Food: Preservative and antioxidant- *range: 10 ppm to 250 ppm**
Cosmetics: Preservative and antimicrobial - *range: 1000 ppm to 10,000 ppm*
Water Treatment: Disinfectant for contaminated water - *range: 50 ppm to 250 ppm**
Therapeutic: - *range: 50 to 200 mg/dose**

Physical Properties

Citricidal® Liquid Extract

Grapefruit Extractives	60%
Glycerin-USP	40%
Total	100%

Citricidal Powder Extract

Grapefruit Extractives	50%
Silicon Dioxide - USP	30%
Glycerin-USP	20%
Total	100%

Chemical Description: Diphenol hydroxybenzene complex
Appearance (liquid): Liquid/heavy viscous
Color (Gardner): 2, Lemon Yellow
Odor: Mild citrus
Specific Gravity (d25 °C): 1.110
Density (lbs./gal.): 9.5
pH (d25 °C): 2.0 - 3.0
Flash Point (°F): 292
Viscosity (Centistoke): 134.91
Molecular Weight: 565
Solubility: Water, alcohol and organic solvents

**International registrations only*

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Citricidal Toxicity

Acute Oral Toxicity	LD_{50} over 5,000 mg/kg of live weight
Chronic Toxicity (Acute oral with continuous feeding and reproduction study for 24 months)	LD_{50} 2,500 mg/kg of live weight (Rats and guinea pigs)
Acute Oral Toxicity (Continuous feeding study with fishmeal for 12 months)	LD_{50} 5,000 mg/kg of live body weight (Adult rats, 12months) LD_{50} 400 mg/kg of live weight (Newborn rats)
Dermal Toxicity	Not a primary skin irritant and is non-corrosive
Carcinogenicity	12 month tests in mice show no carcinogenic effect 24 month test in rats show no carcinogenic effect
Long-Term Inhalation Study	Closed chamber exposure for 8 hours a day, 5 days a week for 90 days - No effect at 100-150 mg/m3 air
Dermal Toxicity Carcinogenicity	2 year studies with rats and mice. No carcinogenic, toxicity or systemic effects seen
Eye Irritation	Full strength - severe irritation with slight corneal iris injury. 0.5%,1% and 2% concentrations produce irritation and moderate erythema
Human Patch Studies	1 % and 2% concentrations produced no irritation or sensitization. 3% concentration produced very mild irritation by allergic humans.

Test Results

The following analytical results illustrate that CITRICIDAL® can have a broad and efficacious range of applications, offering superior performance compared with commonly used antimicrobial agents, while fulfilling standard performance criteria. The following information is representative of additional test results, including safety data, which are available upon request from ***bio/chem.***

Relative Potency of Anti-Microbial Agents

The Minimum Inhibitory Concentration Study is a microbiological assay used to evaluate the relative potency of CITRICIDAL® compared to other antimicrobial agents. This study demonstrates CITRICIDAL® to be a minimum of ten (10X) to one hundred times (100X) more effective than other agents tested against the organisms used in this study.

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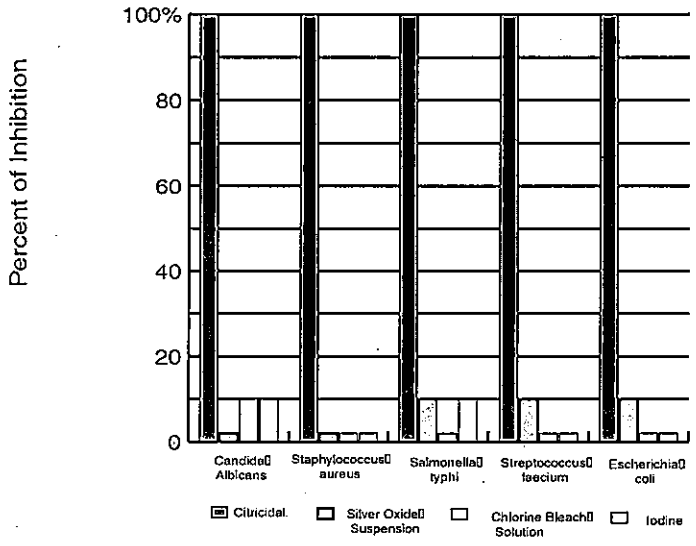
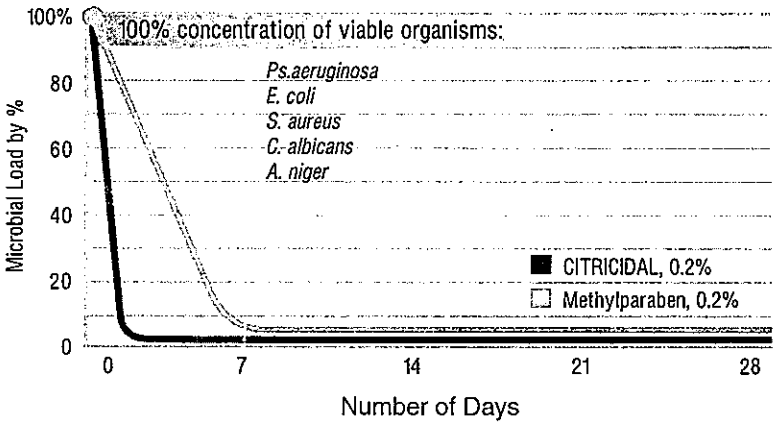
CITRICIDAL



USP Preservative Challenge Test

The USP Preservative Challenge test evaluates the ability of a product to withstand microbial insult. It is designed to determine whether the product is protected from microorganisms, which would alter the quality and integrity of a finished formulation. This study demonstrates that CITRICIDAL® is as effective as methylparaben in meeting the requirements of the USP Preservative Effectiveness Test. It also demonstrates that CITRICIDAL® has a more rapid onset of activity in reducing the concentration of viable organisms. (Please note: CITRICIDAL® is cationic.)

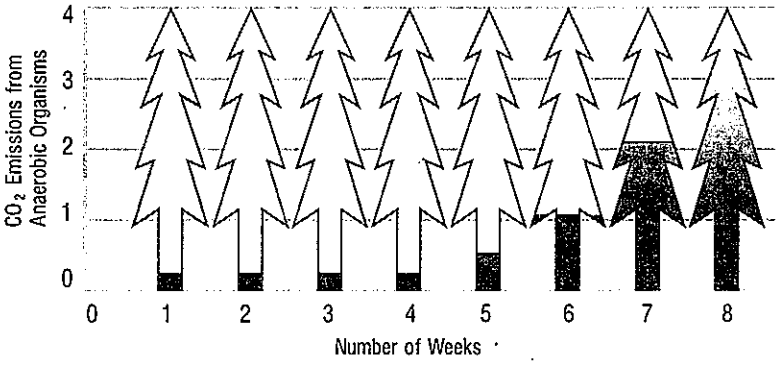


CITRICIDAL

Test Results (Continued)

Biodegradability Assessment of CITRICIDAL®

The biodegradability of CITRICIDAL® Liquid is established by the "Standard Test Methods for Determining the Anaerobic Biodegradation Potential of Organic Chemicals", ASTM Standards, Section II, Water and Environmental Technology, Procedure E 1196-2, pp 879-901, 1993. CITRICIDAL® has an inhibitious effect on carbon dioxide production in an anaerobic digestion system for the first four weeks. At the end of eight weeks, gas production reached the theoretical maximum demonstrating that CITRICIDAL® is biodegradable using accepted testing procedures.



Complete test results available upon request.

CITRICIDAL® Packaging Specifications

Liquid Extract		Powder Extract
5 Gallon Plastic Pail 11" x 14 1/2" x 16" Net wt. 50 lbs. Gross wt. 55 lbs.	55 Gallon Plastic Drum 23 1/2" x 36" Net wt. 500 lbs. Gross wt. 550 lbs	30 Gallon Fiber Drum 16" x 29" Net wt. 110 lbs. Gross wt. 120 lbs

CITRICIDAL® (as a natural extractive) is listed as GRAS (Generally Recognized as Safe) under the Code of Federal Regulations as 21 CFR 182.20. CITRICIDAL® has been tested for safety in both human and animals, including the environment. CITRICIDAL® is considered non-toxic and a non-irritant at dilutions up to 2%. CITRICIDAL is also considered non-corrosive.

CAS NO.: 90045-43-5 **CTFA listing: Grapefruit Extract**

Note: CITRICIDAL® should be handled with care in full strength. Avoid contact with the eyes and avoid breathing vapors at full strength. Any direct contact with the skin should be thoroughly rinsed with water.

CITRICIDAL

CITRICIDAL® Minimum Inhibitory Concentration In-Vitro (MIC)

Gram-negative bacteria	Origin & strain No.		MIC (ppm)
Aerobacter aerogenes	CITM	413	20
Alcalingenes faecalis	A		2000
Brucella intermedia	A		2
Brucella abortus	NCTC	8226	2
Brucella melitensis	A		2
Brucella suis	A		2
Cloaca cloacae	NCTC	8155	6
Escherichia coli	NCTC	86	2
Escherichia coli	ATCC	9663	6
Escherichia coli	NCTC	9001	6
Haemophilus influenzae	A		660
Klebsiella edwardsii	NCTC	7242	6
Klebsiella aerogenes	NCTC	8172	6
Klebseilla pneumoniae	ATCC	4352	6
Legionella pneumoniae	isolate		200
Loefflerella mallei	NCTC	9674	6
Loefflerella pseudomallei	NCIB	10230	20
Moraxella duplex	A		2
Moraxella glucidolytica	A		6
Neisseria catarrhalis	NCTC	3622	660
Pseudomonas capacia	C-175		5000
Pasteurella septica	NCTC	948	2
Pasteurella pseudotuberculosis	C.-G.		200
Proteus vulgaris	NCTC	8313	2
Proteus mirabilis	A		6
Pseudomonas aeruginosa	NCTC	1999	2000
Pseudomonas aeruginosa	ATCC	12055	20,000
Pseudomonas fluorescens	NCTC	4755	2000
Salmonella choleraesuis			50
Salmonella enteritidis	A		6
Salmonella gallinarum			50
Salmonella typhimurium	NCTC	5710	6
Salmonella typhi	NCTC	8384	6
Salmonella paratyphi A	NCTC	5322	6
Salmonella paratyphi B	NCTC	3176	6
Salmonella pullorum	ATCC	9120	6
Serratia marcescens	A		2000
Shigella flexneri	NCTC	8192	6
Shigella sonnei	NCTC	7240	3
Shigella dysenteriae	NCTC	2249	2
Vibrio cholerae	A		200
Vibrio eltor	NCTC	8457	200

Fungi and Yeasts	Origin & strain No.		MIC (ppm)
Aspergillus niger	ATCC	6275	600
Aspergillus fumigatus	ATCC	9197	200
Candida albicans	A		60
Candida albicans	ATCC	10259	60
Epidermophyton floccosum	ATCC	10227	200
Keratinomyces ajelloi	A		200
Monilia albicans			10
Saccharomyces cerevisiae			60
Trichophyton mentagrophytes	ATCC	9533	20
Trichophyton rubrum	A		200
Trichophyton tonsurans	A		200

Gram-positive bacteria	Origin & Strain No.		MIC (ppm)
Bacillus subtilis	NCTC	8236	2
bacillus megatherium	A		60
bacillus cereus	A		60
bacillus cereus var. mycoides	A		60
Clostridium botulinum	NCTC	3805	60
Clostridium tetani	NCTC	9571	60
Corynebacterium acnes	ATCC	6919	60
Corynebacterium diphtheriae	ATCC	6917	60
Corynebacterium diphtheriae	NCTC	3984	60
Corynebacterium diphtheriae	A		60
Corynebacterium minutissium	ATCC	6501	100
Diplococcus pneumoniae	NCTC	7465	60
Lactobacillus arabinosus	CITM	707	66
Lactobacillus arabinosus	ATCC	8014	66
Lactobacillus casei	CITM	707	100
Listeria monocytogenes	ATCC	15313	20
Mycobacterium tuberculosis	A		2000
Mycobacterium smegmatis	NCTC	8152	20
Mycobacterium phlei	A		6
Sarcina lutea	NCTC	196	60
Sarcina ureae	ATCC	6473	2
Staphylococcus aureas	NCTC	7447	2
Staphylococcus aureas	NCTC	4163	2
Staphylococcus aureas	NCTC	6571	6
Staphylococcus aureas	NCTC	6966	2
Staphylococcus aureas	ATCC	13709	2
Staphylococcus aureas	ATCC	6538	2
Staphylococcus albus	NCTC	7292	2
Staphylococcus albus	C.-G.		6
Streptococcus agalactiae	NCTC	8181	60
Streptococcus haemoyticus A	A		20
Streptococcus faecalis	NCTC	8619	200
Streptococcus faecalis	ATCC	10541	60
Streptococcus pyogenes	NCTC	8322	60
Streptococcus viridans			20

Additional Organisms

Giardia lamblia
Entamoeba histolytica
Chlamydia trachomatis
Herpes simplex virus type 1
Influenza A_2 virus
Helicobacter pylori
Campylobacter jejuni

The data presented herein is based on experiments and information believed to be accurate and reliable. However, no warranty is made, either expressed or implied, regarding the accuracy of the results to be obtained from the use of such date. Bio/Chem Research will assume no responsibility for the results of performance in products and applications over which Bio/chem Research has no control.

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CITRICIDAL

Citricidal Grapefruit Seed Extract

What is it? Where did it come from?

Citricidal: Broad Spectrum, Potent Antimicrobial, and Safe

The Citricidal Story

Citricidal was originally developed by a German physicist and immunologist, Jacob Harish, as an antiparasitic. Dr. Harish was finally in the 1960's able to convince researchers at the University of Florida at Gainesville to experiment with the use of grapefruit extract as an alternative to then-current chemicals for the protection of fruit and vegetables. They were quickly won over with GSE's amazing ability to inhibit the growth of not only parasites, but fungi and bacteria as well. Tests conducted by the U.S. Dept. of Agriculture in the early 1980's confirmed that Citricidal, as it was then called, was effective in inhibiting viral strains in cattle and hogs, and was approved for the USDA's Evian Influenza Eradication Program in 1984. Sadly, USDA's promise to investigate further uses for Citricidal in these areas has never materialized.

Since the mid-1980's, the production team at Citricidal® worked closely with Dr. Harish, perfected the manufacturing process, opened new facilities, and pioneered the use of Citricidal® beyond it's original application.

Citricidal® is synthesized from the polyphenolic compounds found in grapefruit seed and pulp. Numerous reactions are involved, including distillation, catalytic conversion, and ammoniation. The active component of Citricidal is a quaternary ammonium chloride(a diphenol hydroxybenzene reacted with ammonium chloride) **similiar** to benzethonium chloride when analysed in accordance with USP XXII/NF XVII. (Benz. Chloride is a powerful germical agent, but is highly toxic to all animal life. See info on toxicity, below)

Residues of pesticides, fungicides and preservatives have always been a concern with regards to our product. The use of these compounds is common in the agricultural and botanical industries in the United States as well as abroad. In addition, the extraction processes involved in botanicals would not necessarily remove these compounds. Therefore, every batch of Citricidal® is certified for the absence of such residues, as well as the absence of Triclosan, a common germicide and preservative. Independent labs have confirmed these results. (see United States Testing Company Report No. 405993, dated 9/8/95). The results show no trace of triclosan, while displaying very strong antimicrobial activity. Every batch of Citricidal is tested and certified free from chemical and heavy metal contamination. And in an attempt to further improve the product, a source of grapefruit seed and pulp from Certified Organically Grown grapefruits has been secured.

To further show the safety of Citricidal, an Acute Oral Toxicity Study was performed(see Northview Pacific Labs Report No. X5E015G, dated 7/6/95). Results showed that Citricidal is considered non-toxic by oral ingestion with an LD_{50} of over 5000 mg/kg of live body weight. This is the equivalent of a 200 lb. person drinking close to 1 lb. of pure Citricidal daily for two weeks, before risking a 50% risk of fatal poisoning. (There are close to 20,000 drops in one pound of Citricidal liquid. The recommended adult dose is 5-6 drops at a time.)

According to the Association of Poison Control Centers, the AMA Physician Reporting System, and the Journal of Emergency Medicine, there have been no reports that Citricidal has ever harmed anyone. In fact, there are thousands of clinical and anecdotal reports that Citricidal has helped many, and enjoys a safety record going back more than 30 years.

Over the years, numerous and differing analytical tests have been performed to determine the active components of Citricidal. The test results have quite often varied, for the following reasons: a.) varying test procedures, b.) different chemicals used in the test procedures producing false positives, c.) different interpretations of test procedures resulting in false positives, and d.) the different background of the chemists involved, organic chemistry vs. inorganic chemistry being an issue. The similarity in molecular weight between Citricidal and both Benzelkonium Chloride and Benzelthonium Chloride has wrongly influenced some(including drug and chemical manufacturers) to assert that Citricidal has been "spiked" with these poisons. (They are both powerful industrial disinfectants, and are even found in some consumer goods in the U.S.) But once again, independent lab tests, and a 30-year track record of safe use as a human therapeutic speak loudly against such slander. More on quaternary compounds here.

"Citricidal®" is the trademark of our professional-strength product, available in both liquid and powder form. "Nutribiotic®" is the trademark of our growing consumer line of products containing Citricidal® in measured quantities, along with other, thoroughly tested ingredients, insuring the highest quality, potency, and safety.

With so many products on the market making so many claims, a measure of skepticism about GSE is understable. But consider that Citricidal® and NutriBiotic® extracts have been used in their present form for some 10 years, all over the world. Virtually all of our bulk-order customers have had the product tested in the laboratory before buying. Even without the advantages afforded the pharmaceutical establishment, Citricidal® and NutriBiotic® have gained wide acceptance. Production for 1998 exceeded 1.5 million pounds. As more uses are discovered, and more testing is done to confirm its safety and efficacy, it is certain that many more questions about agricultural, environmental, and human health will be answered with Citricidal® Grapefruit Seed Extract.

Click here for more Grapefruit Extract information.

Lab reports on grapefruit seed extract, technical data, common uses, feedback from doctors and consumers, FAQ's, dosages, discussions, newsletter, and lots more.
How specific common problems have been treated: candidiasis, parasites, sinusitis, athlete's foot, colds & flu, ulcers, pets and livestock, crops and foodstuffs, applications as germicide, preservative, and preventive.

Click here for Secure Ordering of Grapefruit seed extract products.

GSE liquid concentrate, capsules, tablets, first-aid spray, dental gel, skin cleansers, ear drops, etc. Nutriteam®, Prozone®, NutriBiotic®. Citricidal® liquid and powder concentrates.
Special reduced prices.

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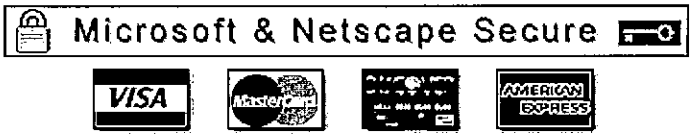

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1 802 388-0661 fax: 815 377-2198
Send email to support@nutriteam.com

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