Item A:
Petition for: **Synthetic substance allowed for use in organic crop production.**

Item B:

1. *Substance common name:*

   CAS No.- 2032207-39-7

   Hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1)

   Registered Tradename- "Tydronium®"

   Generic- "Stabilized Hydronium"

   Other Names: Oxonium trihydrate, sulfate 1:1, Tetra-aqua hydrogen(+1), sulfate 1:1 or Hydrogen ion tetra-hydrate, sulfate 1:1

   Composition: H904. H O4 S

2. *Manufacturer's name and contact information:*

   Tygrus, LLC
   1132 E. Big Beaver Rd.
   Troy, MI 48083
   USA
   Phone: (248) 218-0347 X110
   FAX: (248) 592-7325
   E:mail: lcarlson@tygrus.com

   Tygrus, LLC
   4505 Greenstone Rd.
   Placerville, CA 95667
   USA

Phone: (248) 218-0347 x 108
FAX: (530) 344-7641
E:Mail: thoel@tygrus.com

3. *Intended use of substance:*

Livestock manures- allowance of Hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1) for pH adjustment and stabilizer not below a pH of 5.00. Sulfuric acid, phosphoric acid and citric acid are currently NOP-approved processing aids for pH adjustment in organically processed liquid fish products and some aquatic plant extracts for use in crop production (NOP205.601(j)(7). *Tydronium* would be used in the same way with livestock manures as a processing aid in the production of dehydrated manure for use in organic crop production.

4. *A list of the handling/processing for which the substance will be used:*

To adjust the pH of livestock manures, potentially occurring as high as 8.30 during natural degradation, to not less than pH5.00, prior to dehydrating the solids for final use as a soil amendment allowable for use in organic crop production.

The method of handling this safe and effective acidic material will vary between animal species, diet formulation and respective farm manure handling facilities. Typically, small amounts (0.1 % to 1.00 % v./v.) will be added on a continuous basis via a metering valve or pump off a supply tank. This would occur during manure transport, mixing and storage to decrease the malodorous properties that may be experienced. In cases of long storage times or non-continuous mixing and transport, the acidic material may be added in batch mode but the volume of the acidic

material needed should be consistent with the continuous feed method.

5. *The source of the substance and a detailed description of its manufacturing or process procedures from basic components to final product:*

Hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1) or "Tydronium®" as is the commercial designation is derived from two naturally-occurring substances which are "Food Grade" Sulfuric Acid 66° Be' and "Food Grade" Calcium Hydroxide 45% wgt.

Sulfuric Acid is mostly produced via a typical Contact Process Involving Sulfur Dioxide (SO2) collected by pollution control emission units such as packed bed scrubbers, composite mesh pads or the like that are involved when smelting sulfate-bearing metallic ores. The SO2 at the facilities is first captured in the scrubbers to reduce emissions contributing to acid rain. The resulting "Scrubber Feedstock" from the pollution control scrubbers is further cleansed, purified, concentrated, and used for the production of sulfuric acid.

Calcium Hydroxide Slurry 45% is typically produced by hydrating calcium oxide and can be done in a variety of ways:

Calcium hydroxide adopts a polymeric structure, as do all metal hydroxides. The structure is identical to that of $Mg(OH)_2$ (*brucite structure*); i.e., the cadmium iodide motif. Strong hydrogen bonds exist between the layers.[8]

Calcium hydroxide is produced commercially by treating lime with water:

$$CaO + H_2O \rightarrow Ca(OH)_2$$

The Production process for producing Hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1) is as follows as detailed in US Patent #7,513,987: ***NOTE: See separate attached Folder labeled as "Tydronium Mfg. Method for NOPB Submission".**

6. *A summary of any available previous reviews by State or private certification programs or other organizations of the petitioned substance:*

a) IESCAS Review in NOV 2013 for Chemical Index No. characterization, which resulted in two new classifications for the Tydronium (acidic material) and Tydroxide (basic) substances. Chemical Index No.'s and characterization available from IESCAS.
b) EPA Pre-Submission Review for Bio-Pesticide. **NOTE:*See Pre-Submission Form for Data and Additional Info.**
c) EPA 6-Pack Testing Results included in the EPA Pre-Submission Form.
d) State Registrations for Tydronium® in Formulations as a Soil Amendment- *Attached

7. *Information regarding EPA, FDA and State Regulatory authority registrations including registration numbers.*

a) Under review by EPA and FDA as a bio-pesticide.
b) EPA 6-Pack Testing data shows the chemistry is NON-HAZARDOUS based on dermal, sensitization, ocular, inhalation and ingestion testing results.
c) Not regulated by D.O.T. based on requirements.
d) State Registrations for Tydronium® in Formulations as a Soil Amendment- *Attached

8. The Chemical Abstract Service (CAS) number or other product numbers of the substance and labels of products that contain the petitioned substance- Hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1) Registered Tradename- "Tydronium®"

a) IESCAS/ACS Chemical Index No.-

A division of the American Chemical Society

InventoryExpertService

Phone: 800-631-1804, 614-447-3870
Fax: 614-447-3747
E-mail: answers@cas.org
Web: www.cas.org/products/other-cas-products/client-services/

INVENTORY EXPERT SERVICE REPORT

IES Order Number: 348687-1
Registry Number: Not Registered
CA Index Name:
Hydrogen(1+), triaqua-μ3-oxotri-, sulfate (1:1)

Please print the above CA Index Name on the appropriate page of your PMN.

[X] If this box is checked, CAS has made correction(s) marked in red to your IES order. Please make the same corrections to your PMN before submitting it to the EPA.

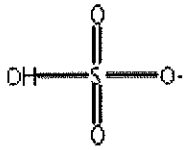
CAS • 2540 Olentangy River Road • P.O. Box 3343 • Columbus, OH 43210-0334 • USA

b) CAS No.- (Via SciFinder)

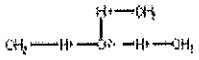
CAS_ServicesDocs[1].pdf
CAS#12501-73-4 Hydrogen(1+), triaqua-μ3-oxotri-.pdf
CAS#2032207-39-7 Hydrogen(1+), triaqua-μ3-oxotri-, sulfate (1-1) copy.pdf
CAS#2032207-39-7 Hydrogen(1+), triaqua-μ3-oxotri-, sulfate (1-1).pdf

CAS Registry Number: 2032207-39-7
$H_9 O_4 . H O_4 S$
Hydrogen(1+), tri$aqua-\mu_3$-oxotri-, sulfate (1:1)

14996-02-2
$H O_4 S$



12501-73-4
$H_2 O_4$



~2 References

CAS Role	Patents	Nonpatents	Nonspecific Derivatives from Patents	Nonspecific Derivatives from Nonpatents
Biological Study	✓			
Properties	✓			
Uses	✓			

Source of Registration: CA

a) *Chemical interactions with other substances, especially substances in organic production:*

Hydrogen (+1), triaqua-μ3-oxotri, sulfate (1:1) used within livestock manures will provide for organically-derived nitrogen compounds to be stable in solution as opposed to being volatilized during the dehydration of manure compounds.
Malodorous generation of the biological breakdown products is greatly reduced and/or mitigated by the pH regulation and buffering of this substance. The introduction of small amounts of this substance to lower the pH to not less than 5.00 will mitigate the rate at which the more volatile forms of nitrogen (ammonia) and organic (carbon based) compounds (fatty acids) are formed.

Manure and dehydrated manure are approved soil amendments by State and Federal fertilizer regulatory agencies when used in conjunction with proper nutrient management practices. IAW NOP 205.203, the soil fertility and crop nutrient management practice standard and the National Organic Program also approves manures.

(b) Toxicity and Environmental persistence of Hydrogen (+1), triaqua-μ3-oxotri, sulfate (1:1)

When Hydrogen (+1), triaqua-μ3-oxotri, sulfate (1:1) is incorporated in correct proportions and dried during the process, its use ranges will be from 0.25-1.50 % v./v. wet weight. Once this substance is added to manure, the protonic portion of the substance is neutralized by the manure and is oxidized form remains as (SO4 -2).

The added polymeric stabilized hydronium facilitates and aids the respiration process in the mitochondrial region, which provides additional protons for the production of energy via the ATP-driven proton pumping mechanism. This is done in a manner,

which is innocuous and safe because of the lack of corrosivity and unique disassociation methodology employed by this technology.

***NOTE: See USEPA 6-Pack Exposure Testing Results.

The added sulfur (S), although negligible, is considered beneficial to the crop. Sulfur, in its oxidized form sulfate (SO_4^{2-}), is an essential nutrient in the formation of chlorophyll and the amino acids within the plant.

The residual oxidized form of sulfate (SO_4^{2-}) in the manure takes on forms and functions within crops to be considered a nutrient as opposed to being a contaminant.

1. FORMS
   - Plants absorb mostly SO_4^{2-}; small quantities of SO_2 can be absorbed by plant leaves
   - Plant S ranges between 0.1 and 0.5% S and varies with plant type:
     → Gramineae (0.18 - 0.19% S in seed)
     → Leguminosae (0.25 - 0.3% S in seed)
     → Cruciferae (1.1 - 1.7% S in seed)
2. Functions
   - S-containing amino acids cystine, cysteine, and methionine, essential components of protein, comprise 90% of plant S.
   - S deficient plants produce less protein and accumulate nonprotein N as NH_2 and NO_3 → leaf NO_3^- accumulates under S deficiency reducing food quality
   - Adequate S improves crop quality by narrowing N/S ratio to 9:1 to 12:1 needed for effective use of N by rumen microorganisms.
   - S is needed for synthesis of chlorophyll and coenzyme A, this being important for oxidation and synthesis of fatty acids and amino acids.
   - S is a component of ferredoxins, an Fe-S protein in chloroplasts. Ferredoxin is important in NO_2^- and SO_4^{2-} reduction and N_2 assimilation by root nodule bacteria.
   - S is responsible for the characteristic taste and smell of mustard and onion plants.

Toxicity and environmental persistence of any other trace material within the Tydronium such as residual metals, is very tightly controlled in the production phase and starts immediately with the raw materials, which are "Food Grade" rated, and on the FDA GRAS List of Chemicals. There are no hazardous reaction by-products that result from the generation of this chemistry and the chemistry generated is listed as "Non-Hazardous" and not subject to listing on the TSCA Registry.

c) *Environmental impacts from its use or manufacture:*

There are no known pollution issues with producing the Tydronium Technology as it is rated as "Non-Hazardous" as verified by 3rd Party Testing, EPA 6-Pack Testing, Independent 3rd Party from A2LA-accredited Testing Facilities and University in-house testing sources. There is no discharge from waste material as there is no emitting to air, water or landfill/deep well injection. The only material generated is a secondary non-hazardous material, which is a "hydronium-catalyzed hydrated calcium sulfate, which will be re-disposition and designated as good, used material that has in excess of at least 7 commercial applications.

(d) Effects on Human health:

Unlike mineral and oxidizing organic acids, and based on recent USEPA 6-Pack Testing, the Tydronium Technology is classified as "Non-hazardous" and has a corrosivity rating very similar to DI water (0.02) and does mot induce amide hydrolysis on plant, animal or human tissue.

*NOTE: Refer to USEPA Six-Pack Data from AFCO/ZEP and Report Summary in the USEPA Pre-Submission Package.

*NOTE: Heavy metals assay attached for product containing Tydronium®

10. Safety information about the substance including a Safety Data Sheet (GHS protocol) and a comprehensive substance report from the National Institute of Environmental Health (NIEH) Studies:

*NOTE: SDS attached for Tydronium®

*NOTE: USEPA 6-Pack testing Data attached.

11. Research information about the petitioned substance, which includes comprehensive substance, research reviews and research bibliographies, which present contrasting positions to those presented by the petitioner in supporting the substance's inclusion on the National List:

*NOTE: Dr. Cole Texas A&M Graduate Paper attached.

*NOTE: Dr. Joseph DeVito ATL R&D Report attached.

*NOTE: Eurofins Report Attached

There have been no contrasting positions presented formally or informally that are available for review at this time. The technology has been presented to Federal, State and Local agencies as well as academic and 3rd Party Reviewers with no negative reviews or concerns at this time.

12. This synthetic substance is necessary for the production and handling of a dehydrated manure based organic soil amendment because:

Hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1) has been shown to be highly effective in minimizing ammonia emissions and odors during our 3 year pilot field test in in testing manure and compost compositions with a well-known organic grower and in conjunction with UC-Davis. He is a major Alpaca manure producer in Northern CA. This beta test site has also shown that this chemistry is highly effective as a protein stabilizer in the manure and pH adjustor on his manure varieties. This test has also included a number of other mineral acids such as Sulfuric,

Phosphoric, Nitric and Hydrochloric to compare against and as controls.

To summarize, we believe that Hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1) is the best choice for the production and handling of a dehydrated manure based organic soil amendment because:

In addition to efficacy and performance tests,

1. The material is much safer to transport, handle and store.

2. Is much more environmentally friendly.

3. This technology is devoid of odor,

4. Non-corrosive to plant/animal/human tissue,

5. Is non-exothermic in aqueous mediums and,

5. Has minimum one-year shelf life stability.

6. The chemistry has shown to be very cost effective and very efficient in usage, as minimal concentrations are needed to be effective.

7. The chemistry is sustainable and is produced from readily available Food Grade constituents.

13) A Commercial Confidential Information Statement which describes the specific required information contained in the petition that is considered Confidential Business Information (CBI).

Tygrus, LLC has published US Patents and PCT disclosures as well as Chemical Index Numbers and CAS No's disclosed in this document and

publicly available. Therefore. We are not pursuing a CBI designation at this time.

1400 Independence Avenue, SW. Room 2648-S, STOP 0268 Washington, DC 20250-0268

September 24, 2018
Tygrus, LLC
1132 E. Big Beaver Rd.
Troy, MI 48083
Sent by email [lcarlson@tygrus.com]

Dear Tygrus, LLC:
Thank you for your petition of August 28, 2018, which requests the addition of Hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1), stabilized hydronium, or "Tydronium®" to the National Organic Program's (NOP) National List of Allowed and Prohibited Substances (National List) as a synthetic substance allowed for use in organic crop production.
We have reviewed your petition according to the procedure described in NOP 3011, National List Petition Guidelines. This procedure provides information such as what can be petitioned, how substances should be petitioned, and what information should be addressed in a petition.
Additional information is needed to complete your petition. To assist you in the development of your petition, we recommend that you modify your current petition to address the following items:

Item A.2 – OFPA Category
We noted that the petition did not include Item A.2 and did not indicate which OFPA category applies to stabilized hydronium. NOP 3011 Section 4.2 "Items to be Included in a Petition,"

indicates that petitions are to contain the following information listed under Item A.2:

For substances petitioned for use in crop or livestock production, eligible substances must contain an active synthetic ingredient in one of the following OFPA categories (7 U.S.C. § 6517(c)(1)(B)(i)):

- *Copper and sulfur compounds;*
- *Toxins derived from bacteria;*
- *Pheromones;*
- *Soaps;*
- *Horticultural oils;*
- *Fish emulsions;*
- *Treated seed;*
- *Vitamins and minerals;*
- *Livestock parasiticides and medicines; and*
- *Production aids.*

Petitioners should indicate which OFPA category applies to their petitioned material.

For the revised petition, please indicate which OFPA category applies to the petitioned material.

Answer- a) The petitioned use of the chemistry is as a manure processing aid primarily for alpaca/llama protein nucleotide extraction and stabilization. As an acidic specialized electrolyte, it will be used as a Production Aid in isolating and stabilizing value-added components from manure sources.

Item B.1 – The Substance's Chemical or Material Common Name

We noted multiple names used for the petitioned substance, including "hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1)," "stabilized hydronium," and the formulated product tradenames "Tydronium" and "Ferocious." Please note that NOP 3011 stipulates at Section 3.2, "Generic substances are eligible for petition; formulated (brand name) products are not eligible." To facilitate the review and reduce potential confusion, in your resubmission we suggest providing clarification on the exact substance(s) being petitioned, including a common name of the substance, if available.

Answer- I apologize for this confusion since the Tydronium chemistry is the active ingredient in the Ferocious formulation and was submitted as an example of the technology being practiced in a commercially-available formulation in the Medical Cannabis industry.

The EXACT substance and common name for the chemistry being submitted is:

Hydrogen(+1), triaqua-μ3-oxotri, sulfate (1:1), stabilized hydronium, or "Tydronium®" as the common, trademarked name. The ACS CAS No. is attached for your review as well as the copy of the issued US Patent from the USPTO describing the chemistry.

Item B.3 – Intended or Current Use

We noted that the stated intended use for stabilized hydronium is "as a processing aid in the production of dehydrated manure for use in organic crop production." However, the product label

included in the petition is for brand name product “Ferocious” with labeled use as “premium cannabis optimizer.” Petition addenda referenced use as biopesticide, plant and soil amendment, or disease suppressant. None of the petition addenda referenced the petitioned use for the substance. Additionally, the petition indicated that sulfuric, phosphoric and citric acids are approved processing aids for pH adjustment of liquid fish products. By way of clarification, please note that liquid fish products were petitioned and ultimately listed on the National List at 205.601(j)(7) as synthetic plant or soil amendments. Neither sulfuric acid, phosphoric acid, nor citric acid is listed on the National List as an allowed plant or soil amendment for use in organic crop production; these acids do appear on the National List in the annotation of liquid fish products, as they relate to pH adjustment of the liquid fish products.

To facilitate the review, we suggest providing clarification on the current and intended use(s) of the petitioned substance.

Item B.7 – Previous Reviews

We noted that the petition addenda included a report on physical and chemical properties and a report on antimicrobial properties of “Tydronium.” A list of states in which the trade name product “Ferocious” is registered as a soil or plant amendment also was provided. The petition did not include reviews relevant to the petitioned use of the substance (i.e., as a manure processing aid).

For the revised petition, please provide a summary of any available previous reviews of the petitioned substance.

Answer- a) Summary of previous reviews- Please see by way of attached files that refer to previous reviews by USEPA/ACS, CAS, US States that have reviewed the Tydronium® chemistry.

**NOTE-We also have exposure and efficacy testing data performed by EPA-Approved analytical labs available upon request. In addition, we have significant Agricultural Testing from Mid Michigan Agronomy/Michigan State University Grad School concerning soil amendment and yield criterion for Soybeans, Roma Tomatoes, Potatoes and Sugar Beets available upon request.*

b) The petitioned use of the chemistry is as a manure processing aid primarily for alpaca/llama protein nucleotide extraction and stabilization.

Item B.8 – Regulatory Authority

The petition indicated that the petitioned substance is currently under review by the EPA and the FDA as a bio-pesticide. We noted that the petition addenda included a pre-submission meeting request

to the EPA listing the material as a 'biochemical pesticide' used as a disinfectant/sanitizer for health-care/institutional/food processing facilities.

Answer- We have been accomplished an EPA Pre-submission meeting (*See notes attached) with the EPA Disinfection Team facilitated by our USEPA consultant/advisor(s), Hon. Dr.

Stephen L. Johnson and our regulatory consultants, Exponent, Inc. (Beth M. Polakoff/Diane Boesenberg) to also apply through the Disinfection Group which we've now done for strategic and technical rationale while we continue to work with Dr. Russell Jones and the Bio-Pesticide Committee to address the additional requirements which is longer term.

A list of states in which the trade name product Ferocious is registered as a soil or plant amendment also was provided. The petition did not include information about EPA, FDA, or State regulatory authority registrations for the petitioned use of the substance (i.e., as a manure processing aid).
For the revised petition, please provide information regarding EPA, FDA, and State regulatory authority registrations, including registration numbers, specific to the petitioned use of the substance. The information provided must confirm that the intended use of the substance is permitted under federal regulations, as applicable.

Answer- a) There are no other active registrations or submissions for Tydronium® chemistry as a "manure processing aid at this time besides the NOPB submission. This technology is platform/disruptive technology and is being also registered for other applications as stated in (b).

(b) Tygrus, LLC is in the process of final pre-review/pre-submission protocol for registrations with USEPA, (Hard Surface Sanitizer) FDA, (F.C.N. registration), USDA, (Soil Amendment) and recently was given registration by Cosmetic INCI Board (International Nomenclature for Cosmetic

Ingredients). We are also pursuing registrations with NSF for water sanitation applications.

Item B.9 – Chemical Abstracts Service (CAS) Number and Product Labels

We noted that the only label included in the petition was for brand name product 'Ferocious' with labeled use of "premium cannabis optimizer." The petitioned material was not listed on the label.

For the revised petition, please provide labels of products that contain the petitioned substance. If a product label does not apply to this substance, please provide a brief explanation. Product specification sheets, product data sheets, non-retail labels, or other product information may be substituted for the product label, if appropriate.

Answer- The 'Ferocious™' horticultural chemistry product contains Tydronium® chemistry at 1.0% v./v. within a formulated product base.

*NOTE: Please see by way of attachment the Technical Process Bulletin, Product Characteristics and R&D label TD-0001 (TD=Technical Development) describing the Tydronium chemistry. Sample labels also included.

Item B.10 – Physical and Chemical Properties

We noted no information was provided regarding potential or known effects on soil organisms, crops or livestock.

For the revised petition, please address this requirement per Section 10(e) of the Procedure National List Petition Guidelines (NOP 3011).

Answer- *Please see attached USEPA 6-Pack Summary Test Results (Raw Data Available by Request) for Dermal, Sensitization, Oral, Ocular and Inhalation exposure. Also, see by way of attachment soil exposure data for CEC (Cationic Exchange Testing). Also, see Agricultural testing attached as a nutrient additive/growth enhancer application.

Tygrus, LLC has now completed testing for the Fathead Minnow and Daphnia water flea water exposure testing at Paragon Laboratories in Livonia, MI. *Please see attached results completed for the aquatic water testing and is attached for your review.

Item B.11 – Safety Information

We noted a Material Safety Data Sheet (MSDS) for Tydronium was provided as a petition addendum. Please provide an MSDS for the petitioned substance (i.e., not a formulated product). Additionally, please include a substance report from the National Institute of Environmental Health Studies.
Per Section 11 of the Procedure National List Petition Guidelines (NOP 3011), if this information does not exist for the substance(s), the petitioner should state so in the petition.

Answer- Please see attached SDS for the Tydronium® chemistry as the petitioned substance. The substance report from the NIEHS does not yet exist for the petitioned product but is under consideration for funded R&D.

Item B.12 – Research Information

We noted that the petition cited a report by Dr. Joseph DeVito; however, this was not included in the petition addenda. A

graduate paper by Dr. Cole and report by Eurofins were included.
For the revised petition, please include all relevant research information.

Answer- Please see attached ATL report by Dr. Joe DeVito re: Tygrus, LLC technology microbial efficacy.

Page 4

Item B.13 – Petition Justification Statement

We noted that the petition cited a pilot field test for minimizing ammonia emissions and odors in manures. You may wish to clarify any nonsynthetic substances, synthetic substances on the National List, or alternative cultural methods that could be used in place of the petitioned synthetic substance. You may also wish to clarify any beneficial effects to the environment, human health, or farm ecosystem from use of the synthetic substance that support its use instead of the use of a nonsynthetic substance or alternative cultural method.

Answer- In addition to efficacy and performance tests,

1. The material is much safer to transport, handle and store.

2. Is much more environmentally friendly.

3. This technology is devoid of odor,

4. Non-corrosive to plant/animal/human tissue,

5. Is non-exothermic in aqueous mediums and,

5. Has minimum one-year shelf life stability.

6. The chemistry has shown to be very cost effective and very efficient in usage, as minimal concentrations are needed to be effective.

7. The chemistry is sustainable and is produced from readily available Food Grade constituents.

Resubmission:
Please note that electronic submission is preferred to facilitate posting of petitions on the NOP website.
Additional information on the petition process is available on the NOP website at How to File a Petition.
If you have any questions, please contact me by phone at (202) 260-9447 or email at clarissa.mathews@ams.usda.gov.
Sincerely,
Clarissa R. Mathews, Ph.D.
National List Manager
National Organic Program

Winterfalls Ranch

Enriching and Nurturing your Horticulture Needs thru Sustainable Agriculture

July 31, 2020

Lawrence R. Carlson CEF
CTO, VP of Quality and Environmental Systems
Tygrus, LLC

Dear Mr. Carlson,

With an evergrowing demand for organic fertilizer and plant foods in the commercial and consumer markets we are faced with the challenge of producing stabilized shelf life for these organic nutrients while at the same time maintaining the integrity, safety, and vitality of the surrounding ecosystem the plants are grown in. Stabilized hydronium provides the solution to both needs.

We produce a liquid fertilizer derived from composted llama manure. One of the challenges we constantly face is shelf life for our products. With our current process we can expect a ~8-month shelf life for 70% of our unopened consumer bottles before the microbes take over. Once the microbes gain ground in our bottles, the product is no longer appealing to our consumer due to the offensive odor.

With the addition of stabilized hydronium to lower the pH we see an increase of the shelf life to ~24 months in 99.6% of our bottles. This allows us to reduce our waste and cost due to recycling of the destabilized product bottles and less energy & resources used to replace the product and bottles. Production line, delivery trucks, etc. Other products used to stabilize, such as citric acid, were not effective and harms the environment when allowed into the ecosystem.

We are currently waiting on NOP certification before we begin using stabilized hydronium in our products for commercial and retail sale. We can also move forward on our CDFA OIM organic certification. CDFA does not allow stabilized hydronium to be used in CA OIM products due to the fact it is not on the NOP approved list.

Another benefit of stabilized hydronium is in fish emulsion or hydrolysate. Phosphoric acid is used in the stabilization of this product. We have found that stabilized hydronium is very effective in stabilizing fish emulsion and at the same time it will not harm the environment the way phosphoric acid can and does due to the overuse and leaching from the fish emulsion. The use of stabilized hydronium in fish emulsion will allow for a reduced footprint in the agricultural and consumer industry.

We have also found that stabilized hydronium is a safe and effective product to bring water down to a correct pH prior to fertilizing plants. Current products contain ammonium sulfate, citric acid, and urea phosphate for example all of which does harm to the environment. The demand for an organic pH down solution has grown in the market and we have some products available for the consumer, but none as safe for the environment as stabilized hydronium.

Bill Prosser
Owner
Winterfalls Ranch
Peruvian Gold Organic Fertilizers

Winterfalls Ranch - Pollock Pines, CA
Mailing Address - P.O. Box 682 El Dorado, CA 95623 • Telephone 530 644-2114 • Fax 530 647-0332

c) *Product Labels containing Substance described herein-*

Ferocious

PREMIUM CANNABIS OPTIMIZER

3334 Rochester Rd. #204 Troy, MI 48083 | Emergency Phone: (231) 412-2420

Hazard Statements: Do not handle until all safety precautions have been read and understood. **Physical Hazards:** May irrita eyes. **Prevention:** When not in use, keep containers tightly closed. **Protection:** Wear protective gloves and eye protection. **First Aid Measures - Inhalation** Not a likely route of exposure. Remove to fresh air if irritation occurs. If symptoms develop, obtain medical attention. **Skin Contact:** Exposure to skin normally does not cause irritation or redness. No toxicity associated with the product being absorbed through skin. Wash exposed areas with water. In the unlikely event of irritation, seek medical advice. **Eye Contact:** This product may cause irritation to the eyes after direct contact with concentrate. Rinse with water for several minutes. Remove contact lenses if present and continue rinsing. **Ingestion:** No toxicity associated with ingestion. Ingesting large volumes may cause minor gastrointestinal distress due to separation of grease and oil in the digestive tract. Drink water to assist in digestion if swallowed. **General Advice:** Handle in accordance with good industrial hygiene and safety practice. **Storage Conditions:** Keep containers tightly closed in a dry, cool, and well-ventilated place. Keep out of reach of children. **Appearance:** clear, colorless, liquid. **Odor:** none to mild. **Disclaimer:** Sellers and/or users of Jungle Control Chemistries are responsible for determining whether any governmental approvals are necessary for their particular claim of us of Jungle Control Chemistries and will not make a claim of use concerning same without such approvals.

BATCH NUMBER
0121815-1-A

NET WEIGHT (lbs.)
8.36

HEALTH	0
FLAMMABILITY	0
REACTIVITY	0
PERSONAL PROTRECTION	A

9. *The substance's physical properties and chemical mode of action including:*

TYGRUS™

BETTER EVERYTHING.

TD-0001

1132 E. Big Beaver Road Troy, MI 48083 | Emergency Phone: (248) 218-0347 x 102

Recommended Use: Reserved for Industrial use only.

Hazard Statements: Do not handle until all safety precautions have been read and understood. **Physical Hazards:** May irritate eyes. **Prevention:** When not in use, keep containers tightly closed. **Protection:** Wear protective gloves and eye protection. **First Aid Measures - Inhalation** Not a likely route of exposure. Remove to fresh air if irritation occurs. If symptoms develop, obtain medical attention. **Skin Contact:** Exposure to skin normally does not cause irritation or redness. No toxicity associated with the product being absorbed through skin. Wash exposed areas with water. In the unlikely event of irritation, seek medical advice**. Eye Contact:** This product may cause irritation to the eyes after direct contact with concentrate. Rinse with water for several minutes. Remove contact lenses if present and continue rinsing. **Ingestion:** No toxicity associated with ingestion. Ingesting large volumes may cause minor gastrointestinal distress due to separation of grease and oil in the digestive tract. Drink water to assist in digestion if swallowed. **General Advice:** Handle in accordance with good industrial hygiene and safety practice. **Storage Conditions:** Keep containers tightly closed in a dry, cool, and well-ventilated place. Keep out of reach of children. **Appearance:** clear, colorless, liquid. **Odor:** none to mild. **Disclaimer:** Sellers and/or users of Tygrus Chemistries are responsible for determining whether any governmental approvals are necessary for their particular claim of use of Tygrus Chemistries and will not make a claim of use concerning same without such approvals.

LOT NUMBER	NET WEGHT (lbs.)
110916-A	495

HEALTH	0
FLAMMABILITY	0
REACTIVITY	0
PERSONAL PROTRECTION	A

SAFETY DATA SHEET

SECTION 1: IDENTIFICATION

1.1 Product Identifier TY-0001-050

Trade Name

Tydronium ™
Acid Electrolyte

1.2 Product Use **Manufacture Use Product (MUP)**
Identified Use Industrial

1.3 Manufacturer/Supplier

Company Tygrus LLC

Address 1132 E. Big Beaver Road Troy, MI 48083

Telephone (248) 218-0347

Email info@tygrus.com

1.4 Emergency Telephone Number **Emergency Phone** (248) 218-0347 x 102

SECTION 2: HAZARDS IDENTIFICATION

2.1 Classification of the Substance or Mixture: Complies with OSHA 29 CFR - 1910.1200 Section (i) "Trade Secrets", B.16 Corrosive to Metals B.16.1.

2.1.1 GHS Classifications and Regulation (EC) No. 1272/2008 (CLP): Corrosive to Metals

2.1.2 Directive 67/548/EEC & Directive 1999/45/EC: Corrosive to Metals

2.2 Label Elements

2.2.1 Label Elements according GHS Classifications and Regulation (EC)

No. 1272/2008 (CLP)
Hazard Pictogram(s)

Signal Word Warning
Hazard Statement Corrosive to Metals

Precautionary Statements Minimize prolonged contact to steel, zinc, magnesium. Low Reactivity to copper, aluminum, stainless steel.

2.2.2 Label Elements

Hazard Symbol Corrosive
Risk Phrases R36/37 Irritating to eyes and skin
Safety Phrases S36/37/39 Wear suitable protective clothing, gloves, and eye/face protection

2.3 Other Hazards
OSHA : Non-hazardous under OSHA Hazard Communication Standard

HMIS
Health: 1
Flammability: 0
Reactivity: 1
Personal Protection: C

HEALTH	1
FLAMMABILITY	0
REACTIVITY	1
PERSONAL PROTECTION	C

WHMIS (Canada): Corrosive to Metals

2.4 Additional Information: No fragrances added

SECTION 3: COMPOSITION/ INFORMATION ON INGREDIENTS

3.1 GHS Classification (EC Classification No. 1272/2008/EC)

Ingredients	Common Name	CAS No.	%W/W
H_2O	Water	7732-18-5	85.0 – 86.0 % W/W
H_9O_4: HSO_4	Hydronium Sulfate [Hydrogen (+1), Triaqua-u3-oxotri, sulfate (1:1)]	2032207-39-7	14.0 – 15.0 % W/W

3.2 ADDITIONAL INFORMATION: NONE

SECTION 4: FIRST AID MEASURES

4.1 Description of First Aid Measures

Inhalation: Not a likely route of exposure. Remove to fresh air if irritation occurs. If symptoms develop, obtain medical attention.

Skin Contact: Exposure to skin normally does not cause irritation or redness. No toxicity associated with the product being absorbed through skin. Wash exposed areas with water. In the unlikely event of irritation, seek medical advice.

Eye Contact: This product may cause irritation to the eyes after direct contact with concentrate. Rinse with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

Ingestion: No toxicity associated with ingestion. Ingesting large volumes may cause minor gastrointestinal distress due to separation of grease and oil in the digestive tract. Drink water to assist in digestion if swallowed.

4.2 Most Important Symptoms and Effects, Both Acute and Delayed

Acute: Ingestion will cause minor distress to the gastrointestinal tract. May cause chemical and mechanical eye irritation with direct contact.

Delayed and Chronic Effects: Expected to be similar to acute exposures.

4.3 Indication of the Immediate Medical Attention and Special Treatment Needed: Treat symptomatically.

SECTION 5: FIREFIGHTING MEASURES

Not flammable or combustible by OSHA/WHMIS criteria. Not sensitive to mechanical impact and static discharge.

Flash Point	Explosive Limits	Temperatures
> 220° C (428° F)	NA Auto-Ignition	NA

5.1 Extinguishing Media

Suitable Extinguishing Media: Use extinguishing media appropriate to surrounding fire conditions.

Unsuitable Extinguishing Media: None known

5.2 Special Hazards Arising from the Substance or Mixture: Containers may rupture from exposure to high temperatures, releasing contents that may be slippery.

5.3 Advice for Firefighters: Suitable protective clothing should be worn in fire conditions. Extinguish preferably with dry chemical, foam or water spray.

5.4 Hazardous Combustion Products: Sulfur Oxides, Calcium Oxides

NFPA 704:	HEALTH HAZARD–BLUE	FLAMMABILITY–RED	INSTABILITY- YELLOW	SPECIAL HAZARD – WHITE
	1	0	1	COR

SECTION 6: ACCIDENTAL RELEASE MEASURES

6.1 Personal Precautions, Protective Equipment and Emergency Procedures: None

6.2 Environmental Precautions: None

6.3 Methods and Material for Containment and Cleaning Up: Rinse area with water. Dispose of material in accordance with local regulations.

6.4 Reference to Other Sections: See Also Section 7, 8, 13

6.5 Additional Information: None

SECTION 7: HANDLING AND STORAGE

7.1 Precautions for Safe Handling: Avoid contact with eyes. Wear acid resistant personal protective gear.

7.2 Conditions for Safe Storage: Store in closed containers between 35°F and 120°F

SECTION 8: EXPOSURE CONTROLS / PERSONAL PROTECTION

8.1 Control Parameters

8.1.1 Occupational Exposure Limits

Substance	CAS No.	LTEL (8hr TWA ppm)	STEL (ppm)	LTEL (8hr TWA mg/m3)	STEL (mg/m3)

OELs are not available for non-listed components.

8.1.3 PNECs and DNELs: No PNECs or DNELs are available for this product. As with all chemical products, users are cautioned to avoid unnecessary exposures.

8.2.2 Personal Protection Equipment

Respiratory Protection: Usually not needed.

Eye Protection: Safety glasses are suggested as good practice.

Hand Protection: Acid resistant gloves.

Skin and Body Protection: Wear acid resistant personal protective gear.

Engineering Controls: No special controls required.

General Hygiene Considerations: Handle according to established industrial hygiene and safety practices.

SECTION 9: PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on Basic Physical and Chemical Properties

	Concentrate
Appearance:	Clear Liquid
Color:	Water White Liquid
Odor:	None to Mild
Odor Threshold:	Not available
pH:	0 - 1.5
Melting Point	NA
Initial Boiling Point:	Not available
Boiling Point:	> 112 °C (233.6° F)
Flash Point:	> 220 °C (428° F)
Evaporation Rate:	1 (Water = 1)
Flammability (solid, gas):	Non-Flammable
Upper/Lower Flammability Limit:	Non-Flammable
Auto-ignition Temperature:	Non-Flammable
Vapor Pressure:	Not available
Vapor Density:	Not available
Relative Density:	Not available
Solubilities:	Infinitely Soluble in water
Partition Coefficient: N-octanol/Water:	Not available
Decomposition Temperature:	Not available
Percent Volatile, wt.%:	0%
Density Target:	1.035 g/mL
Density Range:	1.025 – 1.045 g/ml

VOC Content, wt.%: 0%

SECTION 10: STABILITY AND REACTIVITY

10.1 Reactivity: Chemically active on metals.

10.2 Chemical Stability: Stable under normal conditions. Avoid temperature extremes.

10.3 Possibility of Hazardous Reactions: No hazardous reactions observed.

10.4 Conditions to Avoid: Do not freeze. Do not use above ambient temperature.

10.5 Incompatible Materials: Avoid prolonged contact with metals

10.6 Hazardous Decomposition Product(s): Calcium Oxides and Sulfur Oxides.

SECTION 11: TOXICOLOGICAL INFORMATION

Substance: Tetraaquahydrogen+1 | CAS No. 12501-73-4
Hydrogen Sulfate | CAS No. 14996-02-2

11.1 Information on Toxicological Effects

11.1.2 Mixtures

Effects of Acute Exposure

Ingestion: Not Known. Product may be irritating to gastrointestinal tract.

Inhalation: Not Known. Product may be irritating to nasal tissue.

Skin Contact: Not Known. Prolonged contact may result in mild irritation.

Eye Contact: Not Known. Contact may result in mechanical and chemical irritation.

Corrosivity: None expected.

Repeated Dose Toxicity: Expected to be similar to single exposures.

Carcinogenicity: This product does not contain any carcinogens or potential carcinogens as listed by ACGIH, IARC, OSHA, or NTP.

Mutagenicity: Not available

Toxicity for Reproduction: Not available

11.2 Other Information: None

SECTION 12: ECOLOGICAL INFORMATION

12.1 Toxicity: Not expected to be harmful to aquatic or soil environments.

12.2 Persistence and Degradability: Readily biodegradable organic liquid

12.3 Bio-accumulative Potential: Not available

12.4 Mobility in Soil: Not available

12.5 Results of PBT and vPvB Assessment: Not available

12.6 Additional Information on Eco-toxicity: The product does not add to the AOX-value of effluent water (DIN 38409).

SECTION 13: DISPOSAL CONSIDERATIONS

13.1 Waste Treatment Methods: Disposal should be in accordance with local, state or national legislation. For disposal within the EC, the appropriate code according to the European Waste Catalogue (EWC) should be used. Containers must not be punctured or destroyed by burning, even when empty.

13.2 Additional Information: None

SECTION 14: TRANSPORT INFORMATION

Land transport (ADR/RID) (c)(d): Not classified as dangerous for transport.

U.S. Department of Transportation (DOT) (c)(d): Not classified as dangerous for transport.

Canada Transportation of Dangerous Goods (TDG) (c)(d): Not classified as dangerous for transport.

Sea Transport (IMDG) (c)(d): Not classified as dangerous for transport.

Air Transport (ICAO/IATA) (c)(d): Not classified as dangerous for transport.

(c)– Consult with transport provider. (d) – Check relevant regulations for Special Provisions.

SECTION 15: REGULATORY INFORMATION

15.1 Safety, Health and Environmental Regulations and Associated Hazards for the Mixture

15.1.1 Regulations

TSCA STATUS	A component or components of this products is not listed on the TSCA Inventory of Existing Chemical Substances.
Chemical Name	

15.2 Chemical Safety Assessment: Corrosive

SECTION 16: OTHER INFORMATION

Hazard Statements and Precautionary Statements: Corrosive

Training Advice: None

Additional Information: Replaces all previous editions.

References: RTECS, CAS Registry, EINECS/ESIS, Manufacturer Information

Risk Phrases and Safety Phrases: R36/37 Irritating to eyes and skin
S36/37/39 Wear suitable protective clothing, gloves, and eye/face protection

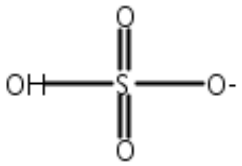
Prepared By **Tygrus Regulatory Affairs**
Email: info@tygrus.com

Creation Date **May 6, 2020**
Revision Date **May 6, 2020**
Print Date **May 6, 2020**
Revision Summary This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS)

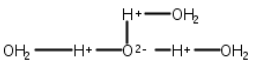
End of Safety Data Sheet

CAS Registry Number: 2032207-39-7
$H_9 O_4 . H O_4 S$
Hydrogen(1+), triaqua-μ_3-oxotri-, sulfate (1:1)

14996-02-2
$H O_4 S$



12501-73-4
$H_9 O_4$



~2 References

CAS Role	Patents	Nonpatents	Nonspecific Derivatives from Patents	Nonspecific Derivatives from Nonpatents
Biological Study				
Properties				
Uses				

Source of Registration: CA

A division of the American Chemical Society

InventoryExpertService

Phone: 800-631-1884, 614-447-3870
Fax: 614-447-3747
E-mail: answers@cas.org
Web: www.cas.org/products/other-cas-products/client-services/

November 14, 2013

Millstone Properties, LLC
ATTN: Mr. Lawrence R. Carlson
P.O.Box 12187
Zephyr Cove, NV 89448-4187

Dear Mr. Carlson:

Thank you for your Inventory Expert Service (IES) order of July 22, 2013.

The processing results are attached.

To ensure efficient processing of your substance(s), it is important that these results are included with your PMN report for when reporting to the EPA.

The cost of the technical processing for your order will be applied to your American Express card.

Thank you for the opportunity to provide this service. If you have any questions, please do not hesitate to contact me.

Sincerely,

Jennifer Moore
CAS Client Services

Enclosure(s)

— CONFIDENTIAL —

Larry Carlson

From: Answers <answers@cas.org>
Sent: Thursday, November 14, 2013 1:21 PM
To: Larry Carlson
Cc: Answers
Subject: IES 348687
Attachments: IES 348687_20131114132157.pdf

Follow Up Flag: Follow up
Flag Status: Flagged

Dear Mr. Carlson,

Please see attached for the results of your Inventory Expert Service order.
Note: This is the only copy you will receive. Please retain for your records.

Regards, Jennifer

Jennifer Moore
CAS Client Services/Inventory Expert Service
CAS, a division of the American Chemical Society
2540 Olentangy River Road
Columbus, OH 43202
Phone: 614-447-3870
Fax: 614-447-3747
www.cas.org/products/other-cas-products/client-services

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InventoryExpertService

Phone: 800-631-1884, 614-447-3870
Fax: 614-447-3747
E-mail: answers@cas.org
Web: www.cas.org/products/other-cas-products/client-services/

INVENTORY EXPERT SERVICE REPORT

IES Order Number: 348687-2

Registry Number: Not Registered

CA Index Name:

Hydrogen(1+), trihydroxy-, sulfate (1:1)

Please print the above CA Index Name on the appropriate page of your PMN.

[X] If this box is checked, CAS has made correction(s) marked in red to your IES order. Please make the same corrections to your PMN before submitting it to the EPA.

CAS • 2540 Olentangy River Road • P.O. Box 3343 • Columbus, OH 43210-0334 • USA

A division of the American Chemical Society

InventoryExpertService

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Fax: 614-447-3747
E-mail: answers@cas.org
Web: www.cas.org/products/other-cas-products/client-services/

INVENTORY EXPERT SERVICE REPORT

IES Order Number: 348687-1

Registry Number: Not Registered

CA Index Name:

Hydrogen(1+), triaqua-μ3-oxotri-, sulfate (1:1)

Please print the above CA Index Name on the appropriate page of your PMN.

[X] If this box is checked, CAS has made correction(s) marked in red to your IES order. Please make the same corrections to your PMN before submitting it to the EPA.

May 12, 2020

Tygrus / EPA meeting:

EPA Attendees:
John Hebert - AD
Kimberly Nesci - BEAD
Demson Fuller - AD
Jack Hall – Risk manager, AD
Cesar Cordero – Acting team lead for efficacy in AD
Laura Parsons – RASSB
Tina Pham – efficacy, AD
Lindsey O'Dell – chem / tox
Susan Lawrence – BEAD
Kristin Willis – AD
Diane Isbell
Jenny Tao
Sue Bartow

Beth: Opening remarks

Objective – introduce the Agency to Tygrus and the new chemistry, hydronium sulphate, initial uses, proof of concept, looking at this as a new antimicrobial. Interested in the agency thoughts on processes and idea of this as a new active ingredient. Want feedback on this as a new AI and the pathway forward.

Slide 4: John Coppolino

Had a meeting with EPA about 18 months ago and have been working on this material ever since. In Q419 was issued a patent. Can share the details on the patent at a later time. Build a factory in Madison Heights Michigan which can make millions of gallons per week. Are getting the facility approved. Over the past year have also been working on medical uses with Dr. Attala and especially against the SARS-CoV-2 virus.

Slide 5 - 7: Shaun

Lindsey – chemistry, the patent will be released to the EPA at an appropriate time. Is the buffering system part of the patent? Also any substances involved in stopping the reaction?

Shaun – the proton from KA1 is retarding the process. There are many homologs. From organic chemistry there are 1 – 2 CAS number for 30 compounds. The process moves fast and moves around. With titration and ICP know the protons and the anions amounts. The material has a different mass spec that sulfuric acid so know it's a new material.

Lindsey – have we completed any GLP chemistry studies?

Shaun – Have some chemistry studies underway, not sure if any are GLP. With the data gap analysis will make sure the complete the appropriate package.

Slide 9 – Dr. Devito

As an acid it is obvious it's a new antimicrobial, though there are many acid based antimicrobials on the market.

Slide 10 – Dr. Devito

Hydronium sulfate can be used as a new AI for environmental surfaces

Slide 11 – Dr. Devito

Early anti-viral activity, directionality towards disinfection activity

Tydracide is the planned trade name

4 sprays per dish

Bleach as a positive control

Tydracide had a nearly 3 log reduction in FCV and a 3 log reduction in HSV1

This is a good way to profile the anti viral activity of tydracide

Slide 12 – Dr. Devito

Planning for the disinfection properties of the product

Slide 13 – Dr. Devito

Tydracide can be classified as a category IV for everything except eye and it's not a skin sensitizer

Concentrated solution is moderately irritating to the eye, sets it apart from other typical acid disinfectants

Slide 14 – Beth

Testing has been done for proof of concept to show there is a viable, new compound here for antimicrobial activity against a number of target organisms.

Very important is the SARS-CoV-2.

Tygrus is further defining the MP, EP and to get a better feel for the concentration of the TGAI including stability testing and what is the stable TGAI and the equilibrium. Will come in and have additional pre-sub meetings with the EPA as work through the data gaps and to update the agency.

Hydronium sulphate is a new compound.

Thoughts on the Agency on whether there is agreement this is a new AI and other ideas:

Feedback on the concept for how want to address data generation overall for new data and potentially bridging to sulfuric acid and sulfates. To work on a strategy and path forward. Will then come back and speak about specifics.

AI thoughts:

Laura Parsons –

Q1: Why do we think it's not a new AI?

Beth – when tygrus came to exponent, it wasn't initially clear. It is a new compound, the question is wha tis the mode of action and where is the activity coming from? Tygrus said there is activity from both. Would love to be a new AI but it increased the data generation and timelines. There are many things that indicate it may not be a new AI. Also depends on how EPA thinks about this. When are looking at an inorganic complex how does the agency determine if it's a new AI?

Laura Parsons –

What they hear is that we want to piggy back on the mineral acids as a bridge?

Beth – yes, in some cases would want to look at bridging to the mineral acids data. There are some similarities and differences, less toxicity, less corrosive, have to do more digging into the mineral acids to argue for that piece, that would be one possible approach.

Laura Parsons –

For RASSB don't have enough information to be able to say one way or another. Will need to do – give some of the bridging arguments about the basic chemistry and toxicity. Need more details before RASSB can say tthis is a new AI, think it probably is especially if has a different corrosion charactertistic than the mineral acids. Use pattern for the mineral acids and this is a broader use pattern than the mineral acids.

Beth –

Part of the next step will be to do what Laura said, go through the mineral acids and make the comparisons and look at the arguments. We have to do more work to be able to come back more definitively on the new AI piece. Inclined that this is new. For the uses / labels we do recognize that are looking for a broader label and more claims than registered for the other products.

John Hebert –

From a regulatory pov, this has a unique CAS# then from a regulatory pov that it is a new AI.

If it is included in other classes for data bridging has to be determined.

Believes it is a new AI based on the CAS# and it's a new compound.

Beth – John / Laura seems to be amenable to looking at the approaches for the other actives and chemistries and with simomilary to come back with a new plan. Then can fulfill the data gaps and if there is opportunity for not having to test or to make sure the agency agrees with the approach.

Demson –

If want to come back in later, what studies do we want to bridge. There are forms that we can fill out and if we believe there are data to use for bridging we can site the information to help RASSB.

Beth – starting with 40CFR 158 (W). Starting with what is required, other areas to fulfill the data requirements. Have a template for the requirements and go through each one and determine if data or bridge. Can the agency share the template they use?

Demson – Yes, they can share the template.

Beth – other questions / thoughts, etc.

John Hebert –

Food uses? Will we have an exemption from tolerance or tolerance limits?

Beth –

Yes, we will likely be looking for an exemption from tolerance. There are things that may be measurable and ubiquitous so a tolerance can be challenging. It is on our mind.

We want to be able to move quickly. It's an exciting chemistry so want to be efficient and it's a difficult time right now. Especially to combat the SARS-CoV-2. Also this could be a low risk compound so anything we can do to move this through to registration would be an objective.

John – Are coming out with expedited process for COVID-19 but does not include new AIs but they would consider this.

Beth – not often get a new AI sot that adds antoher dimension. Will take the Agency guidance into consideration.

John – when would we have a draft label?

Beth – we would love to have one really fast but that may be premature. Perhaps 4 – 6 weeks we will have the strategy and we are moving fast on testing. In about 2 months to talk with the agency again. We would have a better idea of use patterns then. Diane will be working with Tygrus on the microbioloty and a better sense of the testing and labeling. In the next few weeks we will be able to solidify thoughts on timing and further information on a label.

Beth – thank you for yoru time and attention. We hope you are as excited as we are. We are moving quidkly and will prepare the minutes and send those over.

Sue –

Send the minutes to Sue. Sue will type up the attendee list from the EPA.

John –

Sue will be sending out minutes of the meeting and we can work off of those, the EPA minutes are the official minutes. Suggest we stick to using the EPA minutes as official.

Jenny – from the agency perspective, the agency follows and promotes use of in vitro testing for animal testing for eye and sensitization. Acute dermal / oral waiver. If have any questions let them know through Sue. The agency appreciates the reduction in animal testing.

Beth – when we prepare the data gap analysis we will take that into account. We appreciate the reminder. We will also look at bridging for other options for bridging from the mineral acids. If at any time there are questions please reach out to Exponent. We can use Sue as the conduit.

Meeting closed: 3:58 PM

Environmental Impact of Tydracide™ for disinfecting COVID-19 & Comparison to Bleach

Summary
Due to the COVID-19 pandemic there is an extreme level of attention on disinfecting surfaces to reduce the spread of new infections. Tydracide is a novel low toxicity acid that is proven to be more effective at killing COVID-19 viruses than the most widely used solution, sodium hypochlorite or bleach with each is at its recommended disinfection use concentrations. Additionally, Tydracide offers further benefits by reducing toxicity to individuals and the environment, which may allow simplified disinfectant protocols and create new disinfectant applications such as possibly disinfecting N95 respirators for health care personnel.

This whitepaper will discuss Tydracide environmental test results using U.S. EPA (Environmental Protection Agency) Test Procedures focused on Daphnia Magna, a water flea, as a measure of environmental toxicity. The Tydracide LC50 (Lethal Concentration) for daphnia is 1.2667mg/l, which is the concentration which was statistically measured to kill 50% of a daphnia population under specific laboratory conditions.

The recommended usage concentration for disinfecting with Tydracide is 5%. In order to meet the LC50 concentration from 1 gallon of Tydracide-5 it would need to be diluted with 81.7 gallons of water.

Sodium hypochlorite is significantly more lethal to daphnia with a LC50 concentration of 0.033-0.048mg/l. At 500ppm recommended use concentration for disinfecting, 1 gallon of sodium hypochlorite would need to be diluted with 12,346 gallons of water to meet the LC50 concentration. This comparison is summarized in Table 1.

Table 1. Tydracide and Sodium Hypochlorite LC50 for Daphnia and Dilution Factors

Chemical Name	Recommended Disinfection Concentration	Daphnia LC50	Dilution to Meet LC50
Sodium Hypochlorite (bleach)	500ppm or mg/l	0.033-0.048 mg/l	12,346
Tydracide	5%	1.2667 mg/l	81.7

Using the dilution rates from recommended disinfection use concentration to LC50 for daphnia as representative model for the environmental impact of these two chemistries, it can be concluded that sodium hypochlorite is more than 150 time more toxic to the environment than Tydracide.

Introduction

The U.S. EPA utilizes acute toxicity tests for the National Pollutant Discharge Elimination System (NPDES) to identify effluents containing toxic materials in acutely toxic concentrations[1]. LC50 is the testing protocol for acute toxicity used in this process. LC50 is the medial lethal concentration or the concentration of a material in water that is estimated to be lethal to 50% of the test organisms. The LC50 can also be used to estimate acute and chronic toxicity in the receiving water based on appropriate dilution and other factors.

The LC50 provides a concentration measurement result based on statistical testing of lethality to specific organisms in a controlled and reproducible environment. One way to use the LC50 information when applied to pesticides, viracides and other chemicals that may be introduced to the environment, is to consider the dilution rates with water required from shipping or recommended use concentrations of these chemicals in order to meet the LC50 concentration. The higher the dilution rate to meet the LC50 concentration, the more toxic the chemical is to the specific species tested. Using these species as representatives to the overall environmental impact may allow a more general comparison of the environmental impact between specific chemicals.

The LC50 tests can be performed on multiple organisms to provide representative viewpoint of the environmental impact of a particular material when released as an effluent to waters. The most widely used organisms for LC50 testing are Daphnia magma and Daphnia pulex, and the EPA has established a specific test protocol for these organisms under Test Method 2021.0. No single test method or test organism can be expected to satisfy a comprehensive approach to environmental protection[2]. The Fathead minnow is another commonly used species for LC50 tests and the EPA protocol for this species is Test Method 2020.0.

Daphnid is a freshwater microcrustacean invertebrate, commonly known as the water flea, which are a major component of the freshwater zooplankton throughout the world and may be the dominant herbivore in lakes. Species of daphnids include Daphnia magna and Daphnia pulex. Daphnia magma is a lake and pond dweller in waters of western and northern North America[2]. These organisms are an important link in many aquatic food chains and are a significant source of food for juvenile fish species. Daphnids are sensitive to a board range of aquatic contaminants and are widely used for evaluating the toxicity of chemicals. The small size and short life cycles of daphnids simplifies the test protocol with this organism. As result the LC50 concentration for daphnia is one of the most common metrics to determining the environmental impact for a potential chemical release into the environment.

Tydracide LC50 Results and Dilutions

LC50 testing for Tydracide™ was performed by Paragon Laboratories in Livonia, MI. Paragon Laboratories, Inc. is a privately held provider of chemical, physical, and biological testing services. In operation since 1996, Paragon is capable of performing over 225 unique analytical procedures. With significant experience in the application of ASTM, EPA, EN, and Standard Methods, Paragon has developed a reputation as a proven leader across a range of disciplines – including fuels and lubricants, chemicals, and waters – for various industries to meet quality and regulatory standards.

Paragon Laboratories performed LC50 on Tydracide-50 for Daphnia magna per EPA method 2021.0 and for Fathead minnows per EPA method 2020.0. Paragon's results are summarized in Table 2.

Table 2. Tydracide-50 LC50 Concentrations for Daphnia and Minnows

Test Species	**Test Duration**	**LC50 value (grams Tydracide-50/liter)**
Daphnia Magna	48 hours	1.2667
Fathead Minnows	96 hours	1.0946

Tydracide-50 is a concentrated version of Tydracide with a specific gravity of 1.035 grams/ml or 1,035grams/liter. To reach the LC50 concentration of 1.2667 grams of Tydracide-50/liter Tydracide-50 would need to be diluted with 817 parts water to 1 part Tydracide-50 (=1035/1.2667). Similar calculations are shown in Table 3 for 10%, 5%, 2% and 1% concentrations of Tydracide for both Daphnia Magna and Fathead Minnow.

Table 3. Dilution to Meet LC50 for various Tydracide Concentrations

Tydracide Concentrations	Specific Gravity (grams/liter)	Tydracide 50 Content (grams/liter)	Daphnia LC50 Dilution	Minnow LC50 Dilution
Tydracide-50 (50% concentration)	1,035	1,035	817	945
Tydracide-10 (10% concentration; 4:1 dilution)	1,007	207	163	189
Tydracide-5 (5% concentration; 9:1 dilution)	1,003.5	103.5	81.7	94.5
Tydracide-2 (2% concentration; 24:1 dilution)	1,001.4	41.4	32.6	37.8
Tydracide-1 (1% concentration; 49:1 dilution)	1,000.7	20.7	16.3	18.9

The recommended usage concentration for Tydracide for disinfecting surfaces of COVID-19 is (expected) 5%, which is a 9:1 dilution of the more concentrated Tydacide-50. The LC50 dilution

factor for daphnia magna with a 5% concentration of Tydracide is 81.7X. One potential measure of environmental toxicity is a comparison of these LC dilution rates to other COVID-19 viracides such as sodium hypochlorite.

Sodium Hypochlorite LC50 Results and Dilutions
The most widely used viracide for COVID-19 is sodium hypochlorite or bleach.

The daphnia LC50 concentration for sodium hypochlorite is 0.033-.048mg/l[3] or an average of 0.0405mg/l.

Household bleach is normally shipped as a 5,000ppm or mg/l product. Recommended usage is at 500mg/l as a disinfectant (9:1 dilution of concentrate), and at 200mg/l as a sanitizer. The dilution rates to meet the daphnia LC50 is shown in Table 4.

Table 4. Dilution to Meet Daphnia LC50 for various Bleach Concentrations

Sodium Hypochlorite (Bleach) Concentrations	Concentration (mg/l or ppm)	Daphnia LC50 Dilution
Shipping Concentration	5,000	123,457
Disinfecting Concentration	500	12,346
Sanitizing Concentration	200	4,938

References
1 – Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organism Fifth Edition, US EPA, October 2002

2- Biological Test Method: Acute Lethality Test Using Daphnia, Canada Environmental Technology Centre, Report EPA 1/RM/11, July 1990 with 1996 amendments

3- Sodium Hypochlorite/Calcium Hypochlorite (Clorox, Bleach) Chemical Fact Sheet 2/86
http://pmep.cce.cornell.edu/profiles/miscpesticides/methylchloride-xanthangum/hypochlorite/hypochl_prf_0286.html

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EPA-mandated Animal Toxicity Tests Confirm the Safety of Tydracide-50

This report summarizes the performance of Tydracide-50 in a battery of toxicology tests required for EPA registration of chemicals, disinfectants and other antimicrobial products. The toxicology tests (known in the vernacular as the standard "Six-Pack") are performed on small laboratory animals to simulate the human health impact of chemical substances. First published in 1982 and periodically updated, the current EPA guidelines (870.1000) require acute or short-term toxicity testing be performed on all registered chemicals according to their probable routes of human exposure.

Tydracide-50 is a novel product based on the naturally occurring hydronium ion (H_3O^+), which is the defining particle of all acids. Using the proprietary manufacturing processes developed at Tygrus, the hydronium ion can now be produced in its stabilized form (H_9O_4) on a commercial scale. This chemical substance, called Tydronium™, is manufactured as a pure product concentrate and can be formulated for application in various markets. Given the acidic nature of Tydronium™, it is important to conclusively demonstrate that technical grade active ingredient derivative products, like Tydracide-50, behave differently than traditional acids in regards to their overall safety profile.

The following six tests were conducted on Tydracide-50 according to the EPA guidelines: Acute Dermal Toxicity, Acute Oral Toxicity (Up & Down Method), Acute Inhalation Toxicity, Primary Eye Irritation, Primary Skin Irritation, Dermal Sensitization (Local Lymph Node Assay). Testing was performed by Product Safety Laboratories (PDL) of Dayton, New Jersey. PDL is now part of Eurofins Scientific, an international group of laboratories headquartered in Luxembourg that provides certified testing and support services to the pharmaceutical, food, environmental and consumer products industries and to government organizations. Eurofins Scientific is a multinational contract research organization that offers its customers documented, top-of-the-line laboratory services to determine product effectiveness, safety and to support regulatory approval. All six toxicity tests of Tydracide-50 were performed during the second quarter of 2018 using the same lot/batch. The following paragraphs summarize the test results obtained:

An Acute Dermal Toxicity Test was performed by applying a patch containing Tydracide-50 directly to the skin of ten healthy rats (5 male and 5 female). Each patch contained 5000 milligrams (mg) of Tydracide-50 per kilogram (kg) of body weight for each rat. The animals were observed for mortality, signs of gross toxicity, and behavioral changes during the first several hours after application, after patch removal, and then at least once daily thereafter for 14 days. Observations included gross evaluation of skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, somatomotor activity and behavior pattern. Particular attention was directed to observation of tremors, convulsions, salivation, diarrhea, and coma. Individual body weights of the animals were recorded prior to Tydracide-50 application and again on Days 7 and 14, followed by gross necropsy of euthanized animals where tissues and organs of the thoracic and abdominal cavities were examined. All animals remained healthy during the study, gained weight, and there were no adverse findings recorded on any animals except for slight redness of the skin at the dose site of a single male rat that resolved within 24 hours. The data show that for acute dermal toxicity Tydracide-50 has a LD50 (dose at which 50% of the animals show negative effects) above 5000 mg/kg. As such, according to the Global Harmonized System for chemical classification (GHS), Tydracide-50 is a Category 5 substance…i.e. practically non-toxic. The US EPA applies a slightly more restricted four-category rating and Tydracide-50 would be considered a Category 4 product requiring no hazard statements to be present on the product label.

Using related methodologies, an Acute Oral Toxicity study was performed to determine the potential for Tydracide-50 to produce toxicity from a single dose via oral administration. Three healthy female rats (typically more sensitive to chemical substances than males) were dosed at 5000 mg/kg by oral gavage and observed for 14 days as described in the previous paragraph. Despite hypoactivity and irregular respiration following dosing, all animals appeared normal and no gross abnormalities were observed upon necropsy. These data are consistent with assigning Tydracide-50 a GHS Oral Toxicity classification of Category 5 and an EPA classification of Category 4….essentially non-toxic.

Identical results were collected in an Acute Inhalation Toxicity study where five healthy male and five healthy female rats were continuously exposed to Tydracide-50 aerosol (1-4 micron particle size) over a four-hour period at a 5.13 mg/liter concentration. Following exposure, the animals were monitored for signs of gross toxicity and observed for 14 days as described above. All animals survived exposure to the test atmosphere saturated with Tydracide-50 and gained body weight during the study. Immediately following exposure, all rats exhibited irregular respiration, however, all animals recovered and appeared active and healthy for the remainder of the 14-day observation period with no gross abnormalities seen during necropsy.

Tydracide-50 was also evaluated in a Primary Skin Irritation study using the more sensitive rabbit species as a test subject. Methods used were similar to those previously described for the rat. Results showed no skin corrosion from Tydracide-50 exposure and only slight skin irritation following a four-hour exposure that resolved by the study termination at day seven. These data assign Tydracide-50 to the lowest toxicity categories for skin irritation on both GHS and EPA scales (Category 3 and 4, respectively).

Again using a rabbit model, Primary Eye Irritation was measured by instillation of a 100 microliter drop of Tydracide-50 into one eye each of three healthy, naive animals without pre-existing ocular irritation. Ocular irritation was scored by a widely accepted method (Draize scoring) where (at 1, 24, 48, 72 hours and at 4, 7, 10, 14, 17, and 21 days post installation) lesions and variations in the appearance of the cornea, iris and conjunctivae were recorded. Over the first 24 hours following test substance instillation, two treated eyes exhibited corneal opacity and 'positive' conjunctivitis. There was no iritis observed in any treated eye during this study. The overall incidence and severity of irritation decreased gradually with time. Positive irritation cleared from the two treated eyes by Day 21. Minimal conjunctivitis persisted in one treated eye through Day 21 (study termination), all resulting in a total numerical score of 19.7 and classification of Tydracide-50 as moderately irritating to the eye (EPA Category 3, GHS Category 2B).

The final study in the EPA six-pack suite was performed in mice to determine if Tydracide-50 has the capacity to sensitize rodent skin. This test procedure is called the Local Lymph Node Assay (LLNA) and is quite sophisticated. It involves large numbers of mice and directly measures immune cell proliferation in the lymph nodes caused by a chemical applied to the skin. The amount of cell proliferation is assessed by radioactive isotope incorporation into newly synthesized DNA. For the test occasion where Tydracide-50 performance was assessed, a cell stimulation index (SI) of 4.5 was calculated for the positive control substance alpha-Hexylcinnamaldehyde, a moderate dermal sensitizer. The average SI of three Tydracide-50 concentrations (25, 50, and 100%) was 0.98, far below the 3.0 cutoff established to designate a chemical as a contact sensitizer. As such, Tydracide-50 is not considered to be a contact dermal sensitizer and would require no classification by GHS or EPA.

The results from these pivotal studies demonstrate that Tydracide-50 is considered safe according to the "Six-Pack" EPA toxicity testing paradigm. Unlike traditional acid products that are highly corrosive and highly toxic, Tydracide-50 can be assigned the lowest chemical toxicity rating in most categories, and is only moderately irritating to the eyes. Clearly, Tydracide-50 has the potential to be used safely and effectively as a technical grade active ingredient in economically important markets and where environmentally friendly, green chemistries are preferred.

2017 Hydronium / Citric Acid Trials for Crescendo Stabilization

2/17/2017 trials begin. 500ml of Crescendo with pH reduced to 3.8 pH in 500ml flasks with cap hand tightened. Kept in incubator @ 78 degrees for 12 weeks.

	Contol pH 7.0	Citic Acid pH 3.8	Hydronium pH 3.8
w1	bacteria on surface of liquid	No visable bacteria growth	No visable bacteria growth or odor
w2	Heavey bacterial growth on surface of liquid	No visable bacteria growth	No visable bacteria growth or odor
w3	Heavey bacterial growth on surface of liquid	Bacterial growth on surface of liquid	No visable bacteria growth or odor
w4	Heavy bacterial growth on surface of liquid w / offensive odor	Heavy bacterial growth on surface of liquid	No visable bacteria growth or odor
w5	Heavy bacterial growth on surface of liquid w / offensive odor	Heavy bacterial growth on surface of liquid w / offensive odor	No visable bacteria growth or odor
w6	Heavy bacterial growth on surface of liquid w / offensive odor	Heavy bacterial growth on surface of liquid w / offensive odor	No visable bacteria growth or odor
w7	Heavy bacterial growth on surface of liquid w / offensive odor	Heavy bacterial growth on surface of liquid w / offensive odor	No visable bacteria growth or odor
w8	Heavy bacterial growth on surface of liquid w / offensive odor	Heavy bacterial growth on surface of liquid w / offensive odor	No visable bacteria growth or odor
w9	Heavy bacterial growth on surface of liquid w / offensive odor	Heavy bacterial growth on surface of liquid w / offensive odor	No visable bacteria growth or odor
w10	Heavy bacterial growth on surface of liquid w / offensive odor	Heavy bacterial growth on surface of liquid w / offensive odor	No visable bacteria growth or odor
w11	Heavy bacterial growth on surface of liquid w / offensive odor	Heavy bacterial growth on surface of liquid w / offensive odor	No visable bacteria growth or odor
w12	Heavy bacterial growth on surface of liquid w / offensive odor	Heavy bacterial growth on surface of liquid w / offensive odor	No visable bacteria growth or odor

2018 Hydronium Trials for stabilization of fish emulsion with added protien hydrolosate. Ph Reduced to 3.2pH

2018 trials begin. 500ml of fish fertilizer in 500ml beakers with 5ml protein hydrolysate, (Golden Nitro). Incubator @ 78 degrees for 12

	Contol no Hydronium 3.8	**Hydronium pH 3.8**
w1	3.8 No Change	3.8 No Change
w2	Heavey bacterial growth on surface of liquid	3.8 No Change
w3	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change
w4	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change
w5	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change
w6	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change
w7	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change
w8	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change
w9	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change
w10	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change
w11	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change
w12	Heavy bacterial growth on surface of liquid w / offensive odor	3.8 No Change

2019 Hydronium Trials for pH down stabilization

6/24/2019 trials begin. 500ml of tap with 7.6 pH reduced to 6.5 pH in 500ml beakers. Incubator @ 78 degrees for 12 weeks.

	Contol tap water pH 7.6	**Hydronium pH 6.5**
w1	7.6 No Change	6.5 No Change
w2	7.6 No Change	6.5 No Change
w3	7.6 No Change	6.5 No Change
w4	7.6 No Change	6.5 No Change
w5	7.6 No Change	6.5 No Change
w6	7.6 No Change	6.5 No Change
w7	7.6 No Change	6.5 No Change
w8	7.6 No Change	6.5 No Change
w9	7.6 No Change	6.5 No Change
w10	7.6 No Change	6.5 No Change
w11	7.6 No Change	6.5 No Change
w12	7.6 No Change	6.5 No Change

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Jungle Control Potato Trial 2017
Mid-Michigan Agronomy
DeWitt, MI

Objective: To evaluate the effect of Ferocious Plant Optimizer on the growth and yield of potatoes.

The variety, FL2137 was planted on May 31, 2017. Treatments were broadcast sprayed over plots at the desired use rates at 9 application dates. The treatments were applied at 7 day intervals starting on 7/5/2017. Potato yields were measured on October 29, 2017.

Location: DeWitt, MI

Soil Type: Boyer Sandy Loam

Planting Date: 05/31/2017

Emergence Date: 06/10/2017

Harvest Date: 10/29/2017

Variety: FL 2137

Treatment Spray Dates

Application Date	Air Temp
7/5	64
7/12	60
7/19	61
7/27	62
8/3	60
8/10	61
8/17	67
8/24	59
8/30	59

Soil pH: 6.5

Spray Volume: 20 GPA

Treatments:

Material	**Rate**
Untreated Check	---0---
Ferocious	1 % v/v
Ferocious	2 % v/v
Ferocious + Bravo	1 % + 1.5 Pt./A
Ferocious + Bravo	2 % + 1.5 Pt./A

Effects of Foliar Applications of Ferocious on Potato Yield-CWT

Treatment	Rate (% + Pt/A)	Yield CWT* (Hundred weight) US-1	Yield CWT* (Hundred weight) "B"	Yield CWT* (Hundred weight) Total	Phytophthora infestans Pest Incidence (10/28)
Untreated	0	471.5 a	143.0	614.5	17.9 a
Ferocious	1%	547.9 b	153.5	701.5	9.4 b
Ferocious	2 %	558.7 b	131.8	690.4	10.4 b
Ferocious + Bravo	1 % + 1.5	551.8 b	141.6	693.3	4.4 c
Ferocious + Bravo	2 % + 1.5	607.7 b	136.5	744.2	4.3 c

*Hundred weight bags of potatoes

Effects of Foliar Applications of Ferocious on Potato Yield-Number of Tubers

Treatment	Rate (% + Pt/A)	Yield Number of Tubers Total
Untreated	0	142478.1
Ferocious	1%	153731.1
Ferocious	2 %	148649.1
Ferocious + Bravo	1 % + 1.5	152460.6
Ferocious + Bravo	2 % + 1.5	146108.1

Results:

Weekly applications of Ferocious Plant Optimizer on potatoes were evaluated for the effect on potato yield. Ferocious was applied alone and in combination with Bravo fungicide and the treatments were compared to an untreated check. Treatments were applied 9 times at weekly intervals starting on July 5, 2017.

The addition of Bravo fungicide afforded good commercial control of Phytophthora disease organism.

Potato yields were increased by the Ferocious applications at both rates with the 1% rate having the greatest yield increase. Yield increases ranged from 12 to 14%. The addition of Bravo fungicide to the Ferocious increased yields over the untreated check by 12 to 21% primarily due to the improved Phytophthora disease control and the addition of Ferocious.

The total number of potatoes increased with both rates of Ferocious when compared to the untreated check. The addition of the Bravo fungicide to the Ferocious plant amendment did not increase the total number of potatoes when compared to Ferocious alone at the 1% and 2% v/v rate indicating Ferocious assisted to some degree in plant stress management.

To summarize, yields were significantly increased by both rates of Ferocious over the untreated check and the addition of Bravo fungicide increased the yield even more.

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Jungle Control Soybean Trial 2017
Mid-Michigan Agronomy
DeWitt, MI

Objective: To evaluate the effect of Ferocious Plant Optimizer on the growth and yield of soybean.

The variety, Pioneer 92Y51 was planted on May 23, 2017. Treatments were broadcast sprayed over plots at the desired use rates at 9 application dates. The treatments were applied at 7 day intervals starting on 7/5/2017. Soybean vigor was measured on 7/7 and disease suppression of Septoria Brown Spot was evaluated on 8/30. Soybean yields were measured on October 11, 2017.

Location:	DeWitt, MI
Soil Type:	Silty Clay Loam
Planting Date:	05/23/2017
Emergence Date:	05/31/2017
Harvest Date:	10/11/2017
Variety:	Pioneer 92Y51

Treatment Spray Dates

Application Date	Air Temp
7/5	69
7/12	69
7/19	67
7/27	68
8/3	62
8/10	59
8/17	65
8/24	48
8/30	54

Soil pH: 7.9

Spray Volume: 20 GPA

Treatments:

Material	**Rate**
Untreated Check	---0---
Ferocious	1 %
Ferocious	2 %
Ferocious + Quilt	1 % + 20.5
Ferocious + Quilt	2 % + 20.5

Ferocious Treatments Applied to Soybeans

Treatment	Rate (% + oz/A)	Canopy Green Area (7/7)	Septoria Brown Spot (8/30)	Yield (Bu/A)	Test weight lb./bu
Untreated	0	3.3	23.1	43	50.8
Ferocious	1%	4.3	5.2	52.7	51.3
Ferocious	2 %	4.5	7.1	51.1	51.1
Ferocious + Quilt	1 % + 20.5	4.5	3	47.9	50.6
Ferocious + Quilt	2 % + 20.5	4.8	2.8	50.9	51.1

Results:

Weekly applications of Ferocious Plant Optimizer on soybeans were evaluated for the effect on soybean growth and yield. Ferocious was applied alone and in combination with Quilt fungicide from Syngenta and the treatments were compared to an untreated check. Treatments were applied 9 times at weekly intervals starting on July 5, 2017.

Regarding soybean growth and disease suppression there is a trend toward an increase in canopy from the application of Ferocious at both rates. The addition of Quilt to Ferocious at the high rate appears to increase the canopy over the high rate of Ferocious alone. Ferocious significantly reduced the incidence of Septoria Brown Spot, a fungal disease that attacks soybeans as a plant stress manager. Both rates of Ferocious reduced the incidence of the disease. The addition of the fungicide Quilt to the Ferocious provided the best control.

Soybean yields were significantly increased with applications of Ferocious by itself and when the Ferocious was applied with Quilt. Yield increases ranged from 11 to 22% depending on the Ferocious treatment.

To summarize, Ferocious treatments of 1% and 2% applied weekly, 9 times had a positive effect on soybean vigor, disease suppression (Septoria Brown Spot) and grain yield.

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Jungle Control Sugar Beet Trial 2017
Mid-Michigan Agronomy
DeWitt, MI

Objective: To evaluate the effect of Ferocious Plant Optimizer on the yield of sugar beets.

The variety, ACH RR-824 was planted on April 13, 2017. Treatments were broadcast sprayed over plots at the desired use rates at 9 application dates. The treatments were applied at 7 day intervals starting on 7/5/2017. Cercospora suppression was measured on 8/16, 8/23, 9/1 and 9/17. Sugar beet yields were determined on November 18, 2017.

Location:	DeWitt, MI
Soil Type:	Bay City Sugar Beet Loam
Planting Date:	04/13/2017
Harvest Date:	09/18/2017
Variety:	ACH RR-824

Treatment Spray Dates

Application Date	Air Temp (F)
7/5	68
7/12	72
7/19	71
7/27	68
8/3	69
8/10	63
8/17	65
8/24	55
8/30	60

Soil pH: 7.5

Spray Volume: 20 GPA

Treatments:

Material	**Rate**
Untreated Check	---0---
Ferocious	1 % v/v
Ferocious	2 % v/v
Ferocious + Eminent 125 SL	1 % + 13.0
Ferocious + Eminent 125 SL	2 % + 13.0

Effects of Foliar Applications of Ferocious on Sugar Beet Yield

Treatment	Rate (% + Oz./A)	Yield (T/A) *
Untreated	0	15.1 a
Ferocious	1%	20.6 ab
Ferocious	2 %	24.5 a
Ferocious + Eminent 125	1 % + 13.0	22.3 ab
Ferocious + Eminent 125	2 % + 13.0	18.4 bc

*Means followed by the same letter are not statistically different (P=0.10)

Results:

Weekly applications of Ferocious Plant Optimizer on sugar beets were evaluated for the effect on yield. Ferocious was applied alone and in combination with Eminent 125 fungicide. The treatments were compared to an untreated check. Treatments were applied 9 times at weekly intervals starting on July 5, 2017.

When Ferocious was combined with Eminent 125, commercial control of Cercospora was observed.

Sugar beet yields were increased by the Ferocious applications at both rates. Yield increases ranged from 36 to 62%. The addition of Eminent 125 fungicide to the Ferocious increased yields over the untreated check by 47 to 22%. It is interesting to note that the tank-mix of Ferocious + Eminent 125 afforded the best disease control but were not the highest yielding treatments. This suggests that the Ferocious alone is causing yield increases along with plant stress management attributes.

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Jungle Control Tomato Trial 2017
Mid-Michigan Agronomy
DeWitt, MI

Objective: To evaluate the effect of Ferocious Plant Optimizer on growth and yield of tomatoes.

The variety, Roma was planted on June 5, 2017 as transplants. Treatments were broadcast sprayed over plots at the desired use rates at 9 application dates. The treatments were applied at 7 day intervals starting on 7/5/2017. Tomato vigor was measured on 7/7 and disease suppression of Septoria Brown Spot was evaluated on 8/30. Tomato yields were measured on October 11, 2017.

Location:	DeWitt, MI
Soil Type:	Boyer Sandy Loam
Planting Date:	06/05/2017
Emergence Date:	Transplants
Harvest Date:	09/28/2017
Variety:	Roma
Spray Volume:	20 GPA

Treatment Spray Dates

Application Date	Air Temp
7/5	69
7/12	69
7/19	67
7/27	68
8/3	62
8/10	59
8/17	65
8/24	48
8/30	54

Soil pH: 8.5

Treatments:

Material	**Rate**
Untreated Check	---0---
Ferocious	1 % v/v
Ferocious	2 % v/v

Effects of Foliar Applications of Ferocious on Tomato Yield

Treatment	Rate (% + Pt/A)	Yield Mature First Harvest (T/A)	Yield Mature Second Harvest (T/A)	Yield Mature Total (T/A)
Untreated	0	28.7	5.8	24.5
Ferocious	1%	36.9	8.0	44.9
Ferocious	2 %	40.0	10.7	50.7
Ferocious + Bravo	1 % + 1.5	52.7	11.6	64.3
Ferocious + Bravo	2 % + 1.5	59.9	15.6	75.5

Results:

Weekly applications of Ferocious Plant Optimizer on tomatoes were evaluated for the effect on yield. Ferocious was applied alone and in combination with Bravo fungicide and the treatments were compared to an untreated check. Treatments were applied 9 times at weekly intervals starting on July 5, 2017.

Tomato yields were increased by the Ferocious applications at both rates. Yield increases ranged from 28 to 39%. The addition of Bravo fungicide to the Ferocious increased yields over the untreated check by 83 to 108% primarily due to the Phytophthora disease control and positive attributes assisting in plant stress management.

To summarize, yields were significantly increased by both rates of Ferocious over the untreated check.

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TESTING RESULTS

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TYDRONIUM COMPLETELY INACTIVATES HERPES SIMPLEX VIRUS

The attached report contains data collected on Tydronium, an extreme acid manufactured by Tygrus that is non-corrosive (based on Occupational Safety & Health Administration (OSHA) definition), environmentally friendly, and safe for human touch. The experiments were performed by an independent testing organization, Antimicrobial Test Laboratories, LLC (ATL). ATL is a contract research organization that offers its customers documented, top-of-the-line microbiology services to determine product effectiveness and to support regulatory approval.

ATL conducted proof-of-concept testing on the Tydronium product to assess its ability to inactivate human herpes simplex virus. Human herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are responsible for recurrent orolabial and genital infections. Both HSV-1 and -2 are enveloped viruses that share common pathogenic mechanisms and their seroprevalence in the human population can vary depending on geographical location. Although HSV-2 infection is more often associated with genital lesions, the HSV-1 virus can also cause genital lesions in addition to the commonly seen blisters on the face and lips. In fact, HSV-1 is now responsible for up to half of all new cases of genital herpes in developed countries. Besides encephalitis and disseminated neonatal infections, HSV also causes ocular herpes and recurrent ocular HSV-1 infections remain the major cause of viral induced blindness. Although the common symptoms associated with both viruses are usually self-limiting and episodic, HSV infections may be severe in immunocompromised patients (such as those infected with HIV or undergoing cancer treatments). Transmission of herpes simplex virus occurs by contact with secretions from an infected person with either overt infection or asymptomatic excretion of virus. The estimated persistence of the virus particles ranges from 4.5 hours up to 7 days when bodily secretions containing virus are deposited on dry inanimate surfaces. As such, cross-contamination with these viruses through common touch points is a concern in both the healthcare setting and the public environment.

The testing conducted by ATL followed an industry standard protocol approved by the Association of Official Analytical Chemists (AOAC 961.02: Germicidal Spray Method) and adapted for use in evaluating the virucidal efficacy of spray disinfectants. In a Modified AOAC Spray Products Test, a viral inoculum is dried onto carriers, followed by exposure to a test formulation via spray device

for the specified contact time(s). The carriers are neutralized then enumerated using standard cell culture or plaque assay techniques. Log10 and percent reduction values are calculated to determine the effectiveness of the test product relative to control carriers. The AOAC Germicidal Spray Products method for use with spray devices and modified for viruses is recognized by regulatory agencies as an approved method for claim substantiation.

For this study, two different formulations of Tydronium were evaluated and compared to chlorine bleach at a concentration of 500 ppm, which is recommended for general disinfectant use by the CDC. Higher concentrations of bleach are extremely corrosive as well as irritating to the skin, eyes, and mucous membranes. In fact, using higher concentrations of bleach in the AOAC testing protocols is toxic to the cells used for detecting viral infectivity. The results in Table 1 show that both formulations of Tydronium inactivate 99.97% of the exposed virus particles after 5 minutes of contact time, reducing the number of active virus particles by ≥ 3.5 log10 units (~3,000- fold or better). Remarkably, one of the Tydronium formulations is equally active against this virus at only 1 minute contact time and is equivalent to the bleach comparator.

Table 1. Antiviral Activity of Tydronium against Herpes Simplex Virus 1, ATCC VR-260

Test Substance	Contact Time	Log10 Infectious Units/Carrier	% Reduction (compared to control at t= 0)	Log10 Reduction (compared to control at t= 0)
Control	Time Zero	6.48	N/A	N/A
Bleach (500 ppm)	1 minute	≤ 2.98	≥ 99.97%	≥ 3.50
Tydronium 1.0.01	1 minute	≤ 2.98	≥ 99.97%	≥ 3.50
Tydronium 1.5.01	1 minute	5.73	88.22%	0.75
Bleach (500 ppm)	5 minutes	≤ 2.98	≥ 99.97%	≥ 3.50
Tydronium 1.0.01	5 minutes	≤ 2.98	≥ 99.97%	≥ 3.50
Tydronium 1.5.01	5 minutes	≤ 2.98	≥ 99.97%	≥ 3.50

As the lab report demonstrates, the results for Tydronium were extraordinary: a one-minute exposure was sufficient to completely inactivate HSV-1 and reduce the number of active viruses by greater that 3 log10 units. The ability of Tydronium to rapidly neutralize HSV-1 is highly predictive of the product's activity against other viruses of the same family (e.g. HSV-2; HSV-3 [varicella zoster/chicken pox]; Epstein Barr virus [mononucleosis]) and other enveloped viruses such as HIV, Influenza, MERS-CoV (Middle Eastern Respiratory Syndrome Coronavirus), and Ebola virus (hemorrhagic fever).

ANTIMICROBIAL TEST LABORATORIES

Study Report

www.AntimicrobialTestLabs.com - Info@AntimicrobialTestLabs.com - (512) 310-8378

Study Title

Determination of the Efficacy of Tygrus, LLC. Test Substances Delivered via Spray Device Against Herpes Simplex Virus 1

Test Method

Association of Analytical Communities Test Method 961.02
Germicidal Spray Products as Disinfectants Modified for Viruses

Study Identification Number

NG5645-A2

Study Sponsor

Daniel J. Jenuwine
Tygrus, LLC.
1134 E. Big Beaver Road
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Study Contact

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Molosser Group Consulting
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(203) 974-2099

Test Facility

Antimicrobial Test Laboratories
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

History of the Laboratory

Antimicrobial Test Laboratories was launched in 2006 to provide testing services to the antimicrobial industry. The company has grown considerably since then but its focus remains the same: Test antimicrobial agents, test them well, and test them fast! Antimicrobial Test Laboratories operates a 15,000+ square foot facility near Austin, Texas, where hundreds of studies are conducted annually by a staff of friendly, knowledgeable, and experienced microbiologists and virologists.

Laboratory Qualification Statement

Antimicrobial Test Laboratories was founded by microbiologist Dr. Benjamin Tanner. The laboratory ensures consistent, reproducible results by utilizing a well-trained and educated scientific staff who work from a comprehensive system of Standard Operating Procedures, official standard methods from ASTM, AOAC, and other organizations, and custom study protocols. The laboratory provides testing services to dozens of Fortune 500 companies and has been inspected for GLP compliance by the US government.

Scientist Qualifications

Your Study was designed, conducted and reported by: Erika Guin, B.S.

Erika graduated from St. Edward's University with a Bachelors of Science in Biology.

Erika has a strong educational background in microbiology, and is a dedicated and enthusiastic professional. As a Microbiology Associate at Antimicrobial Test Labs she has assisted other microbiologists in the conduct of a wide range of custom studies. Her strong work ethic and interest in comprehensive and accurate testing make her an asset to the laboratory as well as a competent conductor of antimicrobial studies.

If you have any questions about your study, please don't hesitate to contact Erika at:

Erika@AntimicrobialTestLabs.com
or
(512) 310-8378

AOAC Germicidal Spray Products Test: General Information

Formerly known as the Association of Official Analytical Chemists, AOAC International is a globally recognized, third party, not-for-profit association that provides education and facilitates the development of test methods and standards for a wide range of industries. The AOAC Germicidal Spray Test method is a semi-quantitative test method designed to assess the performance of liquid spray disinfectants. The method can be modified to evaluate the virucidal efficacy of spray disinfectants. In a Modified AOAC Spray Products Test, a viral inoculum is dried onto carriers, followed by exposure to a test formulation via spray device for the specified contact time(s). The carriers are neutralized then enumerated using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques. Log_{10} and percent reduction values are calculated to determine the effectiveness of the test product relative to control carriers. The AOAC Germicidal Spray Products method for use with spray devices and modified for viruses is recognized by regulatory agencies as an approved method for claim substantiation.

Laboratory Qualifications Specific to AOAC Germicidal Spray Products Test Method Modified for Viruses

Antimicrobial Test Laboratories has considerable experience in the proper execution of the Modified AOAC Germicidal Spray Products Test Method. The laboratory has performed many modified AOAC germicidal spray tests in order to assess the virucidal efficacy of a broad spectrum of disinfectant products. Each test is performed at Antimicrobial Test Laboratories in a manner appropriate to the test substances submitted by the Study Sponsor, while maintaining the integrity of the study.

Study Timeline

Amended report delivered 19 DEC 2014

Test Substance Information

The test substance was received on 25 NOV 2014. The following pictures were taken after test substance use within a preliminary cytotoxicity screen.

Test Substances Received: Tydronium 1.0.01, Tydronium1.5.01

Test Substances arrived within Study Sponsor provided spray bottles, and were ready to use for the conduct of the study. Test substances were not diluted prior to use in the study.

The Study Sponsor requested 500 ppm bleach control substance was made on site at ATL. It consisted of a concentrated commercial bleach solution diluted in sterile reverse osmosis water.

Test Microorganism Information

The test microorganism(s) selected for this test:

Herpes Simplex Virus 1 (HSV-1), ATCC VR-260

This virus is an enveloped, double-stranded DNA virus of the genus *Simplexvirus*. Clinical signs of infection include small, fluid filled blisters on the lips or mouth (cold sores), fever, a sore throat, and swollen lymph nodes. HSV-1 infection is less stigmatized than HSV-2 (the main cause of genital herpes), but both viral strains can cause genital herpes and be spread by those without active symptoms. Although there are multiple treatments for symptoms such as cold sores, there is currently no cure for HSV-1 and carriers of the virus will continue to have symptomatic outbreaks for the rest of their lives. HSV-1 is common world-wide, and it is estimated that the majority of United States citizens are exposed to or infected by HSV-1 by the time they reach adolescence.

Permissive Host Cell Line Selected for HSV 1: Vero (African Green Monkey Kidney Cells), ATCC CCL-81

Diagram of the Procedure

Summary of the Procedure

- Stock virus is thawed and may be supplemented with an organic soil load, if requested.
- Sterile glass Petri dish carriers (100 mm in diameter) are inoculated with a volume of virus suspension containing an adequate titer to recover a minimum of 4-Log_{10} infectious viruses per carrier. A sufficient number of test and control carriers are prepared.
- Inoculated carriers are dried at room temperature under laminar flow conditions.
- The test substance is prepared according to the Study Sponsor's instructions as requested, and applied to the test carriers using a spray device. The distance, angle, and number of sprays applied are recorded.
- The treated carriers are held for the predetermined contact time(s), and then neutralized in a manner appropriate for the test substance (e.g. dilution and/or gel filtration).
- The control carrier is harvested using an equivalent volume cell culture medium or other suitable buffer.

Summary of the Procedure, Continued

- Following neutralization of test and control carriers, the viral suspensions are quantified to determine the levels of infectious virus using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques.
- Assay trays/plates are incubated for the period most suitable for the virus-host cell system (e.g. 7 days).
- After the incubation period, the assay is scored for the presence/absence of test virus and cytotoxic effects. The appropriate calculations are performed (e.g. Spearman-Karber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- Log_{10} and percent reductions are computed for viral films exposed to the test product relative to the titer obtained for the study control carrier(s), and reported to the Study Sponsor.

Criteria for Scientific Defensibility of a Germicidal Spray Study Modified for Viruses

For Antimicrobial Test Laboratories to consider a Germicidal Spray Products Test Modified for Viruses study to be scientifically defensible, the following criteria must be met:

1. A minimum of 4-$\log_{10}$ infectious viruses are recovered from the virus control carrier.
2. Viral cytopathic effects are distinguishable from cytotoxic effects caused by test substance exposure.
3. Neutralization effectiveness is demonstrated by recovery of comparable levels of infectious viruses from control (e.g. PBS), neutralizer (where applicable), and neutralized test substance.
4. Assay wells designated as sterility controls are absent of infectivity, contamination, and cytotoxicity.

Passing Criteria

AOAC International has defined the passing criteria for the Germicidal Spray test for viruses as:

1. Complete inactivation of the test virus at all dilutions.
2. If cytotoxicity is observed, a ≥3-Log_{10} reduction in viral titer is observed past the level of cytotoxicity relative to the virus control.

Testing Parameters used in this Study

Test Substance Diluent:	N/A (Ready to Use)	Carrier Type:	Glass Petri Dish
Carriers per Test:	1	Number of Sprays:	4
Spray Distance:	6-8 inches	Spray Angle:	45°
Neutralization:	See Study Notes		

Viral Inoculum Volume:	0.2 ml	Carrier Inoculation Area:	100 mm diameter
Carrier Dry Time:	20-34 Minutes	Carrier Dry Conditions:	13.9-18.7°C
Contact Time:	1, 5, 10 Minutes	Contact Conditions:	Ambient
Host Cell Line:	Vero (ATCC CCL-81)	Cell Passage Number:	p. 143
Assay Medium:	2% FBS EMEM	Soil Load:	None
Incubation Period	7 Days	Incubation Conditions:	37°C, 5% CO_2

Study Modifications

No further modifications were made to the method for this study.

Study Notes

The viral inoculum was not diluted from its original stock vials or supplemented in any way prior to application to test and control carriers.

Inoculated carriers were dried at a decreased temperature range (13.9°C – 18.7°C) to reduce viral deactivation during drying.

Test substance was applied to carriers via 4 sprays from Study Sponsor provided spray bottles. The total volume dispensed was approximately 1 ml. A harvest volume of 2 ml of the approprate neutralizer (detailed below) was used to re-capture virus prior to enumeration. Neutralizers were selected after analysis via a preliminary cytotoxicity screen. All chemical neutralizers were coupled with a secondary neutralization consisting of passage through a Sephacryl S-1000 gel filtration column.

Neutralization Method:

1.0.01 Test Substance:	Dey Engley Broth + 0.5M NaOH
1.5.01 Test Substance:	Dey Engley Broth
500 ppm Bleach Control:	Dey Engley Broth
Time Zero Control:	Dey Engley Broth

Study Photographs

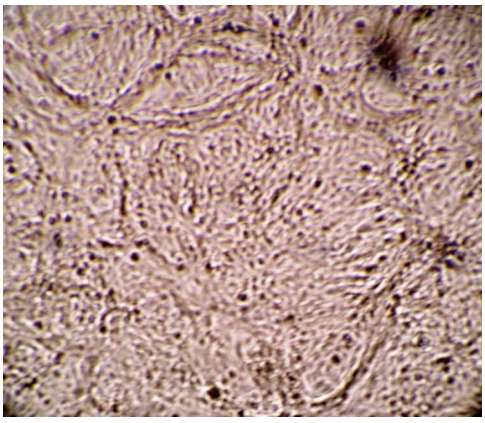


Photo 1. A healthy, undisturbed Vero monolayer after the conclusion of the 7 day assay.

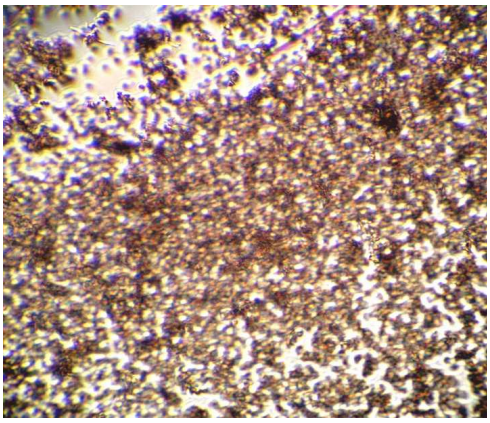


Photo 2. Cytotoxicity within the 10^{-1} well dilution of test substance 1.0.01 plated to permissive Vero host cells. Cytotoxicity often presents as entire sheets of cells sloughing off of the surface of the assay tray well, or as complete destruction of cells within the well, and can vary from cell line to cell line. Cells will also tend to display toxicity via darkening and other morphological changes. Often some remnants of the monolayer will cling to the surface of the well.

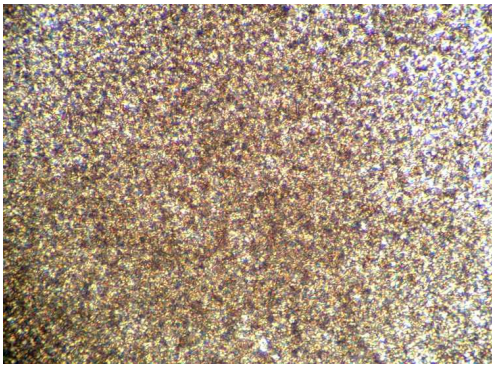


Photo 3. Infected cells after application of HSV 1 treated with the 1.5.01 test substance. Infection often presents as aggregate clumping into grape-like clusters, complete dissociation of the monolayer from the well surface, and refractile rounding of cells.

Control Results

Virus Control Titer:	6.48 $\log_{10}$ per Carrier	Cytotoxicity Titer:	≤2.98 $\log_{10}$ per Carrier
Sterility Controls:	Validated	Neutralization Efficacy:	Validated

Calculations

Viral and cytotoxicity titers ($TCID_{50}$/$TCLD_{50}$ and $TCCD_{50}$, respectively) were determined according to the method developed my Spearman-Karber:

$$-\text{Log}_{10} \text{ of 1st Dilution} - \left(\frac{\text{sum of \% mortality at each dilution}}{100}\right) - 0.5$$

Percent Reduction of Virus is determined according to the following formula:

$$\text{Percent Reduction} = 1 - \left(\frac{C}{B}\right) * 100$$

Where:
B = Log_{10} of Virus Control Carrier
C = Log_{10} of Virus Test Carrier

Results of the Study

Table 1. Antiviral Efficacy of Two Tyrgrus Test Substances Applied via Spray Device against Herpes Simplex Virus 1, ATCC VR-260

Test Microorganism	Contact Time	Test Substance	Log_{10} Infectious Units per Carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
Herpes Simplex Virus 1 ATCC VR-260	Time Zero	Control	6.48	N/A	
	1 Minute	500 ppm Bleach	≤ 2.98	≥ 99.97%	≥ 3.50
		1.0.01	≤ 2.98	≥ 99.97%	≥ 3.50
		1.5.01	5.73	82.22%	0.75
	5 Minutes	500 ppm Bleach	≤ 2.98	≥ 99.97%	≥ 3.50
		1.0.01	≤ 2.98	≥ 99.97%	≥ 3.50
		1.5.01	≤ 2.98	≥ 99.97%	≥ 3.50
	10 Minutes	500 ppm Bleach	≤ 2.98	≥ 99.97%	≥ 3.50
		1.0.01	≤ 2.98	≥ 99.97%	≥ 3.50
		1.5.01	≤ 2.98	≥ 99.97%	≥ 3.50

*"≤" indicates a viral titer at or below the limit of detection for this assay.

The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.

TYDRONIUM RAPIDLY NEUTRALIZES A NOROVIRUS SURROGATE

The attached report contains data collected on Tydronium, an extreme acid manufactured by Tygrus that is safe, non-toxic, and environmentally friendly. The experiments were performed by an independent testing organization, Antimicrobial Test Laboratories, LLC (ATL). ATL is a contract research organization that offers its customers documented, top-of-the-line microbiology services to determine product effectiveness and to support regulatory approval.

ATL conducted proof-of-concept testing on the Tydronium product to assess its ability to inactivate feline calicivirus (FCV). This virus belongs to a large family of non-enveloped viruses called Caliciviridae that share common pathogenic mechanisms and have a wide host range to include both humans and animals. The US EPA has approved the use of FCV as a surrogate for human norovirus testing, as a cell culture assay system has been unavailable to cultivate human norovirus in the laboratory. Norovirus is the most common cause of non-bacterial gastroenteritis in the developed world. In the US, the Centers for Disease Control estimate that norovirus infection is responsible for 19-21 million illnesses, contributing to over 70,000 hospitalizations, and nearly 800 deaths each year. Once infected, norovirus begins to replicate in the small intestine and after one to two days, the typical symptoms associated with acute gastroenteritis begin: nausea, forceful vomiting, watery diarrhea, and abdominal pain. Although these symptoms and the malaise associated with norovirus infections are usually self-limiting, the very young, the elderly, and the immunocompromised can be severely affected. Norovirus is highly contagious and transmission occurs through human contact, consuming contaminated foods or water, and touching contaminated surfaces where virus can persist up to 7 days without a significant drop in viability. As such, cross-contamination with norovirus through common touch points is a concern in both the healthcare setting and the public environment. Norovirus infection outbreaks are more likely to occur in closed or semi-closed environments, such as schools, dormitories, and cruise ships – places where human-to-human contact is common. Indeed, norovirus has often been unfortunately called the cruise ship virus. Currently there are no preventive vaccines or therapeutic treatments available for norovirus infections.

The testing conducted by ATL followed an industry standard protocol approved by the Association of Official Analytical Chemists (AOAC 961.02: Germicidal Spray Method), adapted for use in evaluating

the virucidal efficacy of spray disinfectants and recognized by regulatory agencies as an approved method for claim substantiation. In a Modified AOAC Spray Products Test, a viral inoculum is dried onto carriers, followed by exposure to a test formulation via spray device for the specified contact time(s). The carriers are neutralized and remaining virus is enumerated using standard cell culture or plaque assay techniques.

For this study, two different formulations of Tydronium were evaluated and compared to chlorine bleach at a concentration of 500 ppm, which is recommended for general disinfectant use by the CDC. Higher concentrations of bleach are extremely corrosive as well as irritating to the skin, eyes, and mucous membranes. In fact, using higher concentrations of bleach in the AOAC testing protocols is toxic to the cells used for detecting viral infectivity. The results in Table 1 show that both formulations of Tydronium possess potent antiviral activity. Unfortunately, the laboratory was unable to deposit sufficient amounts of FCV on testing surfaces to conclusively demonstrate a drop in virus activity of ≥3 log10 units, but one of the Tydronium formulations was able to inactivate 99.82% of the exposed virus particles after only 1 minute of contact time (a ≥ 2.75 log10 reduction).

Table 1. Antiviral Activity of Tydronium against Feline Calicivirus, ATCC VR-782

Test Substance	Contact Time	Log10 Infectious Units/Carrier	% Reduction (compared to control at t= 0)	Log10 Reduction (compared to control at t= 0)
Control	Time Zero	5.73	N/A	N/A
Bleach (500 ppm)	1 minute	≤ 2.98	≥ 99.82%	≥ 2.75
Tydronium 1.0.01	1 minute	≤ 2.98	≥ 99.82%	≥ 2.75
Tydronium 1.5.01	1 minute	4.48	94.38%	1.25
Bleach (500 ppm)	5 minutes	≤ 2.98	≥ 99.82%	≥ 2.75
Tydronium 1.0.01	5 minutes	≤ 2.98	≥ 99.82%	≥ 2.75
Tydronium 1.5.01	5 minutes	6.23	No reduction	No reduction

As the lab report demonstrates, the results for Tydronium were extremely promising: a one-minute exposure was sufficient to neutralize FCV. This activity is highly predictive of the product's potency against other viruses of the same family (e.g. norovirus) and against other non-enveloped viral pathogens of human and veterinary importance such as enterovirus D68, adenovirus, parvovirus, coxsackie virus, and porcine arterivirus (porcine reproductive and respiratory syndrome virus).

ANTIMICROBIAL TEST LABORATORIES

Study Report

www.AntimicrobialTestLabs.com - Info@AntimicrobialTestLabs.com - (512) 310-8378

Study Title

Determination of the Efficacy of Tygrus, LLC. Test Substances Delivered via Spray Device Against Feline Calicivirus

Test Method

Association of Analytical Communities Test Method 961.02
Germicidal Spray Products as Disinfectants Modified for Viruses

Study Identification Number

NG5645-A3

Study Sponsor

Daniel J. Jenuwine
Tygrus, LLC.
1134 E. Big Beaver Road
Troy, MI 48083

Study Contact

Joseph DeVito, Ph.D.
Molosser Group Consulting
jdevito@molossergroupconsulting.com
(203) 974-2099

Test Facility

Antimicrobial Test Laboratories
1304 W. Industrial Blvd
Round Rock, TX 78681
(512) 310-8378

History of the Laboratory

Antimicrobial Test Laboratories was launched in 2006 to provide testing services to the antimicrobial industry. The company has grown considerably since then but its focus remains the same: Test antimicrobial agents, test them well, and test them fast! Antimicrobial Test Laboratories operates a 15,000+ square foot facility near Austin, Texas, where hundreds of studies are conducted annually by a staff of friendly, knowledgeable, and experienced microbiologists and virologists.

Laboratory Qualification Statement

Antimicrobial Test Laboratories was founded by microbiologist Dr. Benjamin Tanner. The laboratory ensures consistent, reproducible results by utilizing a well-trained and educated scientific staff who work from a comprehensive system of Standard Operating Procedures, official standard methods from ASTM, AOAC, and other organizations, and custom study protocols. The laboratory provides testing services to dozens of Fortune 500 companies and has been inspected for GLP compliance by the US government.

Scientist Qualifications

Your Study was designed, conducted and reported by: Erika Guin, B.S.

Erika graduated from St. Edward's University with a Bachelors of Science in Biology.

Erika has a strong educational background in microbiology, and is a dedicated and enthusiastic professional. As a Microbiology Associate at Antimicrobial Test Labs she has assisted other microbiologists in the conduct of a wide range of custom studies. Her strong work ethic and interest in comprehensive and accurate testing make her an asset to the laboratory as well as a competent conductor of antimicrobial studies.

If you have any questions about your study, please don't hesitate to contact Erika at:

Erika@AntimicrobialTestLabs.com
or
(512) 310-8378

AOAC Germicidal Spray Products Test: General Information

Formerly known as the Association of Official Analytical Chemists, AOAC International is a globally recognized, third party, not-for-profit association that provides education and facilitates the development of test methods and standards for a wide range of industries. The AOAC Germicidal Spray Test method is a semi-quantitative test method designed to assess the performance of liquid spray disinfectants. The method can be modified to evaluate the virucidal efficacy of spray disinfectants. In a Modified AOAC Spray Products Test, a viral inoculum is dried onto carriers, followed by exposure to a test formulation via spray device for the specified contact time(s). The carriers are neutralized then enumerated using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques. Log_{10} and percent reduction values are calculated to determine the effectiveness of the test product relative to control carriers. The AOAC Germicidal Spray Products method for use with spray devices and modified for viruses is recognized by regulatory agencies as an approved method for claim substantiation.

Laboratory Qualifications Specific to AOAC Germicidal Spray Products Test Method Modified for Viruses

Antimicrobial Test Laboratories has considerable experience in the proper execution of the Modified AOAC Germicidal Spray Products Test Method. The laboratory has performed many modified AOAC germicidal spray tests in order to assess the virucidal efficacy of a broad spectrum of disinfectant products. Each test is performed at Antimicrobial Test Laboratories in a manner appropriate to the test substances submitted by the Study Sponsor, while maintaining the integrity of the study.

Study Timeline

Amended report delivered 23 DEC 2014

Test Substance Information

The test substance was received on 25 NOV 2014. The following pictures were taken after test substance use within a preliminary cytotoxicity screen.

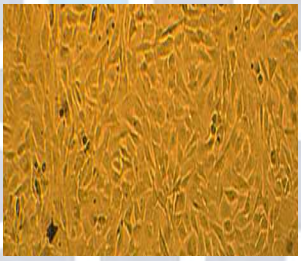
Test Substances Received: Tydronium 1.0.01, Tydronium1.5.01

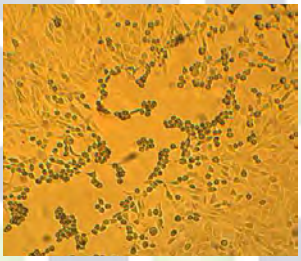
Test Substances arrived within Study Sponsor provided spray bottles, and were ready to use for the conduct of the study. Test substances were not diluted prior to use in the study.

The Study Sponsor requested 500 ppm bleach control substance was made on site at ATL. It consisted of a concentrated commercial bleach solution diluted in sterile reverse osmosis water.

Test Microorganism Information

The test microorganism(s) selected for this test:





Feline calicivirus (FCV), ATCC VR-782

This virus is a non-enveloped, positive-stranded RNA member of the genus *Vesivirus*, and a common cause of respiratory infections in cats. Symptoms of infection in felines include nasal discharge and mouth ulcers. As a member of the *Caliciviridae* viral family, FCV is closely related to human noroviruses, which cause acute gastroenteritis marked by nausea, vomiting, and diarrhea. Unlike human norovirus, however, a simple cell culture assay system is available for FCV. Therefore, feline calicivirus is the US EPA-approved surrogate microorganism for human norovirus label claims. Both FCV and human norovirus are able to remain viable on environmental surfaces for extended periods of time and are resistant to a number of disinfectant actives.

Permissive Host Cell Line Selected for FCV: CRFK (Crandell-Rees Feline Kidney Cells), ATCC CCL-94

Diagram of the Procedure

Summary of the Procedure

- Stock virus is thawed and may be supplemented with an organic soil load, if requested.
- Sterile glass Petri dish carriers (100 mm in diameter) are inoculated with a volume of virus suspension containing an adequate titer to recover a minimum of 4-Log_{10} infectious viruses per carrier. A sufficient number of test and control carriers are prepared.
- Inoculated carriers are dried at room temperature under laminar flow conditions.
- The test substance is prepared according to the Study Sponsor's instructions as requested, and applied to the test carriers using a spray device. The distance, angle, and number of sprays applied are recorded.
- The treated carriers are held for the predetermined contact time(s), and then neutralized in a manner appropriate for the test substance (e.g. dilution and/or gel filtration).
- The control carrier is harvested using an equivalent volume cell culture medium or other suitable buffer.

Summary of the Procedure, Continued

- Following neutralization of test and control carriers, the viral suspensions are quantified to determine the levels of infectious virus using standard cell culture (e.g. $TCID_{50}$) or plaque assay techniques.
- Assay trays/plates are incubated for the period most suitable for the virus-host cell system (e.g. 7 days).
- After the incubation period, the assay is scored for the presence/absence of test virus and cytotoxic effects. The appropriate calculations are performed (e.g. Spearman-Karber) to determine viral titers and levels of test substance cytotoxicity, where applicable.
- Log_{10} and percent reductions are computed for viral films exposed to the test product relative to the titer obtained for the study control carrier(s), and reported to the Study Sponsor.

Criteria for Scientific Defensibility of a Germicidal Spray Study Modified for Viruses

For Antimicrobial Test Laboratories to consider a Germicidal Spray Products Test Modified for Viruses study to be scientifically defensible, the following criteria must be met:

1. A minimum of 4-$\log_{10}$ infectious viruses are recovered from the virus control carrier.
2. Viral cytopathic effects are distinguishable from cytotoxic effects caused by test substance exposure.
3. Neutralization effectiveness is demonstrated by recovery of comparable levels of infectious viruses from control (e.g. PBS), neutralizer (where applicable), and neutralized test substance.
4. Assay wells designated as sterility controls are absent of infectivity, contamination, and cytotoxicity.

Passing Criteria

AOAC International has defined the passing criteria for the Germicidal Spray test for viruses as:

1. Complete inactivation of the test virus at all dilutions.
2. If cytotoxicity is observed, a ≥3-Log_{10} reduction in viral titer is observed past the level of cytotoxicity relative to the virus control.

Testing Parameters used in this Study

Test Substance Diluent:	N/A (Ready to Use)	Carrier Type:	Glass Petri Dish
Carriers per Test:	1	Number of Sprays:	4
Spray Distance:	6-8 inches	Spray Angle:	45°
Neutralization:	See Study Notes		

Viral Inoculum Volume:	0.2 ml	Carrier Inoculation Area:	100 mm diameter
Carrier Dry Time:	12-13 Minutes	Carrier Dry Conditions:	Ambient
Contact Time:	1, 5, 10 Minutes	Contact Conditions:	Ambient
Host Cell Line:	CRFK (ATCC CCL-94)	Cell Passage Number:	p. 196
Assay Medium:	2% FBS EMEM	Soil Load:	None
Incubation Period	7 Days	Incubation Conditions:	37°C, 5% CO_2

Study Modifications

No further modifications were made to the method for this study.

Study Notes

Feline calicivirus was diluted from stock vials into phosphate buffered saline supplemented with 0.01% Triton-X 100 to facilitate spreading across the carrier surface.

Test substance was applied to carriers via 4 sprays from Study Sponsor provided spray bottles. The total volume dispensed was approximately 1 ml. A harvest volume of 2 ml of the appropriate neutralizer (detailed below) was used to re-capture virus prior to enumeration. Neutralizers were selected after analysis via a preliminary cytotoxicity screen. All chemical neutralizers were coupled with a secondary neutralization consisting of passage through a Sephacryl S-1000 gel filtration column.

Neutralization Method:

1.0.01 Test Substance:	Dey Engley Broth + 0.5M NaOH
1.5.01 Test Substance:	Dey Engley Broth
500 ppm Bleach Control:	Dey Engley Broth
Time Zero Control:	Dey Engley Broth

Study Photographs

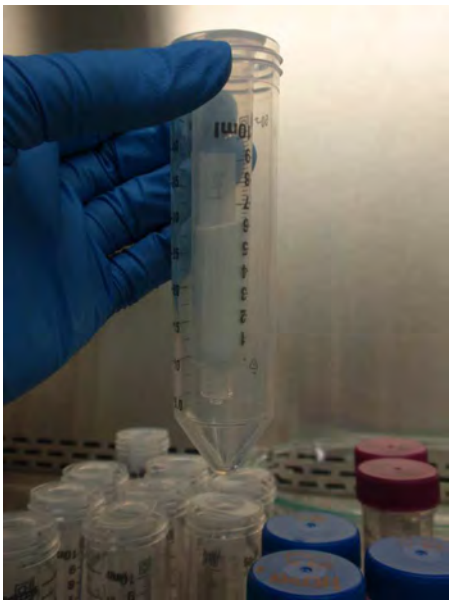


Photo 1. A standard length Sephacryl S-1000 gel filtration column prior to use.

Control Results

Virus Control Titer:	5.73 $\log_{10}$ per Carrier	Cytotoxicity Titer:	≤2.98 $\log_{10}$ per Carrier
Sterility Controls:	Validated	Neutralization Efficacy:	Validated

Calculations

Viral and cytotoxicity titers ($TCID_{50}$/$TCLD_{50}$ and $TCCD_{50}$, respectively) were determined according to the method developed my Spearman-Karber:

$$-\text{Log}_{10} \text{ of 1st Dilution} - \left(\frac{\text{sum of \% mortality at each dilution}}{100}\right) - 0.5$$

Percent Reduction of Virus is determined according to the following formula:

$$\text{Percent Reduction} = 1 - \left(\frac{C}{B}\right) * 100$$

Where:
B = Log_{10} of Virus Control Carrier
C = Log_{10} of Virus Test Carrier

Results of the Study

Table 1. Antiviral Efficacy of Two Tyrgrus Test Substances Applied via Spray Device against Feline Calicivirus, ATCC VR-782 (US EPA-Approved Human Norovirus Surrogate)

Test Microorganism	Contact Time	Test Substance	Log_{10} Infectious Units per Carrier	Percent Reduction Compared to Control at Time Zero	Log_{10} Reduction Compared to Control at Time Zero
Feline Calicivirus ATCC VR-782	Time Zero	Control	5.73	N/A	
	1 Minute	500 ppm Bleach	≤ 2.98	≥ 99.82%	≥ 2.75
		1.0.01	≤ 2.98	≥ 99.82%	≥ 2.75
		1.5.01	4.48	94.38%	1.25
	5 Minutes	500 ppm Bleach	≤ 2.98	≥ 99.82%	≥ 2.75
		1.0.01	≤ 2.98	≥ 99.82%	≥ 2.75
		1.5.01	6.23	No Reduction	
	10 Minutes	500 ppm Bleach	≤ 2.98	≥ 99.82%	≥ 2.75
		1.0.01	≤ 2.98	≥ 99.82%	≥ 2.75
		1.5.01	4.48	94.38%	1.25

*"≤" indicates a viral titer below the limit of detection for this assay.

The results of this study apply to the tested substances(s) only. Extrapolation of findings to related materials is the responsibility of the Sponsor.

GERMICIDAL ACTIVITY OF A NOVEL BIOCIDE AGAINST STAPHYLOCOCCUS AUREUS

The attached report contains data collected on Tydronium, an extreme acid manufactured by Tygrus. The experiments were performed by an independent testing organization, Antimicrobial Test Laboratories LLC, operating a certified facility in Round Rock, Texas. Antimicrobial Test Laboratories (ATL) provides microbiology services to clients that manufacture, supply, and market antimicrobial products. Founded by an infectious disease research scientist, Dr. Benjamin Tanner, with extensive experience in infectious disease transmission, ATL is a contract research organization that offers its customers documented, top-of-the-line laboratory services to determine product effectiveness and to support regulatory approval.

ATL conducted proof-of-concept testing on the Tydronium product to assess its ability to neutralize the growth of Staphylococcus aureus (S. aureus), a gram-positive bacterial pathogen responsible for many common skin and skin structure infections. A highly drug-resistant variant of S. aureus, commonly referred to as MRSA, poses a health threat to patients and healthcare professionals through contact with common touch points in the workplace. Indeed, concerns about cross contamination and spread of S. aureus extend to the community and to other institutional settings as well.

The testing conducted by ATL followed an industry standard protocol approved by the Association of Official Analytical Chemists (AOAC 961.02: Germicidal Spray Method) for assessing the activity of aqueous products to inactivate microorganisms deposited on hard surfaces. The test article listed in the attached report is referred to as “Triton TG”, a provisional product name that has since been replaced with “Tydronium”. Tydronium more appropriately reflects the product’s origin as the hydronium ion (H_3O^+), which is the defining particle of all acids. Using the proprietary manufacturing processes at Tygrus, the hydronium ion can now be produced in its stabilized form (H_9O_4) on a commercial scale for application in various markets. The ability of Tydronium to work as a disinfectant was assessed by ATL using the aforementioned method whereby the S. aureus organism was applied to a series of glass slides (“carriers”) and dried. The carriers containing the dried organism film were then sequentially treated with Tydronium and exposed for predetermined time periods (1, 3 and 5 minutes). After exposure, the carriers were transferred to a liquid growth medium (specifically selected to neutralize the test substance active and to recover any surviving test organisms), incubated and visually examined for the presence or absence of microbial growth.

As the lab report demonstrates, the results for Tydronium were exemplary: a one-minute exposure was sufficient to completely kill S. aureus on all carriers. This independent laboratory study illustrates the potential for use of Tydronium as a novel biocide in the commercial, healthcare and consumer marketplace.

Antimicrobial Test Laboratories

Fast, Reliable Antimicrobial Efficacy Testing

Germicidal Spray Test Study Report NG2666

Client Information

Company Name:	Triton TG	Sponsor:	Triton TG
Sponsor's Phone:	313-670-7450	E-mail:	info@tritontg.com

Test Information

Test(s) Performed:	3, 20 Carrier Germicidal Spray Tests (Study ID NG2666)		
GLP Compliance:	N/A, Non-GLP Study	Performed by:	J. Akins and L. Higgins

Sample Information

Sample(s) Received:	18-May-2011	Test Substance ID:	Stabilized Hydronium H_9O_4
Number of Samples:	1		

Parameters

Microorganism:	*S. aureus* ATCC 6538	Exposure Temp.	Room Temperature (23 ± 2°C)
Subculture Number:	13	Type of Carrier:	Sterile 18x36x1mm Glass Slides
Growth Medium:	AOAC Nutrient Broth	Incubation Temp.:	36.0 ± 1°C
Contact Time:	1 min, 3 min, 5 min	Incubation Time:	~48 hours
Neutralizer Used:	20ml Letheen Broth	Distance Sprayed:	Approximately 6 Inches at a 45° Angle
"Soil" Type:	None	Number of Sprays:	3
Humidity at Exposure:	Not Recorded (~20 - 60%)	Spray Interval:	20 seconds

Controls

Neutralization:	Passed	Growth Control:	Passed
Broth Sterility:	Passed	Agar Sterility:	Passed

Test Results

Confirmation:	No positive carriers recorded	Test(s) valid?:	Yes

Notes: Inoculated test carriers were sprayed from a distance of approximately 6 inches for a total of 3 sprays, followed by a contact time of 1min, 3 min, or 5min. Treated carriers were harvested into 20ml neutralization broth and incubated for approximately 48 hours.

Tests Completed: 21-May-2011 **Report Sent:** 23-May-2011

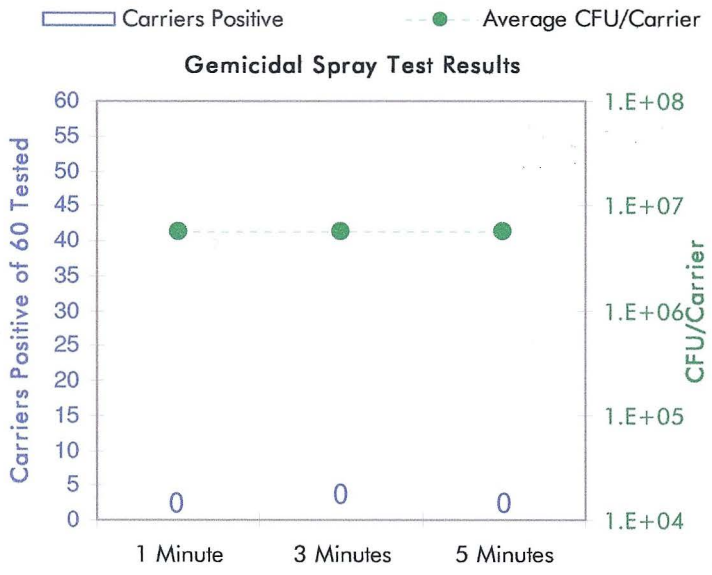
3000 Joe DiMaggio Blvd
Suite 32
Round Rock, Texas 78665

Phone: (512) 310-TEST
E-Mail: info@AntimicrobialTestLabs.com
Web site: http://www.AntimicrobialTestLabs.com

Germicidal Spray Test Study Report NG2666

Test Results for Germicidal Spray Test with Stabilized Hydronium

Microorganism	Test Substance	Contact Time	Average CFU/Carrier	Carriers Positive	Carriers Negative
S. aureus ATCC 6538	Stabilized Hydronium H_9O_4	1 Minute	5.63E+06	0	20
		3 Minutes	5.63E+06	0	20
		5 Minutes	5.63E+06	0	20



Neutralization Control Results		
Broth	Neutralization Inoculum (Average CFU)	Result
Nutrient Broth	32	Pass

3000 Joe DiMaggio Blvd
Suite 32
Round Rock, Texas 78665

Phone: (512) 310-TEST
E-Mail: info@AntimicrobialTestLabs.com
Web site: http://www.AntimicrobialTestLabs.com

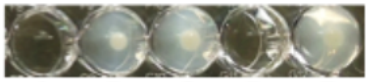
ACTIVITY TESTING OF TYDRONIUM AGAINST ESKAPE PATHOGENS

The spread of antibiotic resistant bacteria is recognized as one of the greatest threats to human health worldwide. The Infectious Disease Society of America has identified a group of bacterial pathogens as the major cause of drug-resistant infections in healthcare facilities. The mnemonic “ESKAPE” was developed to easily identify the organisms that comprise this critical group, namely Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas, and ESBL (Enterobacter and E.coli). Infected patients and healthcare workers can, through contact with common touch points in the workplace, increase the surface bioburden of these pathogens and promote further transmission. Nosocomial infections caused by the ESKAPE pathogens result in significant morbidity, mortality, and healthcare expense. Indeed, concerns about the cross contamination and spread of ESKAPE pathogens extend to the community and to other institutional settings as well.

In April 2014, Tydronium, a product of Tygrus, was tested for its ability to neutralize the growth of ESKAPE pathogens in the laboratory setting. Tydronium is non-corrosive (based on Occupational Safety & Health Administration (OSHA) definition), environmentally friendly, and safe for human touch. Although previous laboratory studies have demonstrated its antibacterial activity against select bacterial species, it is important to demonstrate the product’s effectiveness on contemporary, highly drug-resistant, clinical isolates belonging to the ESKAPE pathogen category. Using methods outlined by the Clinical Laboratory Standards Institute, three concentrations of Tydronium were tested for bacterial growth inhibition. The product was provided by the manufacturer at 3%, 10%, and 25% strength. Each concentration was applied to the culture wells of microtiter trays and representative clinical isolates of the ESKAPE pathogens (both drug-sensitive and drug-resistant) were added to the wells, resulting in final Tydronium concentrations of 2.5%, 1.0% and 0.3%. Each of the culture wells was monitored for bacterial viability to determine the minimum inhibitory concentration (MIC) that could successfully prevent microbial growth. The data in Figure 1 show growth inhibition of a multi-drug resistant Escherichia coli ESBL strain by the Tydronium product to occur at 2.5% strength. Data for Tydronium versus additional ESKAPE organisms (including industry standard quality control strains) are provided in Table 1, along with drug/antibiotic controls. Clinical isolates from other bacteria genera/species were also tested (including Haemophilus influenza, Stenotrophomonas maltophila, Citrobacter freundi, and Serratia marcescens) as part of a comprehensive activity survey of Gram positive and Gram negative organisms that cause clinically relevant infections.

Figure 1. Growth of *E. coli* isolate 1705878 is inhibited by Tvdronium™. Culture wells A-C contain 2.5%, 1.0%, and 0.3% Tvdronium™, respectively. Well D contains growth media only and Well E is the growth control showing bacterial pellet and turbidity with no added test compounds. The minimum inhibitory concentration (MIC) for Tvdronium™ is 2.5%.

Escherichia coli (ESBL)



MIC = 2.5%

A B C D E

Table 1. Activity of Tydronium™ against Bacterial Clinical Isolates. Potency of control drugs represented as minimum inhibitory concentration (MIC- µg/ml). Potency of Tydronium represented as minimum inhibitory concentration (MIC- %). QC- quality control strain; ESBL- extended spectrum β-lactamase; CRE- carbapenemase resistant enterobacter; VRE- vancomycin resistant enterococcus; MDR- multi-drug resistant (to at least 3 or more drugs typically prescribed for treatment).

Bacterial Strains	MIC (µg/ml)			MIC (%)
	Ciprofloxacin	Tigecycline	Tobramycin	Tydronium™
Escherichia coli ATCC25922 (QC)	0.008	0.125	0.5	2.5
Escherichia coli ATCC25922+50% Serum	0.008	0.25	1	2.5
Escherichia coli 1705863	0.016	0.25	1	2.5
Escherichia coli 1705878 (ESBL-MDR)	>128	0.25	64	2.5
Escherichia coli MG1655	0.016	0.5	1	1
Escherichia coli CAG12184	0.004	0.25	1	1
Pseudomonas aeruginosa ATCC27853 (QC)	0.5	8	0.5	1
Pseudomonas aeruginosa 1705886	0.125	8	0.5	1
Pseudomonas aeruginosa 1705904 (MDR)	>128	>32	>128	1
Pseudomonas aeruginosa PAO1	0.125	8	0.5	1
Pseudomonas aeruginosa PAO750	0.004	0.5	0.5	1
Acinetobacter baumannii 1705943	0.25	0.125	0.5	1
Acinetobacter baumannii 1705936 (MDR)	>128	2	8	1
Stenotrophomonas maltophilia 255074	1	0.5	16	1
Klebsiella pneumoniae 1705966	0.008	0.5	<=0.25	2.5
Klebsiella pneumoniae 1705949 (CRE-MDR)	128	0.5	>128	2.5
Citrobacter freundii 255041	0.06	4	32	1
Serratia marcescens 255067	0.016	1	4	2.5
Enterobacter cloacae 17059482	0.03	0.5	0.5	2.5
Staphylococcus aureus 11540 (MRSA-MDR)	128	0.125	1	2.5
Enterococcus faecalis 1069 (VRE-MDR)	1	0.125	>128	1
Enterococcus faecium A6349 (VRE-MDR)	128	0.125	>128	1
Haemophilus influenzae A1950	0.008	0.5	1	1

As an additional assessment of the product's activity, the quality control E. coli strain was tested for Tydronium activity in the presence of heat-inactivated human serum at 50% concentration. The antibacterial potency of Tydronium™ was unaffected by the presence of high serum content. This bacterial strain panel also contains isolates genetically engineered to be defective in common drug efflux systems. The two strains (CAG12184 and PAO750) showed no change in susceptibility to Tydronium compared with their efflux-proficient comparators of the same species. These data, coupled with the potent activity against all strains tested, suggests the action of Tydronium is not subject to common mechanisms of drug resistance.

These laboratory experiments clearly demonstrate the antibacterial activity of Tydronium against recalcitrant clinical isolates of ESKAPE pathogens and other disease-causing bacterial species of contemporary origin and phenotype.

TYDRONIUM REDUCES DISEASE SEVERITY IN TOMATO SEEDLINGS

The attached report contains data collected on Tydronium, an extreme acid manufactured by Tygrus that is safe, non-toxic, and non-corrosive. The experiments were performed in the laboratory of Dr. Pamela D. Roberts at the Institute of Food and Agricultural Sciences (IFAS), University of Florida. Dr. Roberts' research program is focused on plant diseases of agricultural importance and utilizes a 320-acre agricultural research site at the Southwest Florida Research and Education Center to provide data collection resources and a knowledge base for use by all levels of the agricultural industry.

Dr. Roberts and her staff conducted proof-of-concept testing on the Tydronium product to assess its ability to limit damage of tomato plants caused by the bacteria Xanthomonas perforans (see Figure 1). Bacterial spot is a significant disease of both the tomato and pepper where multiple species of xanthomonads can infect and damage seedlings, plants, and fruit. Conditions that favor disease progression are high moisture, high relative humidity (≥ 80%), and warm temperatures (75-90o F). Once detected, field crops are typically destroyed, as there is no curative treatment available. Early and frequent application of copper fungicides and other products can slow the development of bacterial spot during the growing season, but increased environmental concerns and the development of resistance to such commercial pesticides makes management of bacterial spot an ongoing challenge. The economic impact of the disease can be significant, as crop and productivity losses for 2013 were estimated to be greater than $3,000 per acre within the state of Florida alone.

Figure 1. Bacterial Spot Lesions on Fruit and Foliage of the Tomato Plant

The testing conducted by Dr. Roberts followed guidelines established by the University of Florida/ IFAS for experimental design, land preparation, fertility, irrigation, weed management, and insect control. Treatments of tomato seedlings were arranged in a randomized design with four replicates, each plot consisting of 15 plants spaced 18 inches apart within a 21-foot row with 10 feet between each plot and 6 feet between each row. On this test occasion, 22 different product formulations

(commercial and experimental) were applied to seedlings with a high clearance sprayer designed specifically for applications in staked tomato plots. All products were delivered once per week at spray volumes of 60 gallons per acre and at usage rates following manufacturer specifications or as directed by individual study participants. For this test, Tydronium was used at 3 ounces/gallon (approximately 1.5 gallons/acre). Lastly, a suspension of Xanthomonas perforans was used to inoculate plants on two separate occasions at two-week intervals following the third and fifth week of product application. Bacterial spot disease ratings were taken at 7-day intervals and disease severity was assessed over time by visually estimating the percentage of foliage with symptoms of bacterial spot. The measurements of disease severity over the 10-week period were plotted, analyzed, and the area under the disease progress curve (AUDPC) was calculated and used to compare the effects of tested products to the untreated control plants. A less rigorous experimental design for recording and analyzing adjunct damage from a typical fungal disease (e.g. Alternaria early blight) was also performed.

Table 1. Protective Activity of Tydronium against Bacterial Spot Disease in Tomatoes

Test Substance	Type	AUDPC	% Reduction
Control	N/A	238.8	-
Tygrus Tydronium	Novel Single Agent	117.4	50.8%
DuPont Kocide3000/Manzate Prostik	Copper/Fungicide Combo	130.6	45.3%
Albaugh NuCop/Manzate Prostik	Copper/Fungicide Combo	136.6	42.8%
Novozymes Actinovate AG	Biological fungicide	188.4	21.1%
Phyton 27 AG/Manzate Prostik	Copper/Fungicide Combo	232.5	2.6%

The results for Tydronium are extremely promising. Among the various commercial products tested, Tydronium demonstrated the lowest level of disease progression (lowest AUDPC score) and the highest percent of disease reduction (see Table 1 below for exemplar data). The study director conducted a statistical analysis of the recorded observations for bacterial spot disease, and while the data could be more robust, there is a high probability (greater than 92%) that the differences observed among tested products are statistically significant. The studies for typical fungal disease progression were less statistically significant, but Tydronium is among the top five performers in this data set as well. Lastly, no products used in this test occasion were damaging to seedling health or appearance. This study demonstrates proof of concept for using Tydronium to control disease progression in plants. As resistance to pesticides (like copper and other reactive anti-fungal chemicals) increases and environmental regulations become more restrictive, the availability of new, effective, and safer products like Tydronium should be well received by the agricultural industry.

TOMATO (*Lycopersicon esculentum* 'FL 47')
Bacterial spot; *Xanthomonas perforans*

P. D. Roberts and R.E. Sytsma
Department of Plant Pathology
University of Florida/IFAS
SWFREC
Immokalee, FL 34142

Evaluation of compounds for management of bacterial spot in tomato, fall 2011.

Tomato seedlings 'FL47' were transplanted on 12 Sep into Immokalee fine sand at the Southwest Florida Research and Education Center, Immokalee, FL. Treatments were arranged in a randomized design with four replications. Each plot consisted of 15 plants spaced 18 in. apart within a 21 ft row with 10 ft between each plot and 6 ft between each row. Guidelines established by the University of Florida/IFAS were followed for land preparation, fertility, irrigation, weed management and insect control. Sprays were applied with a high clearance sprayer designed specifically for applications in staked tomato plots at 2 mph and at 200 psi. A double drop boom equipped with six nozzles delivered a spray volume of 60 gal/A. A suspension of *Xanthomonas perforans* was used to inoculated plants on 21 Oct and 4 Nov. Disease ratings were taken at 7 day intervals but disease symptoms developed slowly and only ratings with at least a 5% disease severity for the control were included in the report. Disease severity was assessed by visually estimating the percentage of foliage with symptoms of bacterial spot. On the final rating dating, worm damage to plants was severe on the north end of the field and the last rep was removed from analysis. Additionally, damage from fungal diseases (Alternaria early blight) was assessed as 'other' in Table 1. Area under the disease progress curve (AUDPC) was calculated from the severity ratings. Disease severity and AUDPC was analyzed by one-way ANOVA and significant differences between means were separated using LSD. Average monthly high and low temperatures (°F) were 91 and 72 in Sep, 83 and 65 in Oct, and 81 and 60 in Nov. Rainfall totaled 4.0, 8.4, and 0.13 for the same months, respectively.

Treatment 2 and 11 are the same because a water monitor for another purpose was placed in the bed for treatment 11 and irrigation may have been different between these two treatments.

Differences among treatments were difficult to detect and although numbers are presented in the table, the P value is also presented, and most ratings are not significant or highly significant. Some plants seemed to have a decreased disease severity value between the last rating dates (treatments 6, 14, and 17) however was probably due to segregation of the ratings into symptoms of foliar diseases that were more easily distinguished from symptoms of bacterial spot at the end of the season. More repetitions or a more conducive environment might have helped lessen ambiguity. In general, suppression of bacterial diseases by approximately 40% or more compared to untreated control plants, can be considered very good in my opinion. In addition to copper and mancozeb, several treatments look promising in bacterial spot suppression.

Table 1. Treatments, application dates and disease severity ratings of bacterial symptoms on tomato in fall 2011.

			Disease severity[y]			Other[x]
	Treatment (rate/A)	Application dates[z]	22 Nov	5 Dec	8 Dec	8 Dec
1	Untreated		11.1 [w]	16.1 ab	33.3 a	11.7
2	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10	6.5	10.8 bcdef	21.7 abcd	6.0
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10				
3	Manzate ProStik 75 DF 2 lb/A 100 gal water	1,2,3,4,5,6,7,8,9,10	10.6	12.5 abcdef	20.1 bcd	6.0
	Biorend Cu 150 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10				
4	Fosfirend 500 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10	9.9	14.3 abcde	16.7 bcde	4.7
	Biorend Cu 150 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10				
5	Manzate ProStik 75 DF 2 lb/A 100 gal water	1,2,3,4,5,6,7,8,9,10	10.9	13.9 abcde	20.0 bcd	3.7
	Biorend Cu 150 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10				
	Fosfirend 500 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10				
6	NuCop 50 HB 1 lb/A	1,2,3,4,5,6,7,8,9,10	9.5	10.4 bcdef	7.0 e	2.3
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10				
7	NuCop 50 HB 1.5 lb/A	1,2,3,4,5,6,7,8,9,10	8.6	10.1 bcdef	11.0 de	3.0
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10				
8	NuCop 20 HB 2 lb/A	1,2,3,4,5,6,7,8,9,10	6.8	9.3 def	16.7 bcde	5.3
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10				
9	Product X fl oz/A	1,2,3,4,5,6,7,8,9,10	9.5	15.0 abcde	28.3 ab	9.3
10	Product X fl oz/A	1,2,3,4,5,6,7,8,9,10	9.0	10.6 bcdef	16.7 bcde	5.0
	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10				
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10				
11	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10	7.1	9.1 ef	10.0 de	3.0 e
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10				
12	Product X fl oz/A	1,3,5,7,9	10.9	13.7 abcde	14.3 cde	6.0
	Kocide 3000 DF 1.75 lb/A	2,4,6,8,10				
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10				
13	Actinovate AG 3 oz/A	1,2,3,4,5,6,7,8,9,10	10.1	15.4 abc	21.7 abcd	6.0
	Actigard 50 WG 0.33 oz/A	1,2,3,4,5,6,7,8				
14	Actinovate AG 3 oz/A	1,2,3,4,5,6,7,8,9,10	10.1	12.1 abcdef	11.7 cde	8.3
	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10				
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10				
15	Actinovate AG 3 oz/A	1,2,3,4,5,6,7,8,9,10	12.5	14.6 abcde	18.3 bcde	7.0
	Kocide 3000 DF 0.75 lb/A	1,2,3,4,5,6,7,8,9,10				
16	Actinovate AG 6 oz/A	1,2,3,4,5,6,7,8,9,10	10.5	13.4 abcde	11.7 cde	7.0
17	MBI-10605 0.5 v/v	1,2,3,4,5,6,7,8,9,10	12.3	13.3 abcdef	6.7 e	10.0
	Nordox 75 WG 1.25 lb/A	1,2,3,4,5,6,7,8,9,10				
18	MBI-10605 0.5 v/v	1,2,3,4,5,6,7,8,9,10	7.6	12.8 abcdef	20.0 bcd	6.0
	Penncozeb 75 DF 1 lb/A	1,2,3,4,5,6,7,8,9,10				
19	MBI-10605 0.5 v/v	1,2,3,4,5,6,7,8,9,10	8.5	10.0 cdef	10.0 de	5.0
	Penncozeb 75 DF 1 lb/A	1,2,3,4,5,6,7,8,9,10				

	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10				
20	BL-134142-SFXC 3 oz/gal	1,2,3,4,5,6,7,8,9,10	7.0	7.3 f	12.3 cde	4.3
21	Phyton 27 AG 40 oz/100 Gal	1,2,3,4,5,6,7,8,9,10	13.3	15.3 abcd	21.7 abcd	3.7
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10				
22	Serenade Max WP 1 lb/A	1,2,3,4,5,6,7,8,9,10	9.4	11.5 bcdef	11.7 cde	5.0
	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10				
23	Phosmax 1 pt /A	1,2,3,4,5,6,7,8,9,10	10.3	15.9 abc	28.3 ab	5.3
	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10				
24	Phosmax 1 pt /A	1,2,3,4,5,6,7,8,9,10	13.0	17.8 a	23.3 abc	6.7
	Serenade Max WP 1 lb/A	1,2,3,4,5,6,7,8,9,10				
		***P*=**	0.2320	0.0919	0.0014	0.1030

[z] Application dates 1=4 Oct; 2= 11 Oct; 3= 18 Oct; 4= 25 Oct; 5= 1 Nov; 6= 8 Nov; 7= 15 Nov; 8= 23 Nov; 9 =30 Nov; 10 = 6 Dec

[y] Disease severity of bacterial spot as percentage of symptomatic foliage

[x] Disease severity of fungal symptoms (typically early blight caused by *Alternaria*)

[w] Numbers followed by the same letter or by no letters are not significantly different at *P* value in table by LSD.

Table 2. Area under the disease progress curve for bacterial spot.

	Treatment (rate/A)	Application dates[z]	AUDPC
1	Untreated		238.8 ab
2	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10	152.6 cdef
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10	
3	Manzate ProStik 75 DF 2 lb/A 100 gal water	1,2,3,4,5,6,7,8,9,10	192.3 abcdef
	Biorend Cu 150 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10	
4	Fosfirend 500 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10	196.9 abcdef
	Biorend Cu 150 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10	
5	Manzate ProStik 75 DF 2 lb/A 100 gal water	1,2,3,4,5,6,7,8,9,10	204.2 abcde
	Biorend Cu 150 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10	
	Fosfirend 500 cc per 100 liters water	1,2,3,4,5,6,7,8,9,10	
6	NuCop 50 HB 1 lb/A	1,2,3,4,5,6,7,8,9,10	152.6 cdef
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10	
7	NuCop 50 HB 1.5 lb/A	1,2,3,4,5,6,7,8,9,10	149.4 def
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10	
8	NuCop 20 HB 2 lb/A	1,2,3,4,5,6,7,8,9,10	136.6 def
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10	
9	Product X fl oz/A	1,2,3,4,5,6,7,8,9,10	213.6 abcd
10	Product X fl oz/A	1,2,3,4,5,6,7,8,9,10	162.3 bcdef
	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10	
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10	
11	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10	130.6 ef
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10	
12	Product X fl oz/A	1,3,5,7,9	201.8 abcde
	Kocide 3000 DF 1.75 lb/A	2,4,6,8,10	
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10	
13	Actinovate AG 3 oz/A	1,2,3,4,5,6,7,8,9,10	213.2 abcd
	Actigard 50 WG 0.33 oz/A	1,2,3,4,5,6,7,8	
14	Actinovate AG 3 oz/A	1,2,3,4,5,6,7,8,9,10	171.9 abcdef
	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10	
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10	
15	Actinovate AG 3 oz/A	1,2,3,4,5,6,7,8,9,10	218.9 abcd
	Kocide 3000 DF 0.75 lb/A	1,2,3,4,5,6,7,8,9,10	
16	Actinovate AG 6 oz/A	1,2,3,4,5,6,7,8,9,10	188.4 abcdef
17	MBI-10605 0.5 v/v	1,2,3,4,5,6,7,8,9,10	193.1 abcdef
	Nordox 75 WG 1.25 lb/A	1,2,3,4,5,6,7,8,9,10	
18	MBI-10605 0.5 v/v	1,2,3,4,5,6,7,8,9,10	174.1 abcdef
	Penncozeb 75 DF 1 lb/A	1,2,3,4,5,6,7,8,9,10	
19	MBI-10605 0.5 v/v	1,2,3,4,5,6,7,8,9,10	146.5 def
	Penncozeb 75 DF 1 lb/A	1,2,3,4,5,6,7,8,9,10	

	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10	
20	BL-134142-SFXC 3 oz/gal water	1,2,3,4,5,6,7,8,9,10	117.4 f
21	Phyton 27 AG 40 oz/100 Gal	1,2,3,4,5,6,7,8,9,10	232.5 abc
	Manzate ProStik 75 DF 2 lb/A	1,2,3,4,5,6,7,8,9,10	
22	Serenade Max WP 1 lb/A	1,2,3,4,5,6,7,8,9,10	166.1 bcdef
	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10	
23	Phosmax 1 pt /A	1,2,3,4,5,6,7,8,9,10	217.5 abcd
	Kocide 3000 DF 1.75 lb/A	1,2,3,4,5,6,7,8,9,10	
24	Phosmax 1 pt /A	1,2,3,4,5,6,7,8,9,10	252.8 a
	Serenade Max WP 1 lb/A	1,2,3,4,5,6,7,8,9,10	
		***P*=**	0.0842

[z] Application dates 1=4 Oct; 2= 11 Oct; 3= 18 Oct; 4= 25 Oct; 5= 1 Nov; 6= 8 Nov; 7= 15 Nov; 8= 23 Nov; 9 =30 Nov; 10 = 6 Dec

[y] Disease severity of bacterial spot as percentage of symptomatic foliage

[x] Disease severity of fungal symptoms (typically early blight caused by *Alternaria*)

[w] Numbers followed by the same letter or by no letters are not significantly different at *P* value in table by LSD.

ANIMAL MODEL DEMONSTRATES TYDRONIUM IS SAFE FOR SKIN CONTACT

The attached report contains data collected on Tydronium, an extreme acid manufactured by Tygrus. The testing was performed by Eurofins Laboratories (with locations across the US) which is now part of Eurofins Scientific, an international group of laboratories headquartered in Luxembourg that provides certified testing and support services to the pharmaceutical, food, environmental, and consumer products industries and to government organizations. Eurofins Scientific is a multinational contract research organization that offers its customers documented, top-of-the-line laboratory services to determine product effectiveness, safety and to support regulatory approval.

Eurofins Laboratories conducted safety testing on Tydronium using an animal model to study the irritation and corrosiveness of the product when applied topically to the skin. Given the acidic nature of this new product, it is important to conclusively demonstrate that Tydronium behaves differently than traditional acids in regards to it's skin corrosion properties and overall safety profile. The test article listed in the attached report is referred to as "Stabilized Hydronium a.k.a ARS", a provisional product name given by the sponsor at that time (Advanced Mutakinetics Inc., which is one of the precursor companies that led to formation of Tygrus). The name "Tydronium" more appropriately reflects the product's origin as the hydronium ion (H_3O^+), which is the defining particle of all acids. Using the proprietary manufacturing processes at Tygrus, the hydronium ion can now be produced in its stabilized form (H_9O_4) on a commercial scale for application in various markets.

Albino rabbits were used by Eurofins to assess the skin corrosiveness of Tydronium. Rabbit models for skin irritation/corrosion studies are widely known to be ultrasensitive to substances only moderately or minimally irritating to man. Therefore, testing of Tydronium in the rabbit seemed appropriate to demonstrate a wide margin of safety for prediction of skin irritation/corrosion in humans. The traditional dermal patch protocol was used to assess the skin irritation caused by 500 microliters of concentrated Tydronium placed on multiple sites of shaved, intact rabbit skin. Three female rabbits were included in each of three groups tested at 3 minutes, 1 hour, and 4 hours exposure. The study showed no signs of skin corrosion in any test animal, with only minor skin irritation and no edema (swelling)

after 1 and 4 hours of constant exposure. This barely perceptible erythema self-cleared from the 1-hour exposure sites within 24 hours and all animals were totally free of any dermal irritation after seven days.

As the results from this pivotal study demonstrate, concentrated Tydronium is considered non-corrosive to the skin. The results help distinguish this product from traditional acids that are highly corrosive and toxic in their concentrated forms. Clearly, Tydronium has the potential to be used safely and effectively across many technology sectors where extreme acids are required for diverse industrial applications.

eurofins | PSL

DOT SKIN CORROSION STUDY IN RABBITS

TEST METHOD NO.:	P214
STUDY NUMBER:	29553
SPONSOR:	ADVANCED MUTAKINETICS, INC. 4505 Greenstone Road Placerville, CA 95667
TEST SUBSTANCE IDENTIFICATION:	Stabilized Hydronium a.k.a ARS Batch #32210
TEST SUBSTANCE DESCRIPTION:	Colorless Liquid
DATE RECEIVED:	March 31, 2010
EPSL REFERENCE NO.:	100331-19D
DATES OF TEST:	April 6-13, 2010

1. PURPOSE

To determine the corrosive effect of a 3 minute, 1 hour and 4 hour exposure of Stabilized Hydronium a.k.a ARS by the dermal route on the intact skin.

2. PROCEDURE

A group of New Zealand albino rabbits was received from Robinson Services, Inc., Clemmons, NC. The animals were singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cages and was changed at least three times per week. The animal room was temperature controlled and had a 12-hour light/dark cycle. The animals were fed Purina Rabbit Chow #5326 and filtered tap water was supplied *ad-libitum* by automatic watering system.

Following acclimation to the laboratory, a group of animals was prepared by clipping the dorsal area of each animal's trunk free of hair. Three healthy female rabbits without pre-existing dermal irritation were selected for test. Three test sites, each approximately 6 cm^2, were delineated on each animal.

Five-tenths of a milliliter of the test substance was applied to each of the dose sites on each animal and covered with a 1-inch x 1-inch, 4-ply gauze pad. The torso of each animal was wrapped with 3 inch Micropore tape to avoid dislocation of the pads. Elizabethan collars were placed on each rabbit and they were returned to their designated cages. The Elizabethan collars were removed after 4 hours.

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The patches were removed at the appropriate intervals (3 minutes, 1 hour, and 4 hours). The sites were then gently cleansed with a 3 % soap solution followed by tap water and a clean towel to remove any residual test substance. All test sites were evaluated for corrosion for 30-60 minutes after patch removal. Subsequent evaluations were performed approximately 24, 48, and 72 hours and at Day 7 after removal of the 4-hour patch. Corrosion was considered to have resulted if the test substance caused full-thickness necrosis (or ulceration) at the test site in at least one test animal. Full-thickness necrosis is defined as moderate to severe tissue destruction with well-defined dark brown or black discoloration and/or stiffened texture, covering a substantial area. Epidermal sloughing, erythema, edema or fissuring were not considered tissue destruction.

The test sites were also evaluated for skin irritation according to the Draize[1] scoring system (See Table 3) at the same intervals mentioned above. All animals were euthanized by means of a Fatal-Plus® injection at study termination.

3. RESULTS AND CONCLUSION

Individual skin corrosion scores are presented in Table 1. Primary skin irritation scores are presented in Table 2. Primary skin irritation scoring system is presented in Table 3.

No sign of dermal corrosion was noted at any of the treated sites. Based on these results, Stabilized Hydronium a.k.a ARS is considered non-corrosive to the skin when applied as received.

3-Minute Exposure Site

There was no dermal irritation observed at any treated site during this study.

1-Hour Exposure Site

There was no edema observed at any treated sites during this study. One hour after the removal of the one-hour patch, very slight erythema was observed at two treated sites. Irritation cleared from these sites by 24 hours.

4-Hour Exposure Site

There was no edema observed at any treated sites during this study. One hour after the removal of the four-hour patch, very slight erythema was observed at all three treated sites. The overall incidence and severity decreased with time. All animals were free of dermal irritation by Day 7.

---

[1] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

SIGNATURES

Stabilized Hydronium a.k.a ARS

I, the undersigned, declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

Anselmo Villagran

Anselmo Villagran, B.S.
Study Director
Product Safety Laboratories

4/20/10

Date

TABLE 1: INDIVIDUAL SKIN CORROSION SCORES[1]

3-MINUTE EXPOSURE

Animal No.	Sex	1 Hour After Patch Removal	Hours After Removal of Last Patch			Days
			24	48	72	7
3501	F	N	N	N	N	N
3502	F	N	N	N	N	N
3503	F	N	N	N	N	N

1-HOUR EXPOSURE

Animal No.	Sex	1 Hour After Patch Removal	Hours After Removal of Last Patch			Days
			24	48	72	7
3501	F	N	N	N	N	N
3502	F	N	N	N	N	N
3503	F	N	N	N	N	N

4-HOUR EXPOSURE

Animal No.	Sex	Hours After Removal of Patch				Days
		1	24	48	72	7
3501	F	N	N	N	N	N
3502	F	N	N	N	N	N
3503	F	N	N	N	N	N

N = Negative P = Positive

[1] Corrosion - defined as full-thickness necrosis of the dose site.

TABLE 2: PRIMARY SKIN IRRITATION SCORES

ERYTHEMA/EDEMA

3-MINUTE EXPOSURE

Animal No.	Sex	1 Hour After Patch Removal	Hours After Removal of Last Patch			Days
			24	48	72	7
3501	F	0/0	0/0	0/0	0/0	0/0
3502	F	0/0	0/0	0/0	0/0	0/0
3503	F	0/0	0/0	0/0	0/0	0/0
Total		0/0	0/0	0/0	0/0	0/0
Mean		0/0	0/0	0/0	0/0	0/0

1-HOUR EXPOSURE

Animal No.	Sex	1 Hour After Patch Removal	Hours After Removal of Last Patch			Days
			24	48	72	7
3501	F	1/0	0/0	0/0	0/0	0/0
3502	F	1/0	0/0	0/0	0/0	0/0
3503	F	0/0	0/0	0/0	0/0	0/0
Total		2/0	0/0	0/0	0/0	0/0
Mean		0.7/0	0/0	0/0	0/0	0/0

4-HOUR EXPOSURE

Animal No.	Sex	1 Hour After Patch Removal	Hours After Removal of Last Patch			Days
			24	48	72	7
3501	F	1/0	1/0	1/0	1/0	0/0
3502	F	1/0	1/0	1/0	1/0	0/0
3503	F	1/0	1/0	0/0	0/0	0/0
Total		3/0	3/0	2/0	2/0	0/0
Mean		1.0/0	1.0/0	0.7/0	0.7/0	0/0

TABLE 3: PRIMARY SKIN IRRITATION SCORING SYSTEM

Evaluation of Skin Reactions	Value
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 millimeter)	3
Severe edema (raised more than 1 millimeter and extending beyond the area of exposure)	4

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Technical Process Bulletin

No. TD-0001 | This Revision: July 29, 2015 (Initial)

TD-0001 – Liquid Concentrated, Non-hazardous, Non-Corrosive Stabilized Acidic Hydronium Solvent and Electrolyte

1. Introduction

TD-0001 is a patent pending new technical development for use in replacement or partial replacement of conventional, hazardous acidic electrolytes such as hydrochloric, sulfuric, nitric, etc. Unique features and benefits include solubilizing effectivity, non-corrosivity, non-malodorous and non-fuming. It significantly minimizes worker exposure and environmental liability and promotes worker-friendliness. It does not produce severe exothermicity when blended in aqueous mediums like incumbent chemistries. The product does not generate noxious mists and fumes requiring air scrubbing at elevated temperatures. The product exhibits excellent stability and yields no adverse reaction by-products.

2. Operating Summary

CHEMICAL	BATH PREPARATION PER 100 GALLONS
TD-0001	10 % to 100% v./v. (Neat)

OPERATION AND CONTROL	
Concentration* (points)	10.0% to 100% v./v. (Neat)
Time* (seconds)	30-300
pH*	0.00 to 1.50 (1.00 avg.)
Temperature* (°Fahrenheit)	35°F to 150°F

*The actual control ranges used are application specific and will be established through application testing.

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3. The Process
The complete process sequence normally consists of the following steps:

The latitude and applicability of this technology is wide and varied; from permeation applications in geothermal and hydro fracturing wells to surface engineering solutions to specialty applications in institutional, consumer and water remediation. Each user and tester will decide on their own optimal parameters that comply with requirements and meet expectations. Tygrus LLC technical personnel will work with each case study to develop and refine the application.

4. Materials
TD-0001 Testing Reagents and Apparatus - (pH) Use calibrated and NIST traceable pH probes

5. Equipment
Process tank, housing, pumps and piping should be fabricated from 316L or 304L stainless steel or polypropylene. The 316L is preferred for maximum tank life. A secondary choice is 316 stainless steel fabricated with approved welding techniques. CPVC plastic, HDPE, PTFE or HDPP may be used.

Should heating be required or desired, heat exchanger plates or other heating devices should be polished 316L stainless steel. All process circulation pump seals, valve seats, door seals, etc., which come into contact with the process solution and occasional acid equipment cleaners, should be EPDM, Viton or Teflon.

Chemical feed pump parts and other elastomers, which may come into contact with the replenishing chemical, should be EPDM, Viton or Teflon.

All equipment, which will be in contact with TD-0001 or processing solution, should be thoroughly cleaned prior to use with the process. This includes such items as chemical metering pumps, solution tank, spray nozzles, spray zone shields and housings. Our representative can supply a recommended clean-out procedure, which may be followed.

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6. Surface Preparation

Cleaning

Pretreatment, pre-cleaning and/or pre-purging may be needed. A procedure, if required is available and our representative will recommend the proper one for each installation.

Water Rinsing

The rinse, if required, should be overflowed continuously at a rate, which will keep it clean and free from particulates, scum and contamination. D.I. or R.O. final rinsing may be recommended in conjunction with the particular application.

7. Treating with the Tygrus (TD-0001) Processing Solution

Buildup

Recommended buildup is 10 to 100 gallons TD-0001 per 100 gallons of processing solution volume. Specific concentrations are application specific.

Fill the tank about three-fourths full with D.I. water. Add the proper amount of TD-0001 and then add sufficient water to bring the solution up to the working level. Mix thoroughly and adjust to the desired operating temperature.

Operation

Time: 30-300 seconds (Resonance or exposure time)
Temperature: 35°F to 150°F

The solution concentration may be increased or reduced to meet specific line conditions. Our representative will assist in establishing the proper concentration.

Replenishment

TD-0001 will be used for replenishment, depending on the surface area of metal and type of work processed.

8. Testing and Control

pH- -0.00 to 1.50 (1.00 +/- 0.25)

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pH Adjustment
TD-0001 pH Adjuster Toner may be added to raise and stabilize pH value specified range of effectivity.

9. After Treatment
Post Treatment
The surfaces of the substrates treated with TD-0001 may require additional post treatment processes to flush, neutralize, purge and /or seal the contact surfaces. Tygrus LLC technical personnel will advise the appropriate technique and procedure.

10. Storage Requirements
TD-0001 should be stored between 35° and 100° Fahrenheit. If exposed to temperatures outside that range for short periods, the product should be immediately returned to the proper temperature and stirred.

11. Waste Disposal Information
Applicable regulations covering disposal and discharge of chemicals should be consulted and followed.

Disposal information for TD-0001 is given on the Safety Data Sheet.

The processing bath is at pH -0.00 to 1.50. Waste treatment and neutralization may be required prior to discharge.

12. Precautionary Information
When handling the chemical product used in this process, the first aid and handling recommendations on the Safety Data Sheet (SDS) for the product should be read, understood, and followed.

The processing solution is acidic yet is not irritating to skin with little or no eye irritation expected. Avoid contact with skin and eyes. In case of contact follow the recommendations for contact given on the Safety Data Sheet (SDS) for TD-0001.

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Testing Reagents and Apparatus

1. Calibrated pH meter
2. Calibrated pH probe
3. NIST AG pH buffers 1.0, 4.0

Tygrus Indemnification and Disclaimer Clause

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