To Lisa Brines. Ph.D

Agricultural Marketing Specialist

National Organic Program

email: lisa.brines@ams.usda.gov

Hello Lisa.

Please find the revised version according to your letter dated September 27,2010 for the NOP petition to add.

If I have missed something please let me know. I will resend in PDF, the files in table of contents again. I will also send two (2) new updates for your review. One is from EcoCert and the other is from HSE, UK. I will give an explanation of the role of HSE, UK as it relates to EU registration and our status in the EU. Each member state must report to the EU certification body. In the UK it is the HSE that reports and is responsible for compliance.

Regards Tim McCarley

Ahava International Health. 19692 Black Fox Dr. Cottonwood Ca. 96022. E mail tim@ahavainthealth.com Ph. 530-347-0347-
Citrox Ltd UK. www.Citrox.net Ph 011-44-164-224-1777

PETITION TO ADD

Bergamot bitter Orange Powder.

Bergamot Immature oranges. Latin name Citrus aurantium.

TO THE NATIONAL LIST

19692 Black Fox Dr. Cottonwood Ca. 96022

Tele: 530-347-0347

E mail: tim@ahavainthealth.com

Web. www.ahavainthealth.com

Citrox Ltd.

Unit 9 River Court, Brighouse Road, Riverside Park, Middlesbrough TS2 1RT.

Tel. 011-44- 164-224-1777

Web. www.citrox.net

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4. **ATTACHMENT D:** TOXICOLOGY STUDIES SAFETY ETC see *"Evaluation of the aspects of hesperidin, naringin, and other citrus bioflavonoid extracts as food ingredients." 1982 PB82-192931. Prepared for Bureau of Foods. Food and Drug Administration. Department of Health and Human Services. Washington DC. Contract No. FDA 223-78-2100*
5. **ATTACHMENT E:** EFFACY STUDIES GLP AND NON GLP LABRATORIES, FIELD TRIALS PRE AND POST HARVEST APPLICATIONS GLOBALLY.
6. **ATTACHMENT F:** Fish and Sea Food Industry with Citrox products

Petition request to NOP Opening statement.

Petitioned Substance: **Bergamot bitter Orange Powder**. Bergamot oranges. Latin name Citrus aurantium is classified as a food substance by the FDA.

1. Approval for addition to the NOP list for the **Bergamot bitter Orange Powder** which is an ingredient in the following formulas***. Citrox BC concentrate, Citrox Sanitizer 14T, Citrox Detergent 14X, Citrox Processing Aid 14W***. See formulas. All formulas contain the **Bergamot bitter Orange Powder** which is our petitioned substance.

The listed formulas are all based off of Citric Acid and the **Bergamot bitter Orange Powder**.

a. Bioflavonoids derivied from **Bergamot bitter Orange Powder** are classified as a food substance by the FDA. **See Attachment A7**
b. Our request is based on our efficacy data and registration we have achieved globally. There are similar products that are currently registered by the EPA and FSIS that contain the same or similar ingredients in different concentrations and with different active ingredients. **See Attachment B. International Certifications.**
c. Our patent pending formulations are a natural solution to reduce microbial pathogens for food safety.
d. Our patent pending formulation is derived from natural resources and is a non toxic solution to reduce microbial pathogens in food processing facilities which enhances food safety.

e. The **Bergamot bitter Orange Powder** is composed of the flavonoids neohesperdine and naringin. **See A7**

It has been established through the life sciences research office federation of American societies that we consume more bioflavonoids in our daily diet than what is used in our formulations (Ref: Toxicology data) **See Attachment D5**

The two components used to create these formulas are Citric acid and the **Bergamot bitter Orange Powder**.

2. The **Bergamot bitter Orange Powder** is a food ingredient as reviewed and approved by the FDA.

The following quotes are taken from; ***"Evaluation of the aspects of hesperidin, naringin, and other citrus bioflavonoid extracts as food ingredients." 1982 PB82-192931. Prepared for Bureau of Foods. Food and Drug Administration. Department of Health and Human Services. Washington DC. Contract No. FDA 223-78-2100***

Pg.1 " As indicated in the Food, Drug and Cosmetic act 21 USC 321(s), GRAS substances are exempt from the premarketing clearance that is required for food additives.

Pg.1&2. The select committee on GRAS substances of LSRO reviewed and evaluated the available information on hesperdine, naringin and citrus bioflavonoids extracts.

Pg.3. Flavonoids comprise a group of naturally occurring compounds which are among the most ubiquitous in the plant kingdom. They are found in every family and in nearly every species of the higher plants.

Pg.5. Kuhnau (1976) stated that flavonoids are the most common and active antioxidants in our food supply.

Pg.5. Naringin has been accorded GRAS status as a flavoring agent in food (21 CFR 182.20. Office of the Federal Register, 1981) and hesperidin has been given similar status for enhancing and preserving flavor at a level of 30 ppm in flavored milk (Buckely, 1968.)

***Toxicological Studies conducted**.*

Acute Toxicity-" None of the flavonoids administered to experimental animals in a single doses orally, intraperitoneally, or intravenously when possible, produced signs of acute toxicity."

***Short term-** " No significant morphological changes were detected."*

***Long term- 400 days**. " No significant histological changes were reported in the liver"*

Gumbmann et al 1978 fed neohesperidin dihydrochalcone to rats and dogs for more two years with no apparent carcinogenic or Teratogenic effects.

Reproduction- *No adverse effects were noted.*

Carcinogenicity*- No significant differences.*

Teratogenicity- *No effects were reported in reproductive studies.*

Pgs. 15-21

Pg.22 Opinion*.*

"There is no evidence in the available information on hesperidin (purified or hesperdin complex) or naringin that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future."

•The present formulas relates to compositions and their uses in eradication or ameliorating unwanted infestations. Such compositions are aptly employed to reduce the presence of unwanted bacteria or undesirable organisms.

•A variety of flavonoids have been proven to be effective when combined with Citric Acid. This combination is responsible for an enhancement in Log reduction of reducing unwanted organisms.

Need for Action;

Food Safety concerns. Alternate to harsh and or synthetic chemicals currently being used.

•Our formulas are designed to address the need for good hygienic practices in the whole food security chain from per harvest to post harvest. Seed to Fork concept.

•In the last several years science has progressed in its ability to isolate and derive from natural resources components that are just as efficacious and environmentally safe as their synthetic counterparts. Current global trends are to incorporate a safe green approach in the processing of food for decontamination that affords the same or better Log reduction against different bacteria's, fungus, molds and harmful pathogens that are so familiar to the food industry. The current move is away from synthetic and harsh chemicals that pose a health threat to humans and the environment.

•If you will it can be explained as nature fighting nature.

Another industry concern that has risen, is the use of antibiotics and different synaptic harsh chemicals, are the microbes ability to survive and become resistant to these antibiotics and chemicals that have historically been applied for food safety concern.

Some of these common pathogens are various Salmonella, Listeria monocytogenes, Staphylococcus aureus, Campylobacter, E Coli, MRSA, pseudomonas etc.

Millions of Americans become ill each year from something they eat. The exact numbers are unknown as most illness go unreported. The Centers for Disease Control and Prevention calculates that E Coli O157:H7 alone infects over 20,000 Americans a year through contaminated meat and or produce. Recent E Coli O157 illnesses due to fresh cut bagged lettuce, infected Jalapeno peppers etc has been on the rise causing massive recalls and major financial losses to private industry.

These pathogens often require additional means to diminish or eliminate their numbers than the customary approved harsh chemicals currently being used/allowed as Processing wash Aids.

Our formulas which are the subject for approval as a Processing Aids wash complex can play a major role in upgrading or adding to approved Processing Aids to the EPA and FSIS to combat the current food safety security concerns.

For Organic farmers and Pack houses that process organic produce, they currently do not have a Processing Aid that is effective, natural, safe, Non Toxic, Non Tainting. Currently all fresh organic produce, and conventional produce is washed with chlorine based products which are not organic.

"*Antimicrobials are commonly used in the industry to reduce pathogens loads. The most common antimicrobial treatment used is chlorine (sodium hypochlorite) because it is inexpensive. However, failure to optimize the disinfectant properties of sodium hypochlorite i.e., improper pH, concentration, concentration of incoming water reduces its antimicrobial efficacy and can result in offensive and harmful odors/vapors such as chlorine gas and trichloramines".* Ref *" Northcutt.J.K. and MP Lacy 2000. Odor problems associated with chlorine usage in poultry processing plants. Poult.Sci 78 (Suppl.1):47*

With the European REACH accord being enforced, American growers are faced with global changes in uses and restrictions of harsh and or synthetic chemicals.

By placing the **Bergamot bitter Orange Powder** on the NOP list it will afford American growers and processors to have a product that is already approved in the EU and other regions around the world.

Bergamot bitter Orange Powder. Petitioned Substance.

Petition to add to the National list the substance extract from bitter **Bergamot bitter Orange Powder**. Bergamot oranges. Latin name Citrus aurantium.

A* Bergamot bitter Orange Powder *a single substance.

1.The petitioned product is a powder from bitter immature Bergamot oranges. Latin name Citrus aurantium.
2. The dried bitter immature bergamot orange powder contains a broad spectrum of bioflavonoids..and is used to modify the pH of the water.
3. The HPLC is for information only as to the identity of the refined single substance powder.
4. The single substance petitioned is not a formulated product. But a refined powder from bitter oranges/Bergamot oranges. Latin name Citrus aurantium

COMPOSITION/INFORMATION ON INGREDIENT

This product is a natural powder extract from bitter oranges. The main component are the citrus flavanones naringin and neohesperidin.

CAS N°: 72968-50-4

EINECS N°: 277-143-2

HAZARDS INFORMATION

According to EC criteria (67/548/EC) this product is not classified as a dangerous substance neither for environment nor for human health.

In solid form (dust) possible eye and respiratory irritant

TOXICOLOGICAL INFORMATION

Powder extract from bitter oranges obtained only by physical means (natural extract). Main components are the citrus flavanones naringin and neohesperidin of no know toxic properties.

References:

1."Evaluation of the health aspects of hesperidin, naringin and citrus bioflavonoid extracts as food ingredients". Federation of American Societies for Experimental Biology, Bethesda, MD. Prepared for FDA, Washington DC. PB 82-192931. 1982. 2."Monograph on bioflavonoids". Informatic, Inc., Rockville, MD. Prepared for FDA, PB 289 600. 1978.

Petition to add to the National list the substance Bergamot bitter Orange Powder

Item A:

1. **Category.**
   Non organically produced/grown agricultural products allowed in or on processed products labeled as organic. 7CFR § 205.606.

2. **Justification for this category.**
   The petitioned substance is grown and harvested according to organic standards in Italy, Spain and surrounding regions, even though the Bergamot orange orchards are not certified Organic.
   No sewage/sludge, pesticides, herbicides or irradiation is used.
   The product is a natural powder from bitter oranges/Bergamot oranges. Latin name Citrus aurantium.

Item B:

1. **The common name of the substance.**
   Common and Botanical name of plant source.

Bergamot bitter orange. Citrus aurantium

2. **The Manufacturer**.
   Ferrer Group
   EXQUIM,S.A.
   Edifici L'ILLa
   Av. Diagonal,549,5ª Planta 08029 Barcelona, Spain
   E mail. exquim@ferrergrupo.com

   Phone 34-93-504-44-00

3. **The intended or current use of the substance.**

   The petitioned ingredient as an "Intermediate for the manufacture of Non Toxic, Non Hazardous Processing Aids as a water pH modifier in fruit and vegetable wash, meat carcass rinse.

4. **The activities for which the Bergamot bitter Orange Powder is being used and its mode of action.**

The dried bitter immature bergamot orange powder contains a broad spectrum of bioflavonoids and is used to modify the pH of the water.

5. **The source of the substance and a detailed petition description of its manufacturing and processing procedures.**

Process Methodology, citrus fruits

Harvesting of citrus fruit

Whole citrus fruits, such as fruits of citrus aurantium, are harvested when they are still green. The green fruits contain higher levels of flavonoids than yellow fruits harvested at a later stage of development. The sizes of the citrus fruits, used in the processing, are in the range 40 to 60mm.

1. **Processing of citrus fruits**

There are a number of ways of processing fruits to extract the flavonoids. For example the process may involve the selection of fresh or frozen whole fruits. Fruits are frozen to - 20°C to disrupt the cell walls in order to assist the release flavonoids by diffusion. The fruits are kept frozen until further processing. Alternatively, flavonoids may be extracted from fresh or frozen peel issues of fruits, juice vesicle tissue of fruits, flavedo tissue of fruits, albedo tissue of fruits and segment epidermis tissues of fruits.

Thawing of fruits, before processing, is achieved using air ventilated containers which maintains the temperature at the surface of the fruits below 5°C.

Heat transfer is achieved on the batches of frozen fruits and eventually the core of the fruits becomes thawed.

Fruits are the sliced into pieces using a commercial slicer, typically with the dimensions around 5x10x2Omm to facilitate a practical time for the extraction. If pieces of fruit with a larger dimension are used this markedly increases the time of extraction and fruits of smaller dimensions tend to block the filters with finely divided matter.

Extraction of the flavonoids may follow a number of routes. One typical route includes at least four repeated round of extraction in water keeping the temperature below 20°C. The process includes the use of a number of tanks fitted with appropriate stirrers and filters which are filled with the ground citrus fruits and water a temperature below 20°C. the slurry is circulated by means of a stirrer. The mass transfer is increased until the Reynolds number confirms turbulent flow. The resulting juice is transferred to additional tanks filled with citrus fruit and the process of repeated extractions allows the extraction of almost all flavonoids into the mother liquor. In the filtration process the flavonoids are recovered and vegetal residues remain. It is necessary to perform a micro membrane unitrafiltration step in which the juice from the extraction is filtered using membranes fabricated from PSO (Polysulfon) or PVDF (Polyvinylidene Fluride). The membranes allow the passage of flavonoids and retain larger molecules and particles.

In general cut-off values in the range of 20-200 kDa are employed and the temperature is maintained below 10°C.

3. Adsorption of flavonoids to an adsorbent

Absorbents, in a packed column without stirring, selectively retain the desired flavonoids and, typically, commercial adsorbents such as Amberlite XAD7HP and Dowex materials can be used.

When the absorbent material is saturated with the flavonoids it is only necessary to introduce a flow of water to remove substances attached to the surface of the

absorbent material. The flavonoids are eluted from the column using ethanol into an ethanol/water solution contained 0.7% to I% flavonoids.

4. Evaporation of ethanol/water from the solution of flavonoids; spray drying.

A vacuum evaporator maintained at a temperature of around 45°C is used to further concentrate the solution of flavonoids; the concentrated juice is transferred to a spray- drier for evaporation of the water solvent. Nozzles at the top of the spray drier generates small droplets and hot air maintained at 180° C is blown into the device in a counter stream causing evaporation of the mainly water solvent. The temperature value of the exhaust air is around 90° C.

Flavonoid powder is collected from the spray-drier at the end of the process.

Samples of Citrus Aurantium from Spain were analysed by HPLC —DAD (High Performance Liquid Chromatograph-Diode Array Detection)

In general flavonoids absorb UV-visible radiation at approximately 280 nm and 'or 360 nm.

At 280 nm four significant peaks are observed 46.2,50.7,54.3 and 66.5 minutes Fig 1; at 360 nm four main peaks are detected; 50.7,54.3,57.2, and 61.9 minutes (Fig 2).

The data from the HLPC analysis show that the Citrus Aurantium sample include four flavonoles/flavanones and two flavones.

Flavonoids corresponding to five out of the six main peaks from the HPLC-DAD analysis were isolated and analysed by NMR.

Three of the five isolated flavonoids included neoeriocitin, naringin and neohesperidin accounting for 9.388%, 36.212°o and 39.891% of the total flavonoids of the sample(wt/wt).

Sample preparation and HPLC-DAD analysis:

29.4mg sample was dissolved in 10m1 methanol (Sample).

The sample was diluted by a factor 4 and analysed by HPLC-DAD.

Equipment:Shimadzu HPLC, Merck Diode Array Detector, LiChroapher 1OORP-18 Column (No 924112) Oven Temperature 35° C; Injection volume 20 1; flow I ml/min; fixed wavelength; either 280 or 360nm; Scan wavelength 230-500 nm.

Eluent A: 5°o formic acid in water

Eluent B; 50% methanol, 15% acetic acid, in water

See attached documents for the following.

Specification, Raw Materials, Production
Compliance and Traceability Information

- Schematic flow diagram of the extraction process of the bioflavonoid from the oranges.
- Non Genetic Modification certificate.
- Non Genetic Modified certificates for the other raw materials present in the product.
- Non Sewage/Sludge certificates
- No Radiation used certificates

6. **A summary of any available previous reviews of the petition substance by State or private certification programs or other organizations.**
   None to date.

7. **Information regarding EPA, FDA and State regulatory authority registrations.**
   The FDA has approved Citrus pulp/flavonoids as GRAS and as a food substance.
   See Attachment A.

"Evaluation of the aspects of hesperidin, naringin, and other citrus bioflavonoid extracts as food ingredients." 1982 PB82-192931. Prepared for Bureau of Foods. Food and Drug Administration. Department of Health and Human Services. Washington DC. Contract No. FDA 223-78-2100

Food and Drug Administration, HHS § 182.20 GRAS statement/description
Common and Botanical name of plant source.
Bergamot bitter orange. Citrus aurantium

8. **The Chemical Abstract Service (CAS) numbers of the substance**.

CAS N°:72968-50-4
EINECS N°: 277-143-2

9. **The substance's physical properties and chemical mode of action**.
The dried bitter immature bergamot orange powder contains a broad spectrum of bioflavonoids and is used to modify the pH of the water

HPLC 45% means a mixture containing 45% of bioflavonoid and 55% of other matter from extraction of bitter oranges. The bioflavonoids comprised are narangin (about 52%), neohesperidin 28%, poncirin 4%, naringenin 3%, hesperidin 3%, neodiosmin 3%, isonaringin 3%, isocriocrin 2%, other minor to 100 %.

Patent Pending. Confidential information.
PCT/GB2007/002756 and PCT/GB2007/002758 describe particularly effective compositions containing flavonoids. The present invention is based on the finding that certain combinations of agents are particularly effective in eliminating or reducing unwanted bacteria and other undesirable organisms.

Bioflavonoids are extracted from citrus fruits and have been used for years in human health food supplementary vitamin products (for their antioxidant properties). It has been discovered that they possess other interesting properties including the ability to suppress cancer, heart and general circulatory problems. They are also known to possess antiviral, antibacterial and antifungal properties. By blending the bioflavonoids with fruit acids such as citric acid, malic acid and ascorbic acid we have discovered that the antiviral and anti fungal and anti bacterial properties can be enhanced significantly.

:

10. **Safety Information about the substance**.
The FDA has approved flavonoids as GRAS and as a food substance.
See Attachment A.

"Evaluation of the aspects of hesperidin, naringin, and other citrus bioflavonoid extracts as food ingredients." 1982 PB82-192931. Prepared for Bureau of Foods. Food and Drug Administration. Department of Health and Human Services. Washington DC. Contract No. FDA 223-78-2100

11. **Comprehensive reviews and research bibliographies including reviews and bibliographies which present contrasting positions.**
    We are not aware of any research summaries or bibliographies that present a contrasting position.
12. **A petition justification statement: which provides justification for inclusion of a non organically produced agricultural substance onto the National List.**

    The inclusion of the **Bergamot bitter Orange Powder** to the National List as a non organically produced agricultural substance is based on the unavailability of an alternate organic source.

Our justification statement is based off the following FDA review and approval that citrus bioflavonoid extracts are food ingredients: ***"Evaluation of the aspects of hesperidin, naringin, and other citrus bioflavonoid extracts as food ingredients." 1982 PB82-192931. Prepared for Bureau of Foods. Food and Drug Administration. Department of Health and Human Services. Washington DC. Contract No. FDA 223-78-2100***

It is also based off of how the orchards are maintained in their growing regions

The bergamot *Citrus aurantium* subsp. *bergamia* (Risso & Poit.) synonym (*Citrus bergamia* Risso) is the size of an orange, with a yellow color similar to a lemon, and has a pleasant fragrance. The juice tastes less sour than lemon, but more bitter than grapefruit. Citrus bergamot is native to Asia and is commercially grown in Calabria (Italy), in France, and in Ivory Coast. Bergamot grows on small trees which blossom during the winter. The distinctive aroma of the bergamot is most commonly known for its use in Earl Grey tea, though the juice of the fruit has also been used in Calabrian indigenous medicine as an herbal remedy for malaria and its essential oil is popular in aromatherapy applications.

Production mostly is limited to the Ionian coastal region of the province of Calabria in Italy, to such an extent that it is a symbol of the entire region. Most of the bergamot comes from a short stretch of land there where the temperature is favourable. It is also cultivated in Argentina, Brazil and the US state of Georgia, but the quality of the obtained essence is not comparable with the essence produced from the bergamots of Reggio Calabria due to the argillite, limestone and alluvial deposits found there. High quality production is also found on the Southern coast of Turkey, mainly around the town of Antalya. ***REF: Wikpedia***

Soil condition is key to nutrient uptake and quality of the flavonoid production in the fruit. The bergamot grown in the USA is not of the same high quality orange that Citrox Ltd needed for its formulation.

The bergamot orange is not an endangered species. Current and historical information, research or evidence provided shows that the quantities used globally cannot be

obtained organically in the appropriate quality and quantity to fulfill an essential function in a system of organic handling. All bergamot oranges supplied to Ferrer have certificates showing no sewage, sludge, Non GMO and no radiation has been used as fertilizers, herbicides and pesticides.

One of the End uses of the essential oil is approved for use on Organic certified Earl Grey teas.

ANNEX 1

List of NWFP of commercial significance

Category	Key products	Examples of trade data[136]
Essential oils	Essential oils of bergamot, orange, lemon, lime, citrus fruits, nes, geranium, jasmine, lavender or of lavandin, peppermint, other mints, vetiver	World trade is of the order of US$ 1 billion (comtrade 2001), including both the wild as well as cultivated sources. China, Indonesia, Thailand, India and Brazil are the major suppliers of some of the oils. The EC, USA and Japan are the principal import markets, accounting for 72% of the total world imports (FAO, 1995). Plants & parts, pharmacy, perfume, insecticides use nes (HS121190): Export of 517 030 t worth US$660 Mio in 2001. Main exporting countries are China, India and Germany; main importers are USA, India and Germany.

[136]If not specified, data is based on figures provided by the United Nations Statistics Division commodity trade database (Comtrade) as of 26 September 2003. Main exporting countries are ranked according to the export value.

European Production
IAL Consultants estimates that the total production of essential oils by the major producing countries in Western Europe was approximately 33,600 tonnes in 1999. This is an increase of around 17% from year 1998.

France is the major producer of essential oils in Western Europe. Total French production of essential oils in 1999 was around 20,000 tonnes, which

is an increase of around 30% from 1998. The production is valued at around 405 million Euros. The UK's production in 1999 was approximately 7,000 tonnes which is an increase of around 17% from the year 1998.
There is significant production of orange and lemon essential oils in Europe.

Orange oils are mainly produced in Italy and Spain and in the Mediterranean regions.

United States data on Bergamot oranges.

Table 17-U.S. essential oil exports, volume and value, selected oils, 1992-94

Essential oil	1992		1993		1994	
	Volume	Value	Volume	Value	Volume	Value
	Kilograms	$1,000	Kilograms	$1,000	Kilograms	$1,000
Peppermint	1,568,728	52,613.3	1,655,168	53,278.9	2,115,696	66,925.3
Spearmint	644,246	21,254.9	700,752	22,601.6	739,792	24,207.0
Other mint	308,393	7,640.8	164,177	4,236.0	228,689	5,307.3
Bergamot	185,331	2,914.3	180,162	3,540.0	112,176	1,907.2
Lemon	868,772	10,526.7	841,422	11,834.4	818,210	11,928.2
Lime	231,407	5,052.3	196,528	4,057.5	282,917	4,607.7
Orange	3,407,916	10,195.5	3,665,228	11,965.2	4,207,009	17,142.6
Other citrus	647,530	6,272.7	727,524	10,083.0	906,093	12,532.5
Cedarwood, Clove, and Nutmeg	649,976	5,236.7	823,367	5,347.4	883,910	4,736.2
Geranium	58,786	1,565.9	18,130	671.2	39,450	977.4
Jasmine	22,813	62.4	8,603	135.2	4,739	152.5
Lavender	76,481	1,431.7	59,983	1,358.3	73,944	1,712.6
Vetiver	15,933	434.8	10,507	431.7	12,570	503.3
Other essential oils	1,546,812	21,700.7	2,164,095	24,829.8	1,899,838	23,503.4
Total	10,233,124	146,902.7	11,215,646	154,370.2	12,325,033	176,143.2

Source: U.S. Department of Commerce, Bureau of the Census.

13. Confidential Business Information.

None

Evaluation Criteria for Substances added to the National list.

Category 1.

Adverse impacts on humans or the environment?

Petitioned Substance: Bergamot bitter Orange Powder

Documentation.

1. Are there adverse effects on environment from manufacture, use or disposal? §205.600 b.2
   NO

2. Is there environmental contamination during manufacture, use, misuse or disposal? § 6518 m.3
   NO. All of the bergamot pulp is used in the extraction process.
3. Is the substance harmful to the environment? (1)(A)(i);6517(c)(2)(A)i
   NO
4. Does the substance contain List 1,2,3 inerts. New list 4a covers the old list. §6517c(1)(b)(ii)
   Yes. Citrus Pulp CAS 68514-76-1
5. Is there potential for detrimental chemical interaction with other materials used? §6518 m.1
   NO
6. Are there adverse biological and chemical interactions in agro ecosystems? §6518 m5
   NO
7. Are there detrimental physiological effects on soil organisms, crops or livestock? §6518 m5
   NO
8. Is there a toxic or other adverse action of the material or its breakdown products? §6518 m2
   NO.
9. Is there undesirable persistence or concentration of the material or breakdown products in environment? §6518 m2
   NO
10. Is there any harmful effect on human health? §6517 c (1)(A)(i); 6517 c (2)(A)(i); §6518 m.4
    NO
11. Is there an adverse effect on human health as defined by applicable Federal regulations? §205.600 b.5
    NO.
12. Is the substance GRAS when used according to FDA's good manufacturing practices? §205.600 b.5
    YES.

13. Does the substance contain residues of heavy metals or other contaminants in excess of FDA tolerances? §205.600 b.5
    NO.

Category 2.

Is the substance essential for organic production?

1. Is the substance formulated or manufactured by a chemical process? 6502(21)
   NO.
2. Is the substance formulated or manufactured by a process that chemically changes the substance extracted from naturally occurring plant, animal, or mineral sources? 6502(21)
   NO.
3. Is the substance created by naturally occurring biological processes? 6502(21)
   YES.
4. Is there a natural source of the substance? §205.600 b.1
   The Powder is extracted from 100% natural bergamot orange source.
5. Is there an organic substitute? §205.600 b.1
   Even though all bergamot oranges orchards are not organically certified, Ferrer issues certificates guaranteeing No sewage/sludge, No pesticide or heavy metals residues or radiation has been used on the food stock that is used for extraction of the Bergamot bitter Orange Powder.
6. Is the substance essential for handling of organically produced agricultural products? §205.600 b.6
   YES.
   To date there are restricted products such as chlorine Dioxide-restricted use, Chlorine Materials-restricted use under §205.605(b) that are used on organic produce as a processing and handling aid. To date there are not many safer alternates to these harsh chemicals. By the addition of the Bioflavonoids 45% HPLC there will be an alternate natural, safe, non toxic, non hazardous, non genetically modified ingredient that is environmentally safe to fish, fowl, animals and human beings.
7. Is there a wholly natural substitute product? §6517 c(1)(A)(ii)
   The Bergamot bitter Orange Powder is a wholly natural product.
8. Is the substance used in handling, not synthetic, but not organically produced? § 6517 c (1)(B)(iii)
   YES. The Bergamot bitter Orange Powder is wholly natural and the extraction process complies with organic standards.
9. Is there any alternate substance(s)? § 6518 m.6
   NO. Due to growing regions, soil condition where the bergamot oranges are grown there are no substitutes.
10. Is there another practice that would make the substance unnecessary? §6518 m.6
    NO

Category 3.

Is the substance compatible with organic production practices?

1. Is the substance compatible with organic handling? §205.600 b.2
   YES
2. Is the substance consistent with organic farming and handling? §6517 c(1)(A)(iii);6517 c (2)(A)(ii)
   YES.

3. Is the substance compatible with a system of sustainable agriculture? §6518 m.7
   YES.
4. Is the nutritional quality of the food maintained with the substance? §205.600 b.3
   YES
5. Is the primary use as a preservative? §205.600 b.4
   NO.
6. Is the value use to recreate or improve flavors, colors, textures, or nutritive values lost in processing? §205.600 b.4
   NO
7. Is the substance used in production, and does it contain an active synthetic ingredient in the following categories:
   a. Copper and sulfur compounds.
   b. Toxins derived from bacteria.
   c. Pheromones, soaps, horticultural oils, fish emulsions, treated seed, vitamins and minerals.
   d. Livestock parasiticides and medicines.
   e. Production aids including netting, tree wraps and seals, insect traps, sticky barriers, row covers, and equipment cleaners?
      NO to all.

ORGANIC FARMING
ECOCERT

ATTESTATION

for inputs in conformity with (EC) 834/2007 & 889/2008 regulation

Attestation reference: CITROXUK1000n1e -
Number of products: 8

This attestation is issued to the operator below:

CITROX LIMITED
Unit 9 River Court, Brighouse Road, Riverside Park, TS21RT - Middlesbrough
UNITED KINGDOM

Ecocert SA confirms after inspection that the following products:

PRODUCT NAME	CATEGORY	STATUS
Prosino 14wps2	Disinfecting agent (post-harvest)	EU 889/2008 allowed
Progarda 14wp	Disinfecting agent (post-harvest)	EU 889/2008 allowed
Progarda Fl001	Disinfecting agent (post-harvest)	EU 889/2008 allowed
Citrox Bc	Disinfecting agent (post-harvest)	EU 889/2008 allowed
Proalexin Pns001	Disinfecting agent (storage areas)	EU 889/2008 allowed
Phytobac	Disinfecting agent (storage areas)	EU 889/2008 allowed
Procaro Hand Gel	Disinfecting agent (storage areas)	EU 889/2008 allowed
Sosonaturel Hand Gel	Disinfecting agent (storage areas)	EU 889/2008 allowed

comply with (EC) 834/2007 & 889/2008 regulation

Input Service Manager
Esther DEL POZO

Issue date, in L'Isle Jourdain: 25/10/2010
Expiry date: 31/12/2011

Ecocert SA – Capital 440 000€ – Lieudit Lamothe Ouest – 32600 L'Isle Jourdain – France
Tél. +33 (0)5 62 07 34 24 – Fax : +33 (0)5 62 07 11 67 – www.ecocert.com

ORGANIC FARMING
ECOCERT

ATTESTATION

for inputs in conformity with US National Organic Program (NOP)

Attestation reference: CITROXUK1000n2e -
Number of products: 8

This attestation is issued to the operator below:

CITROX LIMITED

Unit 9 River Court, Brighouse Road, Riverside Park, TS21RT - Middlesbrough

UNITED KINGDOM

Ecocert SA confirms after inspection that the following products:

PRODUCT NAME	CATEGORY	STATUS
Prosino 14wps2	Disinfecting agent (post-harvest)	NOP allowed
Progarda 14wp	Disinfecting agent (post-harvest)	NOP allowed
Progarda FI001	Disinfecting agent (post-harvest)	NOP allowed
Citrox Bc	Disinfecting agent (post-harvest)	NOP allowed
Proalexin Pns001	Disinfecting agent (storage areas)	NOP allowed
Phytobac	Disinfecting agent (storage areas)	NOP allowed
Procaro Hand Gel	Disinfecting agent (storage areas)	NOP allowed
Sosonaturel Hand Gel	Disinfecting agent (storage areas)	NOP allowed

comply with the US National Organic Program (NOP)

Input Service Manager

Esther DEL POZO

Issue date, in L'Isle Jourdain: 25/10/2010
Expiry date: 31/12/2011

Chemicals Regulation Directorate

Christopher Ripley

Citrox Limited
Unit 9 River Court
Brighouse Road
Riverside Park
Middlesbrough
TS2 1RT

Date: 09 November 2010

To Whom it may concern,

THE BIOCIDAL PRODUCTS DIRECTIVE 98/8/EC (BPD)
THE CONTROL OF PESTICIDES REGULATIONS 1986 (COPR)

The UK Biocides Competent Authority which is based in the Chemicals Regulation Directorate of the Health and Safety Executive (HSE) can confirm that Disinfectant products (other than those considered to be medicines or veterinary medicines) are regulated under the EU Biocidal Products Directive (BPD).

The BPD active substance review programme is currently in a transition period, as all EU Member States are in the process of transferring all existing active substances from national legislation to regulations under the BPD.

Biocidal products containing notified active substances, which are still in the BPD active substance review programme, can be placed on the EU market until a decision is made whether or not to include the active substance on Annex I of the BPD, however during this transitional period existing national legislation applies.

The UK Biocides Competent Authority have been informed by Citrox Ltd that their disinfectant product ProSino Citrox-BC contains Lactic acid (CAS number: 79-33-4) as the active ingredient and is intended for use in Product Types 2 (Private and public health area disinfectants), 3 (Veterinary Hygiene biocidal products) and 4 (Food and feed area disinfectants), and that ProCaro Citrox-BC, contains Citric Acid (CAS: 77-92-9) and is intended for use in Product Type 1 (Human hygiene biocidal products). Lactic acid was still in the BPD review programme for Product Types 2, 3 and 4, and citric acid for Product Type 1 on 9 November 2010.

In the UK, the existing national legislation HSE deals with is the Control of Pesticides Regulations (COPR). Under this legislation we issue a Notice of Approval once an application for a non-agricultural pesticide has been evaluated and approved. Regulation 3 (2) (e) of COPR, as amended, exempts disinfectants therefore these

2.3 Redgrave Court, Merton Road. Bootle, Merseyside L20 7HS
0151 951 3512 biocides@hse.gsi.gov.uk

products do not require approval from HSE before they can be placed on the UK market.

Consequently, as the active substances in ProSino Citrox-BC and ProCaro Citrox-BC as identified to us by Citrox Ltd are still in the BPD review programme these products can be placed on the UK market and have not needed approval under COPR.

These products will require authorisation under BPD when the active substance in the products is included onto Annex I of the BPD.

Yours Sincerely,

M. Ball.

Martin Ball
On behalf of the Chemicals Regulation Directorate
Health and Safety Executive

Attachment A

United States. Federal and State determinations.

1. Letter from United States Environmental Protection Agency. April 08 2002. Russell S Jones, Phd, Biologist, Chair, Biochemical Classification Committee. Citrox 14W Organic formula containing extract of Bitter Orange, Citric Acid and Ascorbic acid as its active ingredients qualify as biochemical pesticide.
2. Letter from Department of Health and Human Services. Food and Drug Administration. June 12 2003.
   Robert L Martin, Phd. Deputy director, Division of Biotechnology and GRAS Notice Review, HFS-255. Office od Food Additive Safety, Center for Food Safety and Applied Nutrition. GRAS review of ingredients of Citrox. Bitter Orange extract is listed under 21 CFR 182.20 as being GRAS and are regulated for use in food.
3. Letter from State of Maryland Department of Agriculture. February 5 2001. Citrox Crop life. Bitter Orange extract in another one of our formulas as a fertilizer and Nutrient synergist does not require registration under the Maryland Commercial Fertilizer law. Robert Hopkins. Administrative Officer.
4. Letter from the Common wealth of Kentucky. Department of Agriculture. February 28 2001. Division of Pesticides. Citrox Croplife fertilizer which contains the Bitter orange extract is not required to be registered by the Kentucky Division of Regulatory Services.
   Amanda Cloyd. Product Registration Coordinator.
5. Letter from Indiana State Chemist and Seed Commissioner. December 14 2000. Citrox Croplife which contains the Bitter orange extract, for commercial fertilizers does not require registration under Indiana Commercial Fertilizer law.
   Cyndy Anderson. Fertilizer Section.
6. Letter from Illnois Department of Agriculture. May 2 2001. Citrox Croplife which contains Bitter orange extract as a Nutrient Synergist for improving plant health will not require registration under Illinois fertilizer or Soil amendment act.
   Tom Waller. Program Specialist.
7. "Evaluation of the aspects of hesperidin, naringin, and other citrus bioflavonoid extracts as food ingredients." 1982 PB82-192931. Prepared for Bureau of Foods. Food and Drug Administration. Department of Health and Human Services. Washington DC. Contract No. FDA 223-78-2100

A1

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

APR 08 2002

Mr. Henry Kotula
Natural Agricultural Products, Inc.
P. O. Box 526
Bryan, OH 43506

Dear Mr. Kotula:

The Biochemical Classification Committee (BCC) of the Biopesticides & Pollution Prevention Division (BPPD) has reviewed the information submitted and has determined that your product, Citrox 14W Organic (containing extract of Bitter Orange, Citric Acid, and Ascorbic Acid as its active ingredients) qualifies as biochemical pesticide. Should you have any further questions, please call me at the address listed below.

Sincerely,

Russell S. Jones

Russell S. Jones, Ph.D., Biologist, Chair, Biochemical Classification Committee
Biopesticides & Pollution Prevention Division
Office of Pesticide Programs
U. S. Environmental Protection Agency
Ariel Rios Bldg. (7511C)
1200 Pennsylvania Avenue
Washington, DC 20460
703/308-5071
jones.russell@epa.gov

A 2

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
College Park, MD 20740

June 12, 2003

Mr. Henry Kotula
General Manager
Natural Agricultural Products, Inc.
55 E. Washington Street, Suite 401
Chicago, IL 60602

Dear Mr. Kotula:

This is in response to your submission (undated) to the Food and Drug Administration on the product Citrox[1], your letter to Dr. George Pauli dated May 27, 2003, and your telephone conversations with several individuals in the Office of Food Additive Safety concerning the regulatory status of the component ingredients of Citrox. In your submission, you state that Citrox is intended for use as an antimicrobial agent for fruits, vegetables, seafood, meat and poultry and is composed of bitter orange extract, citric acid, malic acid, ascorbic acid, and glycerin. In a telephone conversation on June 3, 2003, you stated to me that bitter orange extract is regulated for use under 21 CFR 182.20.

We have reviewed your submission and, based on your submission and subsequent telephone conversations, we agree that the component ingredients in your formulation are regulated for use in food. The regulations that permit these uses are listed below:

- Bitter orange (flower and peel) is listed under 21 CFR 182.20 (Essential oils, oleoresins (solvent-free), and natural extractives (including distillates) as GRAS when used in accordance with good manufacturing practice.
- Citric acid is listed under 21 CFR 184.1033 as a direct food substance affirmed as GRAS when used in accordance with good manufacturing practice.
- Malic acid is listed under 21 CFR 184.1069 as a direct food substance affirmed as GRAS for use as a flavor enhancer, flavoring agent and adjuvant, and pH control agent in accordance with good manufacturing practice.
- Ascorbic acid is listed under 21 CFR 182.3013 as GRAS when used in accordance with good manufacturing practice.
- Glycerin is listed under 21 CFR 182.1320 as GRAS when used in accordance with good manufacturing practice.

[1]Your submission has been designated as subject file number SBJ 1364. Please refer to SBJ 1364 when referencing this material in future correspondence.

A 2

If you have any further questions concerning this matter, please do not hesitate to contact us.

Sincerely,

Robert L. Martin, Ph.D.
Deputy Director
Division of Biotechnology and
GRAS Notice Review, HFS-255
Office of Food Additive Safety
Center for Food Safety and
and Applied Nutrition

The Wayne A. Cawley, Jr. Building
50 HARRY S. TRUMAN PARKWAY
ANNAPOLIS, MARYLAND 21401
Baltimore/Annapolis (410) 841-5700
Washington (301) 261-8106
Facsimile (410) 841-5914
TTY Users 1-800-735-2258
Internet http://www.mda.state.md.us

STATE OF MARYLAND
DEPARTMENT OF AGRICULTURE

February 5, 2001

Mr. Aaron D. Smith
Natural Agriculture Products Inc
55 E Washington St # 401
Chicago, IL 60602

Dear Mr. Smith:

A review of your product label that you submitted for Croplife Nutrient Synergist does not require registration under The Maryland Commercial Fertilizer Law.

If I can be of any further help, please let me know.

Thank you,
Robert Hopkins
Administrative Officer

A 3

Billy Ray Smith
Commissioner

Office Telephone
(502) 564-7274
FAX - (502) 564-3773
TTY - (502) 564-2075

Commonwealth of Kentucky
Department of Agriculture
Division of Pesticides
100 Fair Oaks Lane, 5th Floor
Frankfort, KY 40601

MEMORANDUM

To: Mr. Aaron Smith
Natural Agricultural Products, Inc.

From: Amanda Cloyd
Product Registration Coordinator

Date: February 28, 2001

Re: CropLife Product

Per our telephone conversation, this particular product is not required to be registered by the Kentucky Department of Agriculture, Division of Pesticides, because it does not make any type of pesticidal claim.

The University of Kentucky, Division of Regulatory Services, registers all fertilizers in this State. Dr. David Terry, Fertilizer Control Official, is the contact person. If you have not already contacted him to discuss this product, you may reach him at:

University of Kentucky
103 Regulatory Services Building
Lexington, KY 40546
(859) 257-2668
Dterry@Ca.Uky.edu

If you have any questions, please feel free to contact me at (502) 564-7274. Thank you.

A 4

A. R. Hanks
State Chemist & Seed Commissioner

Fred Nold
Associate State Chemist & Laboratory Director

H. L. Geiger
Feed Administrator

D. B. Groves
Chief Inspector & Auditor

Office of
INDIANA STATE CHEMIST AND SEED COMMISSIONER
Purdue University • 1154 Biochemistry
West Lafayette, IN 47907-1154
(765) 494-1492 • Fax (765) 494-4331

M. R. Hancock
Fertilizer Administrator

L. W. Nave
Seed Administrator

D. E. Stuff
Pesticide Administrator

C. L. Winer
Accounting & Administrative Assistant

December 14, 2000

Mr. Aaron D. Smith
Natural Agricultural Products Inc.
55 E Washington St #401
Chicago IL. 60602

Dear Mr. Smith:

We are returning herein your recently submitted registration application for commercial fertilizers for CropLife, along with your check #1234 in the amount of $10.

This product does not require registration under the Indiana Commercial Fertilizer Law. If you have any questions please call.

Sincerely,

Cyndy Anderson

Cyndy Anderson
Fertilizer Section

Enclosures

A 5

Illinois Department of Agriculture
George H. Ryan, Governor • Joe Hampton, Director
Bureau of Agricultural Products Inspection
State Fairgrounds • P.O. Box 19281 • Springfield, IL 62794-9281 • 217/782-3817 • TDD 217/524-6858 • Fax 217/524-7801

May 2, 2001

Natural Agricultural Products, Inc.
Mr. Aaron Smith
55 E. Washington St.
Suite 401
Chicago, IL. 60602

Dear Mr. Smith:

Per your letter to Jerry Kirbach, the Bureau of Agricultural Products Inspection responsible for fertilizers and soil amendments reviewed the Crop Life Nutrient Synergist label for registration requirements. The purpose of supplying a vitamin complex in conjunction with directions of use as a foliar application for improving plant health, promoting plant vigor, reducing stress and activating natural plant defense mechanisms will not require registration at this time under the Illinois Fertilizer or Soil Amendment Act before distribution in this State.

If you have any questions, feel free to contact this office at (217) 524-1291 or E-mail: twaller@agr.state.il.us.

Sincerely,
Illinois Department of Agriculture
Bureau of Agricultural Products Inspection

Tom Waller
Program Specialist

A 6

A 7

SCOGS II-3

PB82-192931

EVALUATION OF THE HEALTH ASPECTS OF HESPERIDIN, NARINGIN, AND CITRUS BIOFLAVONOID EXTRACTS AS FOOD INGREDIENTS

1982

Prepared for

Bureau of Foods
Food and Drug Administration
Department of Health and Human Services
Washington, D.C.

Contract No. FDA 223-78-2100

Life Sciences Research Office
Federation of American Societies
for Experimental Biology
9650 Rockville Pike
Bethesda, Maryland 20814

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BIBLIOGRAPHIC DATA SHEET	1. Report No. FDA/BF-82/62	2.	PB82-192931

4. Title and Subtitle

Evaluation of the Health Aspects of Hesperidin, Naringin, and Citrus Bioflavonoid Extracts as Food Ingredients

5. Report Date: 1982

6.

7. Author(s)

8. Performing Organization Rept. No. SCOGS-II-3

9. Performing Organization Name and Address

Life Sciences Research Office
Federation of American Societies for Experimental Biology
9650 Rockville Pike
Bethesda, MD 20014

10. Project/Task/Work Unit No.

11. Contract/Grant No. FDA 223-78-2100

12. Sponsoring Organization Name and Address

Food and Drug Administration
200 C Street, SW
Washington, DC 20204

13. Type of Report & Period Covered: Final

14.

15. Supplementary Notes

16. Abstracts

This report, by a group of qualified scientists designated the Select Committee of GRAS Substances (SCOGS), provides an independent evaluation of the safety of health aspects of hesperidin, naringin and citrus bioflavonoid extracts as food ingredients.

17. Key Words and Document Analysis. 17a. Descriptors

17b. Identifiers/Open-Ended Terms

Approved [illegible] 1982
William B. Wurtzler Date 3/[illegible]/82

17c. COSATI Field/Group

18. Availability Statement

Release unlimited

19. Security Class (This Report) UNCLASSIFIED

20. Security Class (This Page) UNCLASSIFIED

21. No. of Pages

22. Price

NOTICE

This report, one of a series concerning the health aspects of using the Generally Recognized as Safe (GRAS) or prior-sanctioned food substances as food ingredients, is being made by the Federation of American Societies for Experimental Biology (FASEB) under contract no. 223-78-2100 with the Food and Drug Administration (FDA), U.S. Department of Health and Human Services. The Federation recognizes that the safety of GRAS substances is of national significance, and that its resources are particularly suited to marshaling the opinions of knowledgeable scientists to assist in these evaluations. The Life Sciences Research Office (LSRO), established by FASEB in 1962 to make scientific assessments in the biomedical sciences, is conducting these studies.

Qualified scientists were selected as consultants to review and evaluate the available information on each of the GRAS substances. These scientists, designated the Select Committee on GRAS Substances (SCOGS), were chosen for their experience and judgment with due consideration for balance and breadth in the appropriate professional disciplines. The Select Committee's evaluations are being made independently of FDA or any other group, governmental or nongovernmental. The Select Committee accepts responsibility for the content of each report. Members of the Select Committee who have contributed to this report are named in Section VII.

Tentative reports are made available to the public for review in the office of the Dockets Management Branch, Food and Drug Administration, after announcement in the Federal Register, and opportunity is provided for any interested person to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the substances covered by the report. The data, information, and views presented at the hearing are considered by the Select Committee in reaching its final conclusions. Reports are approved by the Select Committee and the Director of LSRO, and subsequently reviewed and approved by the LSRO Advisory Committee (which consists of representatives of each constituent society of FASEB) under authority delegated by the Executive Committee of the Federation Board. Upon completion of these review procedures the reports are approved and transmitted to FDA by the Executive Director of FASEB.

While this is a report of the Federation of American Societies for Experimental Biology, it does not necessarily reflect the opinion of all of the individual members of its constituent societies.

Kenneth D. Fisher, Ph.D., Director
Life Sciences Research Office
FASEB

CONTENTS

I. INTRODUCTION

This report concerns the health aspects of using hesperidin, naringin, and citrus bioflavonoid extracts as food ingredients. It has been based partly on the information contained in a scientific literature review (monograph) furnished by FDA (Bauer, 1978), which summarizes the world's scientific literature from 1920 through 1978. To ensure completeness and currency as of the date of this report, this information has been supplemented by searches of over 30 scientific and statistical reference sources and compendia that are generally available; use of new, relevant books and reviews and the literature citations contained in them; consideration of current literature citations obtained through computer retrieval systems of the National Library of Medicine; searches for relevant data in the files of FDA; and by the combined knowledge and experience of members of the Select Committee and the LSRO staff. In addition, announcement was made in the Federal Register on January 13, 1981 (46 FR 3064-3068) that opportunity would be provided for any interested persons to appear before the Select Committee at a public hearing to make oral presentation of data, information, and views on the health aspects of using hesperidin, naringin, and citrus bioflavonoid complexes as food ingredients. The Select Committee held a hearing on June 22, 1981. Those who requested opportunity to present data, information, and views are identified at the end of this report. The material presented at the hearing has been considered by the Select Committee in reaching its final conclusions.

As indicated in the Food, Drug, and Cosmetic Act [21 USC 321(s)], GRAS substances are exempt from the premarketing clearance that is required for food additives. It is stated in the Act and in the Code of Federal Regulations (Office of the Federal Register, 1981) [21 CFR 170.3 and 170.30] that GRAS means general recognition of safety by experts qualified by scientific training and experience to evaluate the safety of substances on the basis of scientific data derived from published literature. These sections of the Code also indicate that expert judgment is to be based on the evaluation of results of credible toxicological testing or, for those substances used in food prior to January 1, 1958, on a reasoned judgment founded in experience with common food use, and is to take into account reasonably anticipated patterns of consumption, cumulative effects in the diet, and safety factors appropriate for the utilization of animal experimental data. FDA recognizes further [21 CFR 170.30] that it is impossible to provide assurance that any substance is absolutely safe for human consumption.

The Select Committee on GRAS Substances of LSRO reviewed and evaluated the available information on hesperidin, naringin, and citrus bioflavonoid extracts in full recognition of the foregoing provisions. In reaching its conclusions on safety, the

Committee, in accordance with FDA's guidelines, relied primarily on the absence of substantive evidence of, or reasonable grounds to suspect, a significant risk to the public health. This report is intended for the use of FDA in determining the future status of these substances under the Federal Food, Drug, and Cosmetic Act. The Committee anticipates that its conclusions will be reviewed as new information becomes available.

II. BACKGROUND INFORMATION

Flavonoids comprise a group of naturally occurring compounds which are among the most ubiquitous in the plant kingdom. They are found in every family and in nearly every species of the higher plants. Kühnau (1976) reported that about 800 different flavonoids were known and that new members of the group were being discovered "nearly every month." Although the term implies a yellowish coloration, flavonoids may vary in appearance from colorless to red or blue.

The basic flavone structure consists of 1,4-benzopyrone with a phenyl substitution in the 2-position (Fig. 1). Flavonoids differ in the number and position of substitutions on the aromatic rings and in the extent and character of oxidation in the pyrone portion of the molecule. Hydroxyl groups enable flavonoids to combine with sugars to form glycosides, and it is in this form that the flavonoids are usually found in nature. Glucose is the most common prosthetic group, although other sugars, as well as glucuronic and galacturonic acids, have been identified (Herrmann, 1976).

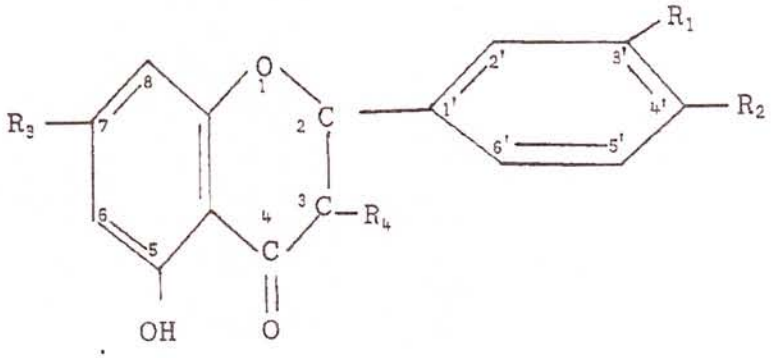

Figure 1. Typical flavonoid structure

Table 1 summarizes the structure of some typical flavonoids found in various foodstuffs.

In 1936, Szent-Györgyi and coworkers (Bentsáth et al., 1936; Rusznyák and Szent-Györgyi, 1936) reported that crude extracts of lemon juice or red peppers were more effective than purified ascorbic acid in alleviating capillary lesions and sustaining the lives of scorbutic guinea pigs. The active principle was tentatively termed vitamin P (for "permeability vitamin"). This was originally thought to be identical with "citrin," a crystalline product isolated from lemon juice (Bentsáth et al., 1936). "Citrin" was later shown to be a mixture of the flavonoids hesperidin, eriodictin (Bruckner and Szent-Györgyi, 1936) and a quercetin-like compound (Robeznieks, 1938). Although these and a

Table 1. Structure of Some Typical Flavonoids*

Flavonoid	R_1	R_2	R_3	R_4	Δ2:3†
Diosmetin	-OH	$-OCH_3$	- OH		Yes
Diosmin	-OH	$-OCH_3$	-ORG§		Yes
Eriodictin	-OH	- OH	- OR¶	- H	No
Eriodictyol	-OH	- OH	- OH	- H	No
Hesperidin	-OH	$-OCH_3$	-ORG§	- H	No
Hesperetin	-OH	$-OCH_3$	- OH	- H	No
Kaempferol	- H	- OH	- OH	-OH	Yes
Naringenin	- H	- OH	- OH	- H	No
Naringin	- H	- OH	-ORG§	- H	No
Quercetin	-OH	- OH	- OH	-OH	Yes
Rutin	-OH	- OH	-ORG§	-OH	Yes

* Adapted from Booth et al. (1958a). Substituent nomenclature refers to Fig. 1

† Double bond

§ RG is rhamnoglucoside

¶ R is rhamnoside

number of other flavonoids have been shown to modify membrane permeability, or to display other biological effects, all have failed to meet the criteria of true vitamins, namely, that they are essential and indispensable food constituents, and that deficiency syndromes are known which can be cured specifically by their administration. Consequently, the Joint Committee on Biochemical Nomenclature of the American Society of Biological Chemists and the American Institute of Nutrition (1950) recommended that the term vitamin P be discontinued. In its place, the less restrictive term "bioflavonoids" has been adopted to encompass the many flavonoids with some form of biological activity. Because bioflavonoids are nonessential food substances with biologic effects, the Committee on Dietary Allowances of the Food and Nutrition Board, (National Research Council, 1980) categorized them as pharmacologic rather than nutritional agents.

A vast, confusing literature has accumulated on the purported pharmacologic or therapeutic effects of one or another bioflavonoid. Willaman (1955), in reviewing the various reports, listed 33 separate types of biologic effects which had been attributed to bioflavonoids, including estrogenic, bactericidal, diuretic, antihistaminic, cathartic, hypotensive, hypertensive, and many others. In the intervening 25 years, claims have been made for still other activities by this group of compounds. Although the early claims of antiscorbutic action have proved unfounded, many of the bioflavonoids are effective antioxidants which protect sensitive, biologically important compounds. Kühnau (1976) stated that flavonoids are the most common and active antioxidants in our food supply, with the possible exception of tocopherols. Unlike the tocopherols, the flavonoids are active in hydrophilic as well as in lipophilic systems. Another general property of flavonoids is their ability to chelate metals, especially copper, to form metal complexes (Kühnau, 1976). Since the oxidation of vitamin C is catalyzed by the presence of copper, the antioxidant and chelating properties of flavonoids may be responsible for their oft-cited "sparing action" of vitamin C.

Three bioflavonoid preparations were given prior-sanctioned status by FDA: naringin, hesperidin, and lemon bioflavonoid complex (lemon peel infusion) (Wulfsberg, 1961a). Use of these substances in amounts up to 1 g daily was authorized in special dietary foods. A subsequent decision by FDA (Wulfsberg, 1961b) accorded prior-sanctioned status to a broader range of citrus preparations; namely, "dried concentrates of water-soluble flavonoids from washed, deoiled, ground peel and pulp of oranges, grapefruit and tangerines." These were authorized in special dietary foods in amounts up to 600 mg daily. Hesperidin complex was stated to be GRAS by FDA when distributed over-the-counter with recommended dosages of no more than 1 g daily (Smith, 1956).

Naringin has been accorded GRAS status as a flavoring agent in food [21 CFR 182.20] (Office of the Federal Register, 1981) and hesperidin has been given similar status for enhancing

and preserving flavor at a level of 30 ppm in flavored milk (Buckley, 1968). Hesperidin was referred to as "apparently a purified product" obtained from orange and lemon peel by use of calcium hydroxide and hydrochloric acid in the extraction and crystallization procedures.

Official food-grade specifications for bioflavonoids have not been established. Sunkist Growers, Inc., a large producer of these preparations, describe their products as follows:

Hesperidin complex: Hesperidin complex is a crude hesperidin preparation obtained by extraction of the albedo of orange peel with calcium hydroxide solution (Beisel, 1981b). On acidification with hydrochloric acid to pH about 4.5, hesperidin precipitates as a crystalline material along with other coprecipitated flavonoids. The precipitate is washed to remove non-flavonoid ballast, and then spray dried. Average hesperidin content is 72%; naringenin-7-rutinoside, 4.4%; isosakuranetin-7-rutinoside, 4.2%; and less than 1% naringenin-7-rutinoside-4'-glucoside, eriocitrin, sinensetin, nobeletin, tangeretin, and several unidentified polymethoxylated flavones. It contains no rutin (Beisel, 1981a). The powdered precipitate is nonhygroscopic; its weight loss on drying is 2-5%; sulfated ash, 2-4%; and methoxyl content, 3.3-3.7% (Nelson, 1980b).

Hesperidin, purified: Purified hesperidin is prepared from hesperidin complex by dissolving the crystals in calcium hydroxide solution and precipitating by acidification with hydrochloric acid (Beisel, 1981b). More than one cycle of dissolution and precipitation may be needed to achieve the minimum hesperidin content of 80% specified for the purified product (Sunkist Growers, Inc., 1978). Most purified product is stated to have a hesperidin content of about 90% (Beisel, 1981b).

Naringin: Naringin is derived from grapefruit peel (Nelson, 1980b). One part in 50,000 parts of water gives a distinct bitter taste, which has about 1/3-1/4 the bitterness of quinine sulfate. It is readily soluble in alcohol, acetone, and hot water. In water at 25°C its solubility is 0.5 g/liter. The naringin content of the preparation is not less than 85% with a weight loss of not more than 10% on drying. The residue on ignition is not more than 2% sulfated ash.

Lemon bioflavonoid complex: This is a hygroscopic dried powder of lemon peel extract containing the water-soluble flavonoids and associated complex extractives (Nelson, 1980b). Beisel (1981a) reported that the Sunkist preparation contains about 35% of mono- and disaccharides [chiefly glucose, fructose, and sucrose (Nelson, 1980c)], 15% ash, 9-12% calcium citrate, 1.5-3.0% citric acid, about 4-7% bioflavonoids and unspecified amounts of inositol and other organic acids. The most plentiful flavonoid is hesperidin, with lesser amounts present of eriocitrin, naringen rutinoside, diosmin, the 7-rutinosides of luteolin and apigenin, and

rutin. The rutin content of this preparation ranges from 240-640 µg/g with an average of 440 µg/g. No free quercetin was detected. A lemon bioflavonoid complex marketed by another firm was reported to contain an estimated 50% carbohydrate and 10-15% flavonoids (Brewster, 1980). The flavonoids were not identified.

Lemon bioflavonoid complex concentrates (LBC concentrates): Although the Select Committee has no information that such products are marketed, animal feeding studies have been conducted with two products designated LBC concentrate (2x) and LBC concentrate (6x) (Beisel, 1981d). LBC concentrate (2x) was prepared by multiple extractions of LBC with isopropanol to preferentially extract flavonoids followed by distillation of the solvent and vacuum drying the residual syrup. LBC concentrate (6x) was prepared by extraction of flavonoids from LBC with methyl ethyl ketone followed by removal of solvent. No information was available on the composition of the products. The concentration factor (2x or 6x) refers to the measured increase in citrate concentration in the extract which presumably was related to an increase in concentration of flavonoids.

Orange, grapefruit, and tangerine bioflavonoid complexes: The Select Committee has no information on the flavonoid composition of the concentrates of water soluble flavonoids derived from oranges, grapefruit, and tangerines, or whether such preparations are currently being marketed. A feeding study has been reported with orange bioflavonoid complex concentrate but the company reporting this study has stated that they have discontinued production of this item (Beisel, 1981d). The orange bioflavonoid complex concentrate was prepared by the same techniques used in the preparation of LBC concentrates.

Citrus peel contains a mixture of flavonoids and related compounds, not all of which may be extracted in the commercial process using water. Analyses of organic solvent extracts have detected the flavonoids listed in Table 2 (Harborne, 1967; Hendrickson and Kesterson, 1965; Horowitz and Gentili, 1960; Maier and Metzler, 1967). The flavonoids generally occur in the peel in their glycosidic forms (Horowitz and Gentili, 1960), and many of the aglycones listed in the table were obtained from enzymatically hydrolyzed extracts. In addition to these flavonoids, numerous chemically related compounds, especially derivatives of phenol, coumarin, and cinnamic acid, have also been identified in citrus peel (Horowitz and Gentili, 1960; Maier and Metzler, 1967).

Beisel (1981a) reported that orange juice reconstituted from the frozen concentrate contains about 3.2 µg rutin/g, reconstituted grapefruit juice less than 1 µg rutin/g, and lemonade about 2.9 µg rutin/g.

The most thorough analyses of flavonoid content in various edible plants appear to be those of kaempferol and quercetin glycosides. The concentrations of these flavonoids have been

Table 2. Flavonoids in Citrus Peel Extracts*

Grapefruit†	Lemon	Orange	Tangerine
Apigenin§	Apigenin	Auranetin	Hesperidin§
Dihydrokaempferol	Apigenin 7-rutinoside	Hesperidin§	Nobiletin
Eriodictyol	Chrysoeriol	Isosakuranetin 7-rutinoside	Tangeretin
Hesperetin	Diosmin§	Naringin	
Hesperidin	Eriocitrin§	Neohesperidin	
Isorhamnetin	Hesperidin§	Nobiletin	
Isosakuranetin§	Isorhamnetin	Rutin¶	
Kaempferol	Limocitrin	Sinensetin	
Naringenin	Limocitrol	Tangeretin	
Naringin§	Luteolin 7-rutinoside	Vitexin	
Neohesperidin	Naringin		
Poncirin	Neohesperidin		
Quercetin	Poncirin		
Rutin¶	Quercetin		

* Flavonoids occur naturally in glycosidic form (Horowitz and Gentili, 1960). The aglycones listed in this table were identified after enzymatic hydrolysis.

† All aglycones were detected in grapefruit endocarp as well as peel after enzymatic hydrolysis (Maier and Metzler, 1967).

§ Flavonoids present in greatest concentrations.

¶ Found in Satsumelo, a hybrid of grapefruit and Satsuma orange (Krewson and Couch, 1948).

determined in numerous fruits and vegetables (Table 3). The flavonoid concentrations are consistently and considerably greater in the leaves, skin, and peel of the various plants than in their deeper tissues (Herrmann, 1976).

The bitter taste of naringin has been used to enhance the piquant flavor of certain beverages and to replace "bitter tonic" preparations. Naringin has also been used as an intermediate in the preparation of certain water-soluble, yellow-red dyes for wool and silk (Kesterson and Hendrickson, 1953). In sharp contrast to naringin, hesperidin is practically tasteless (Wilson and DeEds, 1940). Presumably through its action as an antioxidant, hesperidin has been reported to delay flavor deterioration of milk-based beverages, thereby extending shelf life by 6-12 mo (Nelson, 1980a). Hesperidin also is used as a reagent in the refining and reclaiming of lead and zinc, and as a raw material in the production of the dihydrochalcone of neohesperidin, a nonnutritive sweetener (Nelson, 1980a).

Table 3. Content of Quercetin and Kaempferol Glycosides in Some Vegetables and Fruits Estimated as mg Aglycone/kg Fresh Weight*

Vegetable or Fruit	Quercetin	Kaempferol
Asparagus spears	6.7	0.7
Broccoli	30	6
Tomato	7	0.2
Chives	300	10
Lettuce (Blanco)	31	-†
Lettuce (Valentine)	276	-†
Bell Pepper	63	0
Brussels sprouts	25	40
Cauliflower	1	2
Radish	0	1-8
Leek (9 varieties)	10-25	90-200
Endive (outer leaves)	-†	150
Kohlrabi	<1	<1
Potato	2	1
Apple		
Peel	58-263	<1-7
Remaining tissue	<1-2	0-0.1
Pear		
Peel	28	12
Remaining tissue	<0.1	0

* Adapted from Herrmann (1976)

† Not reported

III. CONSUMER EXPOSURE DATA

Data are sparse on the use of bioflavonoids as food additives. In its 1977 survey, the Committee on GRAS List Survey (1979) queried industry on the use of hesperidin and of lemon bioflavonoid complex. No report on the use of the lemon complex was received. Reports on hesperidin indicated that it had been used as a flavor enhancer in flavored milk products at levels of 30 mg/l and at a level of 1.5% in dietary supplements. The total reported use by industry in 1976 was 420 pounds (190 kg), which corresponds to a per capita daily consumption of 2.5 µg.

Naringin was not included in the 1977 survey. However, an earlier (1970) survey indicated that a total of 3527 pounds (1600 kg) had been used in processed foods (Subcommittee on Review of GRAS List, 1972). This usage corresponds to a per capita daily consumption of 21 µg. Its reported use is shown in Table 4.

Table 4. Levels of Addition of Naringin Extract to Foods by Food Categories (Subcommittee on Review of GRAS List, 1972)

Food Category	Level of Addition mg/kg
Beverages, alcoholic	37.5
Baked goods	61.6
Soft candy	59.4
Gelatin puddings	52.9
Beverages, nonalcoholic	38.4
Frozen dairy products	28.5

Sunkist Growers, Inc., a major producer of citrus bioflavonoids reported their 1980/1981 sales of purified hesperidin to be about 100 kg, and hesperidin complex sales to be about 16,000 kg (Beisel, 1981b). Their estimated annual sales of naringin were about 20,000 kg and of lemon bioflavonoid complex about 15,000 kg (Nelson, 1980a). There are also several other domestic producers of bioflavonoids and sizeable quantities are imported from abroad. Principal use of hesperidin complex and lemon bioflavonoid complex appears to be as special dietary foods. Major use of naringin is for the preparation of chemical derivatives (Beisel, 1981c). A

number of bioflavonoid products are sold over-the-counter as "nutritional supplements," but no data are available on the extent of such usage. Nutrilite Products, a manufacturer of lemon bioflavonoid complex, reported that 31 products containing bioflavonoids were purchased in Southern California from grocery, drug, and health food stores and from direct sales and mail order houses (Cupello, 1981). Health food stores were the most common marketing source, representing the source of supply for more than half the products obtained. Mail order and direct sales organizations provided most of the remaining products. Fourteen of the 31 preparations contained no rutin fortification and 30 to 1000 mg lemon bioflavonoid complex (commercial source not stated). Rutin content of the tablets ranged from 0-42 µg, and averaged 12 µg/tablet. Recommended daily dose was 1 tablet for 74% of the 31 preparations, 1-2 tablets for 10%; 3, 4, or 6 tablets were recommended for 3% each of the preparations.

Rough calculations suggest that the per capita intakes of these bioflavonoids in natural sources are many times the amounts added to food or employed as "nutritional supplements." Kühnau (1976) has estimated that the average total flavonoid intake from a normal mixed diet in the United States is approximately 1 g/d. Accurate data on the intake of individual flavonoids are not available because of the complex mixture of these compounds in the fruits and vegetables normally consumed. Kühnau (1976) has calculated that approximately 160-175 mg of 4-oxoflavonoids would be consumed daily with a normal diet, and that approximately one-third of this amount would be obtained from fruit juice. This chemical group includes the most common bioflavonoids (hesperidin, naringin, diosmin, etc.) found in citrus fruits. Brown (1980) has estimated that perhaps 50 mg (quercetin equivalents) of promutagenic glycosides are included in the daily diet.

Orange juice represents the single most important source of hesperidin in the average American diet. About 10.5 million tons (9.6×10^9 kg) of oranges were produced in the United States in 1978/1979 (U.S. Department of Agriculture, 1980). About 330 thousand tons were exported, leaving 10.2 million tons (9.3×10^9 kg) for domestic consumption. Assuming half this amount consists of orange juice containing 375 mg hesperidin/kg (range 150-600 mg/kg) (Hendrickson and Kesterson, 1965), the daily per capita amount of hesperidin available for consumption would be about 44 mg from this source alone. This is probably an underestimate since the rag and pulp components eaten with the fresh fruit contain considerably higher concentrations of hesperidin than does the juice.

The juice of Florida grapefruit has been reported to contain 0.02-0.03% naringin (Kesterson and Hendrickson, 1953) and that of California grapefruit, about 0.06% (Poore, 1934). Assuming half the grapefruit is juice, the juice equivalent of grapefruit produced in the United States in 1978/1979 (U.S. Department of Agriculture, 1980) was about 1.1×10^9 kg. With an average naringin content of 0.04%, the daily per capita amount of naringin available for consumption from grapefruit was about 5.6 mg.

Reconstituted orange juice from the frozen concentrate contains about 3 µg rutin/g or about 550 µg in a 6 oz serving (Beisel, 1981a). This amount of lemonade would contain about 500 µg rutin. Reconstituted grapefruit juice contains <1 µg/g juice. Vegetables appear to be a more important food source of rutin than citrus fruits. Based on the composition data given in Table 3 and average serving size data (Pao, 1981), and assuming quercetin is present as rutin and no rutin is lost in table preparation, an average serving of tomatoes (raw) would provide about 1.1 mg rutin; potatoes (baked), 0.4 mg; lettuce, up to 20 mg depending on variety; Brussels sprouts, 5.3 mg; bell peppers (raw), 5.1 mg; and asparagus spears, 1.5 mg. Herrmann (1976) reported that the outer dry skins of colored onions (Allium cepa L.) contained 2.5-6.5% quercetin mainly in the free form. The outer and inner epidermis of the first three non-dried scales (Stuttgarten Riesen variety) contained 24,000 and 540 mg total quercetin per kg, respectively, in the form of glucosides; concentrations in the fourth to eighth scales were 10,600 and 400 mg/kg, respectively.

IV. BIOLOGICAL STUDIES

Absorption and metabolism

Most flavonoids are present in food as β-glycosides, and must be hydrolyzed before they can be absorbed (Kühnau, 1976). Enzymes splitting these glycosidic bonds are not normally present in digestive secretions or in the intestinal wall (Griffiths and Barrow, 1972). However, these flavonoid glycosides are extensively hydrolyzed by intestinal flora. Scheline (1968) reported that hesperidin incubated with rat cecal contents was rapidly converted to its aglycone (hesperetin) and was further metabolized to m-hydroxyphenylpropionic acid (m-HPPA). These products do not occur in germ-free animals and their formation is completely inhibited by antibiotic sterilization of the intestine (Griffiths and Barrow, 1972). Both the bacterially-generated aglycone and its catabolic products may be absorbed. The relative percentage of the glycoside which escapes bacterial destruction depends on the activity of the bacterial flora and on the degree of hydroxylation of the flavonoid molecule. Increased hydroxylation appears to increase the compound's susceptibility to bacterial degradation, e.g., luteolin, quercitrin, and rutin are preferentially subject to destruction by intestinal microorganisms (Brown and Dietrich, 1979). Kühnau (1976) estimated that approximately half of the daily flavonoid intake is absorbed from the gut as the aglycone.

After absorption, flavonoids are quickly bound in the liver as glucuronides and/or sulfate conjugates which are excreted as such in urine, or more often, in the bile (Kühnau, 1976). Biliary excretion into the intestine again exposes the flavonoid conjugate to possible bacterial degradation.

The major metabolic product of hesperidin and related flavonoids in rats and rabbits is m-HPPA (DeEds, 1968). 3,4-Dihydroxyphenylpropionic acid is the first metabolic product but this is rapidly subjected to microbiological dehydroxylations and methylations to form various compounds in addition to m-HPPA. Booth et al. (1958a) detected the following compounds in the urine of a rabbit given 330 mg/kg hesperidin by stomach tube: hesperetin, hesperetin glucuronide, m-HPPA, 3,4-dihydroxyphenylpropionic acid, 3-methoxy-4-hydroxyphenylpropionic acid, m-hydroxycinnamic acid, m-hydroxyhippuric acid, m-hydroxybenzoic acid, and 3-methoxy-4-hydroxybenzoic acid.

Honohan et al. (1976) administered hesperetin-3-^{14}C (1.7 mg/kg body wt) to rats and found rapid absorption and subsequent excretion of radioactivity. They estimated that the intestinal absorption was more than 90% of the administered dose. This estimate was based upon the radioactivity detected in the urine, tissues, and expired air of the animals. Nearly 40% of the administered radioactivity was expired as carbon dioxide. Primary metabolic products found in the urine were m-HPPA, 3,4-dihydroxyphenylpropionic acid, and 3-methoxy-4-hydroxyphenylpropionic acid. No intact flavonoid was detected.

In a single human volunteer, the major product found after hesperidin ingestion was 3-hydroxy-4-methoxyphenylhydracrylic acid, suggesting a species difference between man and rodents (Booth et al., 1958a).

The major metabolic product of naringin given to rats by stomach tube or subcutaneously was p-HPPA, rather than the meta form found after administration of hesperidin and other flavonoids. Small amounts of p-hydroxycinnamic acid and of p-hydroxybenzoic acid and its ethereal sulfate were also detected (Booth et al., 1958b), suggesting that some of the 3-carbon side chain of p-HPPA had undergone β-oxidation. When naringin was fed to a human volunteer, only the aglycone, naringenin, and its glucuronide could be detected in the urine. The failure to find evidence for splitting the naringin molecule in man was unexpected, since both quercetin and hesperetin undergo further breakdown.

Rutin undergoes extensive hydrolysis to its aglycone, quercetin, by intestinal microflora (Scheline, 1968). Quercetin in turn is subject to further bacterial degradation. Gugler et al. (1975) fed 4 g quercetin to human volunteers but detected none in the blood or urine at any of the intervals studied (20 to 540 min). They concluded that less than 1% of the dose had been absorbed. Only 53% of the ingested dose could be recovered in the feces, indicating extensive breakdown in the gut. Analysis for quercetin metabolites was not performed. Booth et al. (1956) had previously shown that at least four metabolites were excreted after oral ingestion of rutin or quercetin by man, rat, rabbit, and guinea pig. When rabbits were given 2 g quercetin, 195-285 mg of identifiable metabolites were found in the urine.

Acute toxicity

Singleton and Kratzer (1973) characterized the toxicities of the common plant flavonoids as "negligible." Hesperidin complex, and lemon bioflavonoid complex (Sunkist Growers, Inc.'s products) were administered by stomach tube to groups of 10 young, male Long-Evans rats (Primorganics, Inc., 1955). No deaths occurred with doses of 16.0 g/kg body wt. During a 72-h observation period, the rats appeared well and ate and drank in normal fashion. A lemon bioflavonoid complex and a lemon-orange flavonate glycoside were similarly administered to young rats at maximum levels of 24 g/kg body wt. The compositions of these preparations were not described. Again, no deaths occurred and no ill-effects were apparent (Primorganics, Inc., 1956). The Select Committee is not aware of other reports concerning the acute oral toxicity of citrus bioflavonoids. Singleton and Kratzer (1973) claimed that studies with various flavonoids, including naringin, showed no acute toxicity, and DeEds (1968) has stated that: "None of the flavonoids administered to experimental animals in single doses orally, intraperitoneally, or intravenously when possible, produced signs of acute toxicity."

Short-term studies

Citrus bioflavonoids were fed for 4 or 8 wk to 6-8-wk-old chicks at dietary levels of 0.5-5.0% (approximately 0.5-5.0 g/kg body wt) (Deyoe et al., 1962). The bioflavonoid composition was not stated, but hesperidin would presumably be the major member present. Normal growth, efficiency of feed conversion, and mortality occurred with dietary levels of 2.5% bioflavonoids; a marked reduction in growth and feed utilization was noted at the 5% level.

Guinea pigs were fed 10-20 mg rutin daily (about 15-30 mg/kg) for 8 wk at which time they were sacrificed (Griffith, 1955). The animals gained weight normally, showed no abnormal signs, and revealed no pathological changes at necropsy.

Wilson and DeEds (1940) fed rats up to 1.0% hesperidin or naringin in a standard diet (about 1 g/kg body wt) for 200 d. Although this level of naringin should have imparted a bitter taste to the diet, the food was not rejected by the rats. There was no significant difference between control and experimental rats in food intakes, growth curves, blood sugar levels, or visceral weights. No significant morphological changes were detected in the livers, hearts, spleens, adrenals, and testes of rats receiving hesperidin. Tissues of animals receiving naringin were not examined histologically.

Long-term studies

In a 400-d flavonoid feeding study, groups of 16 female weanling Sprague-Dawley rats were fed diets containing 2.5% (about 2-5 g/kg body wt/d) lemon bioflavonoid complex (LBC), LBC concentrate (2x), LBC concentrate (6x), hesperidin, naringin, or orange bioflavonoid complex concentrate (Patterson, 1960). At 70-75 d, half of the rats in each group were necropsied and groups of 8 animals were continued on each of the diets until 400 d had elapsed. Mean body weight of the group fed LBC was slightly (3%) but significantly less ($P<0.05$) than that of the control group at 75 d. Mean kidney:body wt ratios for all treated groups were less than the ratio for the control group but were significantly less ($P<0.05$) only for the animals fed naringin (9.8%), hesperidin (8.6%), and orange bioflavonoid complex concentrate (6.3%). Liver:body wt ratios were significantly greater for the groups fed LBC concentrate (2x) (9.7%), or LBC concentrate (6x) (17.1%), than the corresponding ratio for the control group. Histopathological examinations of organs at 75 d were made only for animals fed LBC concentrates. The pathologist reported that examination of kidney slices indicated a mild form of hydronephrosis. No significant histological changes were reported in the liver tissues.

Patterson (1961) reported the results of the examination of the rats (8 per group) that continued to be fed LBC, hesperidin, or bioflavonoid complex until killed at 400 d. No significant differences were found in body weights among treated or control

groups. Hematocrit, hemoglobin level, white cell count, percent polymorphonuclears and lymphocytes in the experimental and control animals did not differ significantly. There were no significant differences between treated and control groups in mean organ:body wt ratios for the thymus, heart, lungs, spleen, kidney, liver, or uterus, except for a 13.7% increase in liver:body wt ratio for the group fed orange bioflavonoid complex. Histopathological examination of the heart, spleen, kidney, liver, and lower left jaw revealed no abnormal changes.

Patterson (1962) reported the results of examination of the rats (8 per group) that were fed naringin, LBC concentrate (2x), or LBC concentrate (6x) until killed at 400 d. Mean body weight of the group receiving naringin was significantly ($P<0.05$) lower (10.8%) than that of the controls at necropsy. Consumption of the naringin diet was also lower, possibly attributable to its bitter flavor. No significant changes were found in hematological parameters in any of the treated groups. Differences found in lung:body wt ratios were associated with respiratory infections which occurred to various extents in all groups. Histological examination revealed a slight fatty metamorphosis (of an unnamed organ, presumably the liver) in four of the eight rats in the LBC concentrate (6x) group.

Quercetin and its derivatives (quercitrin, dihydroquercetin, and rutin) and neohesperidin dihydrochalcone, a citrus derived bioflavonoid, have been subjected to long-term animal feeding tests. Wilson et al. (1947) maintained six male and six female albino rats (strain not stated) on a diet containing 1% rutin (about 1 g/kg/d) for 400 d. Growth records were discontinued after 150 d, at which time weight records were normal. Necropsy examination at 400 d revealed no striking changes in any of the experimental rats compared with controls. A slight irregularity was noted in the vacuolation of adrenal cortical cells but was deemed to be of doubtful significance.

Ambrose et al. (1952) fed groups of 10 weanling rats, five of each sex, (strain not stated) diets containing 0.25, 0.5, or 1% quercetin or quercitrin (approximately 250, 500, and 1000 mg/kg/d) for 410 d. No abnormalities could be detected as judged by growth, food consumption, red and white blood cell counts, hemoglobin estimation, organ weights, or histopathological examination of adrenals, kidneys, spleen, liver, heart, thyroid, lungs, pancreas, stomach, small intestine, and bladder.

Booth and DeEds (1958) fed albino rats (strain not stated) dietary levels of dihydroquercetin as high as 1% (about 1 g/kg/d) for 450 d. Growth, food intake, organ weights, and gross and microscopic appearance of tissues did not differ significantly from controls. A high incidence of respiratory infections was noted in both control and experimental groups.

Gumbmann et al. (1978) fed neohesperidin dihydrochalcone to rats and dogs for more than 2 yr with no apparent carcinogenic or teratogenic effects.

A number of clinical studies have been reported in which bioflavonoid preparations have been given daily for periods up to 5 yr with no reported side-effects or toxic reactions. The preparations have been employed in a wide variety of conditions, generally those characterized by increased capillary fragility (Fostvedt, 1956). The usual maintenance dose of hesperidin has been 150-600 mg daily (about 2.5-10 mg/kg), together with equal amounts of vitamin C, and often in combination with lemon bioflavonoids, hesperidin methylchalcone, or other compounds. The diversity of therapeutic mixtures and dosages as well as the anecdotal and uncontrolled nature of the clinical reports complicate any evaluation of the therapeutic efficacy of these bioflavonoids. However, it is impressive that toxic effects have not been reported even when large doses were administered for many months. Van Buskirk (1946) reported no adverse effect on one individual susceptible to prolonged, severe bleeding, who had received from 1-4 g hesperidin daily for 2 yr and from 10-16 g daily for an additional 2 yr.

Mutagenicity

Recent reports have demonstrated the mutagenicity of several flavonoids which have been detected in certain citrus fruits (Bjeldanes and Chang, 1977; Brown et al., 1977; Sugimura et al., 1977; MacGregor and Jurd, 1978; Hardigree and Epler, 1978). All investigators utilized Salmonella typhimurium strains TA-98 and -100 as the test organisms. Bjeldanes and Chang (1977) found quercetin to be mutagenic without activation to both strains, as well as to TA-1538. Microsomal activation significantly increased its mutagenicity. The authors stated that the mutagenic activity of quercetin was 1-3 orders of magnitude less than the highly potent mutagens aflatoxin B_1 and 2-aminofluorene. Sugimura et al. (1977) confirmed the mutagenicity of quercetin and found that kaempferol was also a strong mutagen. The activities of both flavonoids were comparable with those of the known mutagens, o-aminoazotoluene and 4-aminobiphenyl with the TA-98 strain of S. typhimurium and with 3'-methyl-4-dimethylaminoazobenzene with the strain TA-100. MacGregor and Jurd (1978) and Hardigree and Epler (1978) also reported that quercetin and kaempferol were mutagenic without metabolic activation and that activation markedly enhanced their mutagenicity. Hardigree and Epler (1978) found quercetin to be mutagenic in strain D4 of Saccharomyces and in Escherichia coli as well as in S. typhimurium. Hardigree and Epler (1978) and Mortelmans and Griffin (1981) found rutin to be weakly mutagenic to S. typhimurium strains TA-98 and -100 with metabolic activation, but MacGregor and Jurd (1978), Brown and Dietrich (1979), and Tamura et al. (1980) reported no mutagenic activity. Hardigree and Epler (1978) noted that quercetin was detected after metabolic activation in their rutin

samples; the latter three groups of investigators found that treatment of rutin with mixed glycosidases was required for development of mutagenic activity.

Luteolin, diosmetin, hesperetin, hesperidin, naringin, and eriodictyol were nonmutagenic with the S. typhimurium assay with and without activation (Brown et al., 1977; MacGregor and Jurd, 1978).

Reproduction

Hesperidin complex, lemon bioflavonoid complex, and naringin were fed to mice in a study of the effect of flavonoids on fertility (Palmer and Patterson, 1954). It was estimated that daily flavonoid consumption ranged from 1.3-3.6 g/kg body wt. In a control period prior to adding bioflavonoids to the diets, the number of litters born to the three groups of nine females selected for treatment with naringin, hesperidin complex, and lemon bioflavonoid complex were 6, 8, and 8, respectively. After receiving the bioflavonoid diets, the number of females giving birth to litters was 9, 8, and 6, in the respective groups. Some of the rats from each treatment group were returned to the control diet, caged with males, and the number producing litters noted. However, the results reported in different tables in the report appear conflicting, and the Select Committee attached no significance to this aspect of the study. No adverse effects were noted in animals continued on the diets for various periods and then killed at the following times: lemon bioflavonoid complex diet, 176 d; hesperidin complex diet, 158 d; naringin diet, 219 d.

In another reproduction study, diets containing 4% (up to 10 g/kg body wt) LBC concentrate (2x), 2% LBC concentrate (6x), or 2% orange bioflavonoid complex concentrate were fed to groups of one male and two female weanling Sprague-Dawley rats which were caged together (Call and Patterson, 1960). After reaching puberty, the males were rotated among the cages. Ratios of the number of females that bore litters to the number fed the respective diets were: controls, 6/6; LBC concentrate (2x), 4/4; LBC concentrate (6x), 4/6; and orange bioflavonoid complex concentrate, 5/5. Mean number of days from start of the experiment to birth of litters was: controls, 67.3; LBC concentrate (2x), 56.8; LBC concentrate (6x), 77.8; and orange bioflavonoid complex concentrate, 62.0.

Wilson et al. (1947) maintained 15 female, 3-mo-old, albino rats (strain not specified) for 1 mo with a diet containing 1% rutin (about 1 g/kg/d). During this period they, together with control female rats from the same litter, were mated with the same males. Four litters were born to the females eating the rutin diet and two litters to the control females. Several of these litters were allowed to grow until weaning. No difference could be detected in the size or activity of the young from the two groups. The investigators also reported that the length of the estrus cycle in rats receiving the 1% rutin diet did not differ from that of control rats.

Carcinogenicity

No carcinogenic effects were reported from feeding rats with 1% rutin for 400 d (Wilson et al., 1947), 1% quercetin or quercitrin for 410 d (Ambrose et al., 1952), or 1% dihydroquercetin for 450 d (Booth and DeEds, 1958). However, Pamukcu and coworkers (1980) reported that quercetin is an intestinal and bladder carcinogen. They fed male and female weanling albino rats (Norwegian strain) a grain diet containing 1,000 ppm quercetin (about 100 mg/kg/d) for 14 mo. Weight gains and survival times were slightly less than those of the controls. Twenty of 25 rats fed quercetin developed multiple intestinal tumors of the ileal segment, seven fibro-adenomas, four adenomas, and nine adenocarcinomas. Three adenocarcinomas displayed mesenteric metastases. Five of the 25 rats developed bladder transitional cell carcinomas. No similar tumors were detected in control rats fed the grain diet.

Saito et al. (1980) found no significant difference in the incidence of tumors between control and quercetin-fed mice. Six-wk-old mice (ddY strain) of both sexes were fed pelleted diets containing 2% quercetin throughout their lifespan. Animals in both test and control groups developed leukemia and tumors of the lung, forestomach, mammary gland, adrenal, and soft tissues. However, four liver tumors were found only in quercetin-treated males and three uterine and two salivary tumors only in quercetin-treated females. The authors also called attention to a heart spindle cell sarcoma, an unusual tumor in mice, which developed in one quercetin-fed male and had metastasized to the liver, kidney, pancreas, forestomach, and diaphragm.

More recently, the carcinogenicity of quercetin and rutin was examined in an inbred ACI strain of rats (Hirono et al., 1981). Rats were fed diets containing 1% or 5% quercetin or 5% rutin for 540 d, or 10% quercetin or 10% rutin for 850 d. There was no significant difference in the incidence of tumors between the experimental groups and the control groups fed a normal basal diet.

In another study, Hosaka and Hirono (1981) utilized pulmonary bioassay to investigate the lung tumor response to quercetin in strain A mice. No significant differences in the incidence and multiplicity of lung adenomas were observed between mice fed diets containing 5% quercetin for 23 wk and those fed the basal diet. No metastases were observed.

Teratogenicity

No teratogenic effects were reported in reproductive studies with rutin by Wilson et al. (1947). Similarly, Gumbmann et al. (1978) could find no teratogenic effects in rats fed the synthetic bioflavonoid neohesperidin dihydrochalcone in a three-generation reproduction and teratology study.

Other studies

Flavonoids have been reported to have inhibitory effects on various enzyme systems, including hyaluronidase, histidine decarboxylase, xanthine oxidase, and succinoxidase (Griffith, 1955; Rodney et al., 1950). Of special interest is the finding that quercetin and other flavonoids hydroxylated in 5,3',4'- or 5,3',4',5'-positions inhibit o-methyltransferase which normally inactivates epinephrine and norepinephrine (Axelrod and Tomchick, 1959; DeEds, 1968). This enzymatic inhibition is demonstrable at flavonoid concentrations of 10^{-5} M, levels which may be present in body fluids under normal nutritional conditions (Kühnau, 1976). Prolongation of catecholamine action by this mechanism might account for some of the vascular effects which have been attributed to bioflavonoids. Varma and Kinoshita (1976) reported that a large number of flavonoids were highly active inhibitors of aldose reductase, an enzyme implicated in cataract formation in diabetes. Quercetin and quercitrin 2-acetate are the most potent inhibitors of aldose reductase thus far reported, inhibiting enzyme activity by 50% at levels of 10^{-7} and 4×10^{-8} M, respectively. Preliminary studies by the investigators indicated that these flavonoids prevented or delayed formation of cataracts in diabetic animals. Wattenberg et al. (1968) investigated the capacity of several flavonoids to induce increased aryl hydrocarbon hydroxylase activity (AHH) in the liver and lung of the rat. Tangeretin and nobiletin were found to be active inducers. Rutin was reported to have very weak AHH-inducing capacity (Wattenberg, 1980).

V. OPINION

Hesperidin is found in all citrus fruits as well as in a number of the fruits and vegetables commonly consumed. Naringin is found in relatively large amounts in grapefruit and in other citrus fruits as well. The amount of each of these bioflavonoids normally consumed in citrus fruit and in other dietary items is several orders of magnitude greater than that added to foods as flavoring agents.

Acute toxicities of purified hesperidin, hesperidin complex, and naringin are extremely low. Short-term (200 d) and long-term rat feeding studies with purified hesperidin and naringin at levels up to 2.5 g/kg body wt/d have revealed no adverse effects. Both compounds have been shown to be nonmutagenic in microbial systems, and no mutagenic flavonoids have been identified as constituents of hesperidin complex. Reproduction studies conducted with a limited number of mice consuming about 2.5 g/kg body wt of hesperidin complex or naringin daily indicated no adverse effect on fertility. Hesperidin complex, and purified hesperidin to a much lesser extent, have been used prophylactically and therapeutically for a variety of disorders, and is freely available without prescription. Hesperidin preparations have not proved toxic even when doses of several grams have been used daily for months or years. The Select Committee recommends that food grade specifications for hesperidin and hesperidin complex be developed.

In view of the foregoing, the Select Committee concludes that:

> There is no evidence in the available information on hesperidin (purified or hesperidin complex) or naringin that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when they are used at levels that are now current or that might reasonably be expected in the future.

The acute toxicity of lemon bioflavonoid complex is very low. No adverse effects were observed in a 400 d rat feeding study with lemon bioflavonoid complex fed at a level of 2.5 g/kg/d. Concentrates of lemon bioflavonoids included in the same study were associated with a mild form of hydronephrosis in animals necropsied at 75 d, but no significant histopathology was noted in animals necropsied at 400 d. Studies reporting a reduction in the number of rats bearing litters after being fed diets providing 2.5 g/kg body wt/d of lemon bioflavonoid complex or 5 g/kg/d of a lemon bioflavonoid complex concentrate were not considered to demonstrate a reduction in fertility in view of the very limited

number of animals involved. Lemon bioflavonoid complex contains rutin which can be hydrolyzed in vitro by intestinal bacteria to liberate quercetin. Whether such hydrolysis occurs in humans is not known. Quercetin has been shown to have mutagenic activity in microbial systems but investigators disagree on the mutagenicity of rutin. Conflicting reports have recently appeared concerning the carcinogenicity of quercetin. One group of investigators has reported a greater incidence of intestinal and bladder tumors in rats fed diets containing 0.1% quercetin than in rats fed a control diet. Another group of investigators has reported the occurrence of unusual tumors (but no overall increase in incidence of tumors) in mice fed 2% quercetin in their diet. Other investigators have failed to find increased incidence of tumors in rats fed diets providing 1, 5, or 10% quercetin. Although the weight of currently available data suggests noncarcinogenicity of quercetin, the definitive settlement of the issue merits further attention. Long-term feeding studies of rutin at levels of 1, 5, and 10% in the diet of rats have not demonstrated an increased incidence of tumors.

Information available to the Select Committee indicates the major use of the lemon bioflavonoid complex is as a component of special dietary foods. Food grade specifications for the complex should be developed. The amount of quercetin potentially derivable from the rutin present in the recommended daily intake of these foods is orders of magnitude lower than that present in glycosidic form in the vegetables, fruits, and fruit juices commonly consumed daily. Thus, hazard from consumption of quercetin glycosides can be little affected by the intake of lemon bioflavonoid complex. However, in the opinion of the Select Committee, questions exist about whether consumption of rutin from other food sources by humans results in exposure to quercetin.

Accordingly, the Select Committee concludes that:

> There is no evidence in the available information on lemon bioflavonoid complex that demonstrates, or suggests reasonable grounds to suspect, a hazard to the public when it is used at levels that are now current or that might reasonably be expected in the future.

No information was available to the Select Committee on the commercial production, marketing, composition, or animal feeding studies with dried concentrates of water-soluble flavonoids from washed, deoiled, ground peel and pulp of grapefruit and tangerines. No significant changes were observed in rats receiving about 2.5 g/kg/d of orange bioflavonoid complex concentrate in 75 d- or 400 d-feeding studies nor were adverse effects reported in reproduction studies in which the rats consumed up to 5 g/kg/d. However, the Select Committee has no information on the composition, production, or consumption of orange bioflavonoid complex concentrate.

In view of the foregoing, the Select Committee concludes that:

In view of the deficiency of relevant data, the Select Committee has insufficient information upon which to base an evaluation of dried concentrates of water-soluble flavonoids from washed, deoiled, ground peel and pulp of oranges, grapefruit, and tangerines.

VI. REFERENCES CITED

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VII. SCIENTISTS CONTRIBUTING TO THIS REPORT

1. Members of the Select Committee on GRAS Substances:

Joseph F. Borzelleca, Ph.D., Professor of Pharmacology, Medical College of Virginia, Health Sciences Division, Virginia Commonwealth University, Richmond, Virginia.

Harry G. Day, Sc.D., Professor Emeritus of Chemistry, Indiana University, Bloomington, Indiana.

Samuel J. Fomon, M.D., Professor of Pediatrics, College of Medicine, University of Iowa, Iowa City, Iowa.

Bert N. La Du, Jr., M.D., Ph.D., Professor, Department of Pharmacology, University of Michigan Medical School, Ann Arbor, Michigan.

John R. McCoy, V.M.D., Professor of Comparative Pathology, New Jersey College of Medicine and Dentistry, Rutgers Medical School, New Brunswick, New Jersey.

Gabriel L. Plaa, Ph.D., Professor and Chairman, Department of Pharmacology, University of Montreal Faculty of Medicine, Montreal, Canada.

Michael B. Shimkin, M.D., Professor Emeritus of Community Medicine and Oncology, School of Medicine, University of California, San Diego, La Jolla, California.

Ralph G.H. Siu, Ph.D., Consultant, Washington, D.C.

Marian E. Swendseid, Ph.D., Professor of Nutrition, School of Public Health, University of California, Los Angeles, California.

John L. Wood, Ph.D., Professor Emeritus, Department of Biochemistry, University of Tennessee Center for the Health Sciences, Memphis, Tennessee.

George W. Irving, Jr., Ph.D., (Chairman), Life Sciences Research Office, Federation of American Societies for Experimental Biology, Bethesda, Maryland.

2. LSRO staff:

Kenneth D. Fisher, Ph.D., Director
Frederic R. Senti, Ph.D., Associate Director
Richard G. Allison, Ph.D., Staff Scientist
Sue Ann Anderson, Ph.D., Staff Scientist
Herman I. Chinn, Ph.D., Senior Staff Scientist
John M. Talbot, M.D., Senior Medical Consultant

Report submitted by:

February 19, 1982
Date

George W. Irving, Jr., Chairman
Select Committee on GRAS Substances

PUBLIC HEARING ON HESPERIDIN, NARINGIN, AND
CITRUS BIOFLAVONOID EXTRACTS
HELD JUNE 22, 1981*

The following individuals made presentations:

1. C. Gordon Beisel, Director of Research and Development, Products Group, Sunkist Growers, Inc., Ontario, CA.

2. Dickson R. Loos, Counsel for Sunkist Growers, Inc., Ontario, CA.

3. James M. Cupello, Ph.D., Technical Director, Nutrilite Products, Inc., Buena Park, CA.

4. John S. Leland, General Counsel for Nutrilite Products, Inc., Buena Park, CA.

A written statement was also submitted after the meeting by Dr. Cupello.

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* A transcript of the hearing is available from Ace Federal Reporters, Inc., 444 North Capitol Street, Washington, DC 20001.

Attachment B

International Certification

1. Letter from Soil Association Certified Limited. Confirms that Citrox BC and Citrox 14W are compliant against the requirements for non organic aids and or additives as described in the Soil Association organic standards for use in conjunction with SA certified organic food products. May 6 2008. Philip Skentelbery. Certification officer (processors).
2. Letter from EcoCert France. Citrox ProCaro, Anti oxidant HXT001, citrus aurantium amara extract can be used in the formulation of cosmetics certified according to the ecological and organic standards.
3. Certificate of Accreditation. The International Organic Accreditation Service Inc. Citrox Bio Gro New Zealand Ltd. Has been duly evaluated and found to be in compliance with the International Federation of Organic Agriculture Movements (IFOAM). Activities covered: Crop production, Livestock, Wild products, Processing and handling, Input manufacturing, Retail, Certification transference, Grower Groups. January 1 2006. IFOAM Accredited.
4. Letter from OFG, Organic Farmers & Growers. Citrox Pro Alexin PNS 001, Pro Alexin PEL 001. Products approved for use in Organic systems. February 2 2009. Andrew Withington. Quality Systems Supervisor.
5. Austria Bio Garantie. Products that are certified by the ABG, bear the code number of AT-N-01-BIO. All of the submitted formulas are classified as BioPesticides and can be used in organic systems under the BPR.

All of the following formulas contain the Bitter orange which is our petitioned substance.
Citrox products submitted for certification and registration are as follows:

Info X gen certificates.
ProSino 14WPS2 = Surface sanitiser
ProSino 14XP = Surface cleaner (foaming)
ProGarda 14WP = Produce wash (fruits & veg, dairy, etc)
ProGarda 14T = Produce wash (fruits & veg etc) containing a surfactant
ProGarda FL = Food & beverage preservative (=Citrox BC concentrate)
ProGarda 14TP = Surface cleaner (low foaming)
PoAlexin PNS001 = Pre harvest treatment

EU Bio-Regulation (EEC) No. 834 / 07 idgF
Austrian IdgF food code, chapter A8
Association policy BIO AUSTRIA
ABG (AT-N-01-BIO)

6. State of Israel. Ministry of Agricultural and Rural Development. Plant Protection and Inspection Services. Department of Chemistry (Pesticides). Compostions of the products was found in compliance with the Israeli Organic Standards and with NOP standard. June 19 2008. Ethel Shafrut. Head of registration Department. Pesticides.
7. Greece. Ministry of Economics and Finance. Food sector A. Citrox BC compound can be circulated in the Greek market without demanding any special approval of circulation. July 31 2007
   Director of General chemistry
8. Letter from the New Zealand Food Safety Authority (NZFSA) concerning Citrox as a Processing Aid and food additive. In our view the bitter orange extract is a food ingredient. Under Standard 1.3.3 Processing Aids, food (which would include citrus extracts) and additives in Schedule 2 of the standard 1.3.1 may be used as Processing Aids. September 7 2007. Jean van den Beuken. Programme Manager (Composition).
9. BioGro New Zealand. Certified Organic certification for Citrox BC and Citrox BioKlenz. December 1 2008
10. BioGro New Zealand. Certified Organic certification for Citrox BioAlexin. December 1 2008.
11. New Zealand Food Safety Authority. (nzfsa) Formulas approved
    a. Citrox SurfaceSan . Surface sanitizer approval.
    b. Citrox EnviroFoam (Citrox 14X) Heavy duty cleaner.
    c. Citrox PWT. Potable Water Treatment.
    d. Citrox BC. Processing Aid.
    e. Citrox BioAlexin (was BioAlexin Plus). Fertilizer additive.
    f. Citrox BioKlenz. Processing Aid.
    g. Citrox ProAlexin. Plant Nutrient Synergist. Fertilizer additive.
    h. Citrox SaniWash (Citrox 14T). Heavy Duty Cleaner.
12. DEFRA. Department for Environment Food and Rural Affairs. Exotic Disease Prevention and Control Division.
    a. DEFRA Approval of Disinfectants: Citrox BC for Foot and Mouth. TADP 0046 February 18 2005
    b. Exotic Disease Prevention and Control Division. June 1 2005. Approval of Disinfectants: Citrox BC for the disease of Poultry. TADP 0046.
    c. DEFRA. Approval of Disinfectants: Citrox BC. June 6 2005 TADP 0046.
       General Purpose. PASSED
       Tuberculosis. PASSED
    d. DEFRA. Approved disinfectants Order 2006. Labeling of products newly approved for sale in England and Scotland. TADP 46.
       Foot and Mouth Disease. Scotland

Disease of Poultry. Order 2003. Scotland.
Tuberculosis Order England, Scotland and Wales.

B 1

Andrew Best

From: Philip Skentelbery [PSkentelbery@soilassociation.org]
Sent: 06 May 2008 11:42
To: citrox@btconnect.com
Cc: abest@citrox-products.co.uk
Subject: Confirmation of product's compliance

Dear Andrew & Ian,

Citrox BC & Citrox 14W+ compliant

Thank you very much for your email and the information supplied.

I am pleased to confirm that these products have been verified by Soil Association Certification Ltd (SA Certification) against the requirements for non-organic processing aids and/or additives, as described in the Soil Association organic standards. I can confirm that the named product is acceptable for use in conjunction with SA certified organic food products.

Please note that this confirmation of compliance with SA standards in no way constitutes approval, certification or other endorsement of the aforementioned product by SA Certification. As such the product may not be marketed or promoted in connection with the Soil Association or SA Certification.

Please do not hesitate to contact us should you require any further information regarding 'compliant products'.

Kind regards,

~~~~~~

Philip Skentelbery
0117 914 2411
Certification officer (processors)
www.soilassociation.org/certification

~~~~~~

B 2

ECOCERT France SAS

CHECKING OF RAW MATERIALS CONFORMITY ACCORDING TO THE ECOLOGICAL AND ORGANIC COSMETIC STANDARDS

If complying, this raw materials can be used in the formulation of cosmetics certified according to the ecological and organic standards. The applicant is responsible for the compliance of its products with general regulation.
THIS DOCUMENT IS NOT AN ORGANIC CERTIFICATE

Raw materials containing phenoxyethanol and parabens will not be accepted any more at the end of 2008.

Application n° 1443 Company: CITROX LTD

Commercial Name	INCI Name	Function	Origin	Conformity	Comments
ProCaro Anti-Oxidant HXT001	Citrus Aurantium Amara Extract (and) Citric Acid (and) Olea Europeae Extract (and) Water (and) Glycerin	**Anti-oxidant**	10% Plant Ingredient / 90% Natural Origin	OUI / YES	

Drawn up in l'Isle Jourdain, valid from 01/01/2008 Valid until 31/12/2008

Valérie LEMAIRE
Cosmetic Department

SAS au Capital de 1 226 200 € - BP 47 - 32600 L'ISLE-JOURDAIN
Tva intracommunautaire n° FR 614 299 66 787 00316
Tel. 05 62 07 34 24 Fax 05 62 07 11 67
E.mail : info@ecocert.fr
CREDIT MUTUEL 02200 00021537240 85 - SIRET 423 388 787 00316 - APE 743 B

B 3

Certificate of Accreditation

The International Organic Accreditation Service Inc.

responsible for the implementation of the IFOAM Accreditation Programme
attest that

Bio-Gro New Zealand Ltd

Has been duly evaluated and found to be in compliance with the International Federation of Organic Agriculture Movements (IFOAM) Basic Standards (2002) and IFOAM Criteria For Programmes Certifying Organic Agriculture and Processing (2002) and is accordingly deemed to be

IFOAM Accredited

Signed: (Executive Director) Date: January 1, 2006
Expires: December 31, 2006

Contract No.: 23 Contract expires June 30, 2009

Scope of Accreditation:

Programme(s) of the Certification Body:	Bio-Gro private standards and seal programme
Activities of this programme covered:	Crop production, Livestock, Wild products, Processing and handling, Input manufacturing, Retail, Certification transference, Grower Groups.
Activities of this programme not covered:	Aquaculture, Fibre processing, wild game and marine products
Programmes not covered:	USDA NOP; NZ Domestic Programme; Water; Salt; Health & Bodycare

For additional conditions and limitations of this certificate see reverse

Litho. in U.S.A.

Limitations and Conditions of Accreditation Certificate

- This certificate is issued to **Bio-Gro New Zealand, Level 9, 75 Ghuznee St., Wellington 6031, New Zealand** and is nontransferable.
- The accreditation is limited to the certification programme operated by the accredited entity that is listed on the front page of this certificate.
- The accreditation is limited to those areas of activity currently actively being certified within the certification programme and which are also covered by the IFOAM Basic Standards or the IFOAM Criteria for Certification Programmes. The activities covered are listed on the front page of this certificate.
- The accreditation is at all times subject to the terms and conditions of the accreditation contract, duly executed between IOAS and the accredited entity.
- The accreditation is based on the assessment of compliance with the IFOAM Basic Standards and the IFOAM Criteria for Organic Certification Programmes.
- This certificate is not a warranty, either expressed or implied, of organic or other quality of any product certified by the accredited entity. It testifies that the certification programme named above was evaluated and, subject to the conditions of accreditation, it's procedures and policies were found to conform with the IFOAM Criteria for Certification Programmes and it's standards to be in accord with the IFOAM Basic Standards. The accreditation process does not provide a guarantee against failure of the certification process to prevent intended or unintended contamination of product. The guarantee is limited to the professional execution of the accreditation process.
- This certificate contains two pages and is only valid when it is numbered, signed and dated by the International Organic Accreditation Service Inc.

B 3

The certificate is issued by the International Organic Accreditation Service of 102 ½ 1st Ave South, Suite 4, Jamestown, ND 58401, USA.
Tel: 1 701 252 4070; Fax: 1 701 252 4124; Email: Info@ioas.org

ORGANIC FARMERS & GROWERS
Organic Certification UK2

B 4

Mr C Ripley
Citrox Ltd
Unit 9, River Court
Brighouse Road
Riverside Park
Middlesbrough
Cleveland
TS2 1RT

Organic Farmers & Growers
The Old Estate Yard
Shrewsbury Road
Albrighton
Shrewsbury
SY4 3AG

Telephone: 01939 291800
Fax: 01939 291250

Date: 17/02/2009

Dear Mr Ripley,

I am happy to enclose an OF&G Evaluation Scheme Certificate listing products from your company that we have approved for use in organic systems.

The certificate is valid for the coming year. Please check it carefully for errors and return it to me with amendments, if any errors are found.

The basis for approval for the products is as follows (OF&G Control Manual references are given where appropriate):

Plant Disease Control

Pro Alexin PNS 001- Product Approved for Use in Organic Systems
Pro Alexin PEL 001 - Product Approved for Use in Organic Systems

Please keep us informed if you make any changes to the labels and leaflets or make reference to OF&G on your website. If you use reference to OF&G on labels etc please gain our approval prior to final print to save costly changes.

If you wish to add more products to the certificate, or if the current product specifications change in any way, you must complete a copy of the Evaluation Scheme Specification Sheet and send it to us for assessment. The cost is £32+Vat for each new product.

Should you require any additional information, please contact your Certification Officer on 0845 330 5122 ext 232

Yours sincerely,

Andrew Withington - Quality Systems Supervisor

B 4

ORGANIC FARMERS & GROWERS EVALUATION SCHEME

Certificate of Evaluation for Compliance

This is to certify that the products or services listed below comply with the OF&G Standards and are approved for use in Organic Systems:

Citrox Ltd

Unit 9, River Court Brighouse Road Riverside Park Middlesbrough Cleveland TS2 1RT
Tel: 01642 241777

Category
Plant Health Products

Product Approved for Use in Organic Systems
Pro Alexin PNS 001
Pro Alexin PEL 001

Registration Number:	UKE0478	Renewal Month:	02 (February)
Certificate Expiry Date:	28/2/2010	Date Issued:	17/2/2009

Signed by:..... Katie Owens - Certification Officer

Suche ...

Home English

The ABG
- The Company
- Mission Statement
- The team
- Services
- Terms and Conditions
- Accreditation
- Networks
- News
- Locations

Organic Farming
Bio-processing, trade
International
NOP
Guidelines
Online Services
Orders
Consumer Info
Links
Contact

AUSTRIA BIO GARANTIE
Österreichs führende Bio-Kontrollstelle

Customer Portal
Electronic certificates
Organic farmers start
Organic Entry
VerarbeiterInnen
AgroVet

The Company

Austria Bio Garantie - the leading bio-control of Austria

Whether for Austria, Europe or the U.S.:
We are your partner when it comes to organic certification.

The Austria Bio Garantie GmbH (ABG) was founded in 1993 as a bio-control. Task is to control and certification of organic products: from the biological original product through to the final processor.
The ABG is working on behalf of the food authorities. Products that are certified by the ABG, bear the code number of AT-N-01-BIO.

Stations of success

- 1994: Recognition by the food authorities in all provinces
- 1998: Accreditation of ABG by the Federal Ministry for Economic Affairs (EN 45011)
- 2003: recognition as a NOP certification (organic standards of the USA) by the U.S. Department of Agriculture (USDA)
- since 1998 member of IFOAM (International Federation of Organic Agriculture Movements).
- Today: control agreements with around 10,700 organic farmers, and 1000 processors, and international cooperation with inspection and certification bodies.

Approximately 11,600 establishments offer in the production, processing and trade area and the best service to our claim, has led us to Austria's leading organic inspection done! With 40 service employees and about 100 bookkeeper, we serve our clients from two offices covering areas in Austria and neighboring countries.

ABG: Non-Profit Non-profit company with the best service and international contacts

The ABG is a non-profit non-profit company with the highest quality standards and a strong service orientation. With our service department, best-trained and experienced staff KontrollorInnen that come from practice, we guarantee our clients not only competent inspection and certification activities and the best service! Our lean organizational structure and our identity as a non-profit, non-profit organization, our customers benefit from best price-performance ratio.

Internationally Top

Cooperation with leading bodies in Europe and excellent international contacts make us the ideal partner when it comes to international trade of organic foods.

Austria Bio Garantie GmbH
Königsbrunnerstraße 8
A-2202 Enzersfeld
Tel: +43 (0) 2262 / 672212
Fax: +43 (0) 2262 / 674143
Email: nw@abg.at
Web: www.abg.at

B 5

B 5

BioGarantie/InfoXgen.com

For the mentioned product(s) was furnished a proof of GMO-free production. The mentioned enactments/directives for the single products have been observed. This could be the 2 mentioned guidelines as well as further international and also privately organised guidelines.

• interpretation of the interdiction of the use of genetic engineering in the production and manipulation of organic food (created by ALOG - Arbeitsgemeinschaft Lebensmittel ohne Gentechnik) regulation (EC) Nr. 2092/91 idgF Schweizerischen Bio-Verordnung (SR 910.18)

• Codex-Guidelines for the definition of "GMO-free " according to the Austrian Codex Alimentarius (according to the decree of the Austrian Ministry for social security and generations, dated 7th, march 2001, GZ. 32.048/10-IX/B/1/01)

• validity of this declaration of assurance: until 28.02.2010

The admission was given based on the information that the enterprise supplied to InfoXgen.

Hundsbichler GMBH Österreichische Laberzeugung

ID-Nummer: 301040

B 5

PRODUKTSUCHE

Suche nach Produkt

Bereich: Alle

Produkt:

Produktbereich: ---

Status: ---

Erlaubt Nach: ---

Suche nach Anbieter

ID-Nummer:

Name: hundsbichler

Land: ---

-PLZ:

Ort:

Suche

Druckauswahl: Alle auswählen Hinzufügen Leeren Anzeigen Drucken

	Anbieter	Adresse	Kategorie	Handelsbezeichnung	
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Reinigungs- und Desinfektionsmittel	ProGarda 14T
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Zusatzstoffe/ Anhang VIII A	ProGarda FL
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Reinigungs- und Desinfektionsmittel	ProSino 14TP=14TAH
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Pflanzenhilfsstoffe	ProAlexin

<<<>>> [Seite 2 von 2] Treffer: 14

B 5

PRODUKTSUCHE

Suche nach Produkt

Bereich: Alle

Produkt:

Produktbereich: ---

Status: ---

Erlaubt Nach: ---

Suche nach Anbieter

ID-Nummer:

Name: hundsbichler

Land: ---

-PLZ:

Ort:

Suche

Druckauswahl: Alle auswählen Hinzufügen Leeren Anzeigen Drucken

	Anbieter	Adresse	Kategorie	Handelsbezeichnung	
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Reinigungs- und Desinfektionsmittel	ProSino 14WPS2
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Reinigungs- und Desinfektionsmittel	ProSino 14XP=14XAH
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Enzyme (LM Zusatzstoffe)	Lab-Pulver
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Enzyme (LM Zusatzstoffe)	BioRen Naturlab Pulver
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Enzyme (LM Zusatzstoffe)	Labextrakt flüssig
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Reinigungs- und Desinfektionsmittel	ProGarda 14WP/CP
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Enzyme (LM Zusatzstoffe)	Labpaste
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Enzyme (LM Zusatzstoffe)	Sacco Käse-, Sauermilch- und Joghurtkulturen

B 5

☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Enzyme (LM Zusatzstoffe)	getrocknete Kälbermägen
☐		Hundsbichler GMBH Österreichische Laberzeugung	Sportplatzweg 5, 6336 Langkampfen	Enzyme (LM Zusatzstoffe)	BioRen Naturlab flüssig

<<<>>> [Seite 1 von 2] Treffer: 14

B 5

21.10.2009

InfoXgen.com

Für das/die genannte/n Produkt/e wurde der Nachweis erbracht, dass bei der Herstellung keine gentechnische Verfahren eingesetzt wurden. Es werden die beim jeweiligen Produkt angeführten Verordnungen/Richtlinien erfüllt. Dies können die beiden angeführten Richtlinien sein, sowie weitere internationale und privatrechtliche Richtlinien.

- **Interpretation des Verbotes der Anwendung von Gentechnik in der Erzeugung und bei der Verarbeitung von biologischen Lebensmitteln (erstellt von ALOG - Arbeitsgemeinschaft Lebensmittel ohne Gentechnik) Verordnung (EWG) Nr. 2092/91 idgF Schweizerischen Bio-Verordnung (SR 910.18)**

- **Codex-Richtlinie zur Definition der "Gentechnikfreiheit" gemäß Österreichischem Lebensmittelbuch**
  (gemäß Erlass des BM für soziale Sicherheit und Generationen vom 7.März 2001, GZ. 32.048/10-IX/B/1/01)

- **Gültigkeitszeitraum der Zusicherungerklärung:** bis 28.02.2010

Die Aufnahme des/r Produkte/s in die Datenbank erfolgt aufgrund der Angaben, die das Unternehmen gegenüber der InfoXgen gegeben hat.

Hundsbichler GMBH Österreichische Laberzeugung

ID-Nummer: **301040**

Adresse:

Sportplatzweg 5
6336 Langkampfen

Kontakt:

Telefon: 05372/62256
Email: office@hundsbichler.com
Homepage: www.hundsbichler.com

Produkte dieses Herstellers:

Reinigungs- und Desinfektionsmittel

Handelsbezeichnung: ProSino 14WPS2

Zusatzinformation: Desinfektionsmittel – tauchen, besprühen und vernebeln

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

B 5

21.10.2009

InfoXgen.com

Für das/die genannte/n Produkt/e wurde der Nachweis erbracht, dass bei der Herstellung keine gentechnische Verfahren eingesetzt wurden. Es werden die beim jeweiligen Produkt angeführten Verordnungen/Richtlinien erfüllt. Dies können die beiden angeführten Richtlinien sein, sowie weitere internationale und privatrechtliche Richtlinien.

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- **Gültigkeitszeitraum der Zusicherungerklärung:** bis 28.02.2010

Die Aufnahme des/r Produkte/s in die Datenbank erfolgt aufgrund der Angaben, die das Unternehmen gegenüber der InfoXgen gegeben hat.

Hundsbichler GMBH Österreichische Laberzeugung

ID-Nummer: **301040**

Adresse:

Sportplatzweg 5
6336 Langkampfen

Kontakt:

Telefon: 05372/62256
Email: office@hundsbichler.com
Homepage: www.hundsbichler.com

Produkte dieses Herstellers:

Reinigungs- und Desinfektionsmittel

Handelsbezeichnung: ProSino 14XP=14XAH

Zusatzinformation: stark schäumender Desinfektionsreiniger

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

B 5

21.10.2009

InfoXgen.com

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- **Gültigkeitszeitraum der Zusicherungerklärung:** bis 28.02.2010

Die Aufnahme des/r Produkte/s in die Datenbank erfolgt aufgrund der Angaben, die das Unternehmen gegenüber der InfoXgen gegeben hat.

Hundsbichler GMBH Österreichische Laberzeugung

ID-Nummer: **301040**

Adresse:

Sportplatzweg 5
6336 Langkampfen

Kontakt:

Telefon: 05372/62256
Email: office@hundsbichler.com
Homepage: www.hundsbichler.com

Produkte dieses Herstellers:

Reinigungs- und Desinfektionsmittel

Handelsbezeichnung: ProGarda 14WP/CP

Zusatzinformation: Progarda 14WP: Waschzusatz Obst und Gemüse offenporig – natürliche Keimkontrolle
ProGarda 14CP: natürliche Keimkontrolle Milchprodukte

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

Reinigungs- und Desinfektionsmittel

Handelsbezeichnung: ProSino 14TP=14TAH

Zusatzinformation: nicht schäumender Desinfektionsreiniger

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:
ABG (AT-N-01-BIO)

B 5

InfoXgen.com

Für das/die genannte/n Produkt/e wurde der Nachweis erbracht, dass bei der Herstellung keine gentechnische Verfahren eingesetzt wurden. Es werden die beim jeweiligen Produkt angeführten Verordnungen/Richtlinien erfüllt. Dies können die beiden angeführten Richtlinien sein, sowie weitere internationale und privatrechtliche Richtlinien.

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- **Codex-Richtlinie zur Definition der "Gentechnikfreiheit" gemäß Österreichischem Lebensmittelbuch**
  (gemäß Erlass des BM für soziale Sicherheit und Generationen vom 7.März 2001, GZ. 32.048/10-IX/B/1/01)

- **Gültigkeitszeitraum der Zusicherungerklärung:** bis 28.02.2010

Die Aufnahme des/r Produkte/s in die Datenbank erfolgt aufgrund der Angaben, die das Unternehmen gegenüber der InfoXgen gegeben hat.

Hundsbichler GMBH Österreichische Laberzeugung

ID-Nummer: **301040**

Adresse:

Sportplatzweg 5
6336 Langkampfen

Kontakt:

Telefon: 05372/62256
Email: office@hundsbichler.com
Homepage: www.hundsbichler.com

Produkte dieses Herstellers:

Reinigungs- und Desinfektionsmittel

Handelsbezeichnung: ProGarda 14T

Zusatzinformation: Waschzusatz Obst und Gemüse geschlossene Schale – natürliche Keimkontrolle

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

Reinigungs- und Desinfektionsmittel

Handelsbezeichnung: ProSino 14TP=14TAH

Zusatzinformation: nicht schäumender Desinfektionsreiniger

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

B 5

21.10.2009

InfoXgen.com

Für das/die genannte/n Produkt/e wurde der Nachweis erbracht, dass bei der Herstellung keine gentechnische Verfahren eingesetzt wurden. Es werden die beim jeweiligen Produkt angeführten Verordnungen/Richtlinien erfüllt. Dies können die beiden angeführten Richtlinien sein, sowie weitere internationale und privatrechtliche Richtlinien.

- **Interpretation des Verbotes der Anwendung von Gentechnik in der Erzeugung und bei der Verarbeitung von biologischen Lebensmitteln (erstellt von ALOG - Arbeitsgemeinschaft Lebensmittel ohne Gentechnik) Verordnung (EWG) Nr. 2092/91 idgF Schweizerischen Bio-Verordnung (SR 910.18)**

- **Codex-Richtlinie zur Definition der "Gentechnikfreiheit" gemäß Österreichischem Lebensmittelbuch**
  (gemäß Erlass des BM für soziale Sicherheit und Generationen vom 7.März 2001, GZ. 32.048/10-IX/B/1/01)

- **Gültigkeitszeitraum der Zusicherungerklärung:** bis 28.02.2010

Die Aufnahme des/r Produkte/s in die Datenbank erfolgt aufgrund der Angaben, die das Unternehmen gegenüber der InfoXgen gegeben hat.

Hundsbichler GMBH Österreichische Laberzeugung

ID-Nummer: **301040**

Adresse:

Sportplatzweg 5
6336 Langkampfen

Kontakt:

Telefon: 05372/62256
Email: office@hundsbichler.com
Homepage: www.hundsbichler.com

Produkte dieses Herstellers:

Zusatzstoffe/ Anhang VIII A

Handelsbezeichnung: ProGarda FL

Zusatzinformation: ProGarda FL001 Zusatzstoff Lebensmittel – natürliche Keimkontrolle
ProGarda FL002 Zusatzstoff Milch – natürliche Keimkontrolle
ProGarda FL005 Zusatzstoff Getränke – natürliche Keimkontrolle
ProGarda FL006 Zusatzstoff Fleischverarbeitung – natürliche Keimkontrolle

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

Reinigungs- und Desinfektionsmittel

Handelsbezeichnung: ProSino 14TP=14TAH

Zusatzinformation: nicht schäumender Desinfektionsreiniger

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF

B 5

B 5

21.10.2009

InfoXgen.com

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- **Interpretation des Verbotes der Anwendung von Gentechnik in der Erzeugung und bei der Verarbeitung von biologischen Lebensmitteln (erstellt von ALOG - Arbeitsgemeinschaft Lebensmittel ohne Gentechnik) Verordnung (EWG) Nr. 2092/91 idgF Schweizerischen Bio-Verordnung (SR 910.18)**

- **Codex-Richtlinie zur Definition der "Gentechnikfreiheit" gemäß Österreichischem Lebensmittelbuch**
  (gemäß Erlass des BM für soziale Sicherheit und Generationen vom 7.März 2001, GZ. 32.048/10-IX/B/1/01)

- **Gültigkeitszeitraum der Zusicherungerklärung:** bis 28.02.2010

Die Aufnahme des/r Produkte/s in die Datenbank erfolgt aufgrund der Angaben, die das Unternehmen gegenüber der InfoXgen gegeben hat.

Hundsbichler GMBH Österreichische Laberzeugung

ID-Nummer: **301040**

Adresse:

Sportplatzweg 5
6336 Langkampfen

Kontakt:

Telefon: 05372/62256
Email: office@hundsbichler.com
Homepage: www.hundsbichler.com

Produkte dieses Herstellers:

Reinigungs- und Desinfektionsmittel

Handelsbezeichnung: ProSino 14TP=14TAH

Zusatzinformation: nicht schäumender Desinfektionsreiniger

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

B 5

21.10.2009

InfoXgen.com

Für das/die genannte/n Produkt/e wurde der Nachweis erbracht, dass bei der Herstellung keine gentechnische Verfahren eingesetzt wurden. Es werden die beim jeweiligen Produkt angeführten Verordnungen/Richtlinien erfüllt. Dies können die beiden angeführten Richtlinien sein, sowie weitere internationale und privatrechtliche Richtlinien.

- **Interpretation des Verbotes der Anwendung von Gentechnik in der Erzeugung und bei der Verarbeitung von biologischen Lebensmitteln (erstellt von ALOG - Arbeitsgemeinschaft Lebensmittel ohne Gentechnik) Verordnung (EWG) Nr. 2092/91 idgF Schweizerischen Bio-Verordnung (SR 910.18)**

- **Codex-Richtlinie zur Definition der "Gentechnikfreiheit" gemäß Österreichischem Lebensmittelbuch**
  (gemäß Erlass des BM für soziale Sicherheit und Generationen vom 7.März 2001, GZ. 32.048/10-IX/B/1/01)

- **Gültigkeitszeitraum der Zusicherungerklärung:** bis 28.02.2010

Die Aufnahme des/r Produkte/s in die Datenbank erfolgt aufgrund der Angaben, die das Unternehmen gegenüber der InfoXgen gegeben hat.

Hundsbichler GMBH Österreichische Laberzeugung

ID-Nummer: **301040**

Adresse:

Sportplatzweg 5
6336 Langkampfen

Kontakt:

Telefon: 05372/62256
Email: office@hundsbichler.com
Homepage: www.hundsbichler.com

Produkte dieses Herstellers:

Pflanzenhilfsstoffe

Handelsbezeichnung: ProAlexin

Zusatzinformation: Pflanzenhilfsstoff zur Stärkung der Abwehr von schädlichen Mikroorganismen im Boden und auf der Pflanze.

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

Zusatzstoffe/ Anhang VIII A

Handelsbezeichnung: ProGarda FL

Zusatzinformation:
ProGarda FL001 Zusatzstoff Lebensmittel – natürliche Keimkontrolle
ProGarda FL002 Zusatzstoff Milch – natürliche Keimkontrolle
ProGarda FL005 Zusatzstoff Getränke – natürliche Keimkontrolle
ProGarda FL006 Zusatzstoff Fleischverarbeitung – natürliche Keimkontrolle

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF

Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

Reinigungs- und Desinfektionsmittel

Handelsbezeichnung:	ProSino 14TP=14TAH
Zusatzinformation:	nicht schäumender Desinfektionsreiniger

Erlaubt bei:

EU Bio-Verordnung (EWG) Nr. 834/07 idgF
Österreichischer Lebensmittelkodex, Kapitel A8 idgF
Verbandsrichtlinie BIO AUSTRIA

Zertifiziert von:

ABG (AT-N-01-BIO)

B 5

B 6

TRANSLATION FROM HEBREW

State Of Israel

Ministry of Agriculture and Rural Development – Plant Protection and Inspection Services.
Department of Chemistry (Pesticides). www.ppis.moag.gov.il

To:
Uri Rosenberg
AGRON Ltd.
P.O.Box 2196
Rehovot 76121

19.June 2008

Re: Citrox Proalexin, Citrox Progarda

I confirm receiving additional data. The documents that were submitted were checked by us.

The composition of the products was found in compliance with the Israeli Organic Standard and with NOP standard.
We recommend the head of Plant Protection and Inspection Services to approve conduction of trials with the products Citrox Proalexin and Citrox Progarda , following the approval of the tox committee.

Ethel Shafrut,
Head of registration Department
Pesticides

משרד החקלאות ופיתוח הכפר – השרותים להגנת הצומח ולביקורת
הקריה החקלאית ת.ד. 78, בית דגן 50250 טל. 03-9681572 פקס. 03-9681582
WWW.PPIS.MOAG.GOV.IL
תחום כימיה (תכשירי הדברה)

ט״ז סיון תשס״ח
19 יוני 2008

לכבוד
אורי רוזנברג
אגרון בע״מ
ת.ד. 2196
רחובות 76121

א.נ.,

הנדון: **Citrox Proalexin, Citrox Progarda**

אני מאשרת את קבלת ההשלמות. המסמכים שהוגשו נבדקו.

הרכב התכשירים נמצאו מתאים לסטנדרט הישראלי וסטנדרט ה- NOP .
אנו ממליצים למנהל השרותים להגה״צ ולבקורת לאשר ביצוע ניסויים בתכשירים Citrox Progarda ו- Citrox Proalexin בכפוף להחלטת הועדה לאישור תכשירים ניסיוניים.

בברכה
אטל שפרוט
מנהלת המחלקה לרישוי
תכשירי הדברה

העתק:
מ. פרוינד
ר. אשכנזי
תיקי התכשירים

B 6

TRANSLATION

B 7

ΕΛΛΗΝΙΚΗ ΔΗΜΟΚΡΑΤΙΑ

ΥΠΟΥΡΓΕΙΟ ΟΙΚΟΝΟΜΙΑΣ & ΟΙΚΟΝΟΜΙΚΩΝ

Ministry of Economics and Finance
Secretariat of Tax and customs issues
Buro of General Chemist office
Food Sector A'
Address: A. Tsoxa 16
Postal Code: 11521
Info: D. Chrysafidis
Tel: 2106479405
Fax: 2106467725

Athens, July 31st 2007

Protocol Nr. 3114370/1330/2007

To: POLYPAN
POLYPAN GROUP SA
2b Lefkados & KYprou 5 str
18346 Moschato

SUBJECT: Circulation of Product CITROX BC
RE: Your document dated 13/6/2007

Answering to your request, we would like to inform you that the product CITROX BC compound of natural flavor of citrus with organic acids as was declared and with ingredients: citrus natural flavor, Organic acids: malic acid (E296), citric acid (E330), ascorbic acid (E300), carrier: glycerin, water,
is according to the remarks and regulations of national and EU legislation and can be circulated in the greek market without demanding any special approval of circulation.

The Director of General chemistry

ΑΚΡΙΒΕΣ ΑΝΤΙΓΡΑΦΟ

ΙΩΑΝΝΗΣ ΧΡΟΝΑΙΟΣ

Εσωτ. Διανομή :
Δ/νση Τροφίμων

ΜΑΡΙΝΑ ΚΟΡΔΩΘΗ

Β 7

ΥΠΟΥΡΓΕΙΟ ΟΙΚΟΝΟΜΙΑΣ & ΟΙΚΟΝΟΜΙΚΩΝ

ΓΕΝΙΚΗ ΓΡΑΜΜΑΤΕΙΑ ΦΟΡΟΛΟΓΙΚΩΝ & ΤΕΛΩΝΕΙΑΚΩΝ ΘΕΜΑΤΩΝ
ΓΕΝΙΚΗ ΔΙΕΥΘΥΝΣΗ ΓΕΝΙΚΟΥ ΧΗΜΕΙΟΥ ΤΟΥ ΚΡΑΤΟΥΣ
ΔΙΕΥΘΥΝΣΗ ΤΡΟΦΙΜΩΝ
ΤΜΗΜΑ Α'

Ταχ. Διεύθυνση: Αν. Τσόχα 16
Ταχ. Κώδικας: 115 21
Πληροφορίες: Δ. Χρυσαφίδης
Τηλέφωνο : 210 64 79 405
Τέλεφαξ : 210 64 67725

Αθήνα 31 Ιουλίου 2007

Αρ.Πρωτ.:3014370/1330/ 2007

Π Ρ Ο Σ : POLYPAN
ΠΟΛΥΠΑΝ ΓΚΡΟΥΠ Α.Ε.Ε.
28 Λευκάδος & Κύπρου 5
183 46 Μοσχάτο

ΘΕΜΑ : Κυκλοφορία προϊόντος σας CITROX BC

ΣΧΕΤ. : έγγραφό σας από 13/6/2007.

Απαντώντας σε σχετικό αίτημά σας, σας ενημερώνουμε ότι το προϊόν CITROX BC μίγμα φυσικού αρώματος νεραντζιού με οργανικά οξέα με την επισήμανση που μας κατατέθηκε και με συστατικά : Αρωματικός παράγοντας : φυσικό άρωμα νεραντζιού, Οργανικά οξέα : μηλικό οξύ (Ε296). κιτρικό οξύ (Ε330), ασκορβικό οξύ (Ε300), Φορέας : γλυκερίνη, νερό, είναι σύμφωνο ως προς την επισήμανση με τις διατάξεις της Εθνικής και Κοινοτικής Νομοθεσίας και μπορεί να κυκλοφορήσει στην Ελληνική αγορά χωρίς να απαιτείται ιδιαίτερη έγκριση κυκλοφορίας.

Ο ΠΡΟΪΣΤΑΜΕΝΟΣ ΤΗΣ ΔΙΕΥΘΥΝΣΗΣ

ΑΚΡΙΒΕΣ ΑΝΤΙΓΡΑΦΟ

ΙΩΑΝΝΗΣ ΧΡΟΝΑΙΟΣ

Εσωτ. Διανομή :
Δ/νση Τροφίμων

B 8

7 September 2007

Clive Morrison
Cebec Group NZ Ltd
Email clive.morrison@cebecgroup.com

Dear Clive

Citrox as a processing aid and a food additive

Thank you for your questions emailed to New Zealand Food Safety Authority (NZFSA) and Food Standards Australia New Zealand (FSANZ) about permitted uses of *Citrox* brand sanitiser under the Food Standards Code (the Code). This letter follows an email to you from Dean Stockwell to confirm that we have met to consider our views. We note that *Citrox* is a brand name of a product that has active ingredients that are: a bitter orange extract, citric acid, ascorbic acid, malic acid, and glycerine.

In our view, from the information supplied, the bitter orange extract is a food ingredient, and the remaining ingredients are food additives that are listed in Schedule 2 of Standard 1.3.1 Food Additives of the Code. These food additives are permitted to be used in a wide range of foods as listed in Schedule 1 of Standard 1.3.1 and would subject to good manufacturing practice and but must not be added to foods where stated that a food must not contain additives in Schedules 2, 3 , and 4, unless specific permission is given.

Under Standard 1.3.3 Processing Aids, food (which would include citrus extracts) and additives in Schedule 2 of Standard 1.3.1 may be to be used as processing aids in addition to substances listed in tables in the standard. It is important to note that under the definition of processing aids, substances used as processing aids must not perform a technological function in the final food and they are generally absent or present in trace amounts. Food additives, however, perform a technological function in the final food and remain in the food or its by-products do.

In our view, there appears to be three situations that cover the use of sanitising antimicrobial products, such as *Citrox*:

1. To sanitise plant and equipment

This is outside the scope of the Code, but products may be approved by NZFSA for use as a sanitiser under the Animal Products legislation. These are listed in the register which can be viewed at (note listings for *Citrox*).

http://www.nzfsa.govt.nz/animalproducts/registers-lists/manual15/index.htm

2. To sanitise food or surfaces of food with no technological effect in the final food (ie use as a processing aid)

In our view, the substances in *Citrox* may be used to wash product surfaces under the Standard 1.3.3 Processing aids, provided that no residues remain that are capable of performing a technological function in the final food. This is consistent with the definition of processing aid. This may be achieved by applying a sanitiser and rinsing or using a sanitiser that inactivates with time such as chlorine.

3. To sanitise food or food surfaces that results in a technological effect in the final food (ie use as a food additive)

Food additives listed in Schedule 2 of Standard 1.3.1 Food Additives, including those in *Citrox* may be added to foods listed in Schedule 1 of Standard 1.3.1 that permit the use of Schedule 2 additives. However, it should be noted that Schedule 1 does not permit food additives to be added to fresh meats, fish, fruit and vegetables and limited additives to juices. Furthermore, where food is treated with Schedule 2 food additives (eg acidity regulators to product surfaces) that remain active in the final food, would need to be listed in the ingredients list (ie with class name (eg acidity regular) and additive names (critic acid, glycerine, ascorbic acid, malic acid)) and food ingredient (bitter orange extract). The name of the product could also be required to reflect the treatment.

In our view, it likely that the ingredients in *Citrox* are active on treated surfaces until rinsed off or after further processing or cooking. In this case rinsing off after a suitable contact time may result in residues that have no technological effect in the final food which could be considered as a processing aid use. Similarly where *Citrox* remains in a liquid final product is it likely to be active and hence there would need to be permission to used as a food additive.

I am happy to arrange a meeting to discuss this further if needed.

B 8

Yours sincerely

John van den Beuken
Programme Manager (Composition)

Cc Dean Stockwell, General Manager, FSANZ, Wellington
Lennox Vellenkoop, Programme Manager (Animal Products), New Zealand Standards, NZFSA

B 9

BioGro New Zealand

INPUT FOR ORGANIC PRODUCTION CERTIFICATE

The company named below is certified to supply the listed products in accordance with the requirements of the BIO-GRO New Zealand Organic Standards, to the requirements of BioGro's IFOAM Accredited Programme, and to the requirements of the USDA National Organic Program (NOP).

Licensee Company Name	**Citrox (NZ) Ltd**
Location of Facility (physical address)	**361 Remuera Road** **Remuera** **Auckland** **New Zealand**

Sector Description	Product List	Certification Status
Input Supplies	**Crop Management Products**	
	Citrox BC – 5 L, 20 L	**Permitted**
	Citrox BioKlenz – 1 L, 5 L, 20 L, 200 L	**Permitted**

Note: BioGro certification of the above products is subject to them being used in compliance with all relevant regulatory, industry and market requirements.

BioGro Number	**5126 CO1**	Certificate Number	**# 2 of 3**
First Certified from	**01 December 2008**	Certification Valid from	**01 December 2008**
		Certification Valid to	**30 November 2009**

Signed by Certification Committee .....

Signed by Director .....

Issuing Office
BioGro New Zealand Ltd
P O Box 9693, Marion Square,
Wellington 6141, New Zealand
Tel +64 4 801 9741 Fax +64 4 801 9742
Email: info@biogro.co.nz

B 10

BioGro New Zealand

INPUT FOR ORGANIC PRODUCTION CERTIFICATE

The company named below is certified to supply the listed products in accordance with the requirements of the BIO-GRO New Zealand Organic Standards, to the requirements of BioGro's IFOAM Accredited Programme, and to the requirements of the USDA National Organic Program (NOP).

Licensee Company Name	**Citrox (NZ) Ltd**
Location of Facility (physical address)	**361 Remuera Road Remuera Auckland New Zealand**

Sector Description	Product List	Certification Status
Input Supplies	**Soil and Plant Nutrition Management Products**	
	Citrox BioAlexin – 250 mL , 1 L, 5 L, 20 L, 200 L	**Permitted**

Note: BioGro certification of the above products is subject to them being used in compliance with all relevant regulatory, industry and market requirements.

BioGro Number	**5126 CO1**	Certificate Number	**# 1 of 3**
First Certified from	**01 December 2008**	Certification Valid from	**01 December 2008**
		Certification Valid to	**30 November 2009**

Signed by Certification Committee .....

Signed by Director .....

Issuing Office
BioGro New Zealand Ltd
P O Box 9693, Marion Square,
Wellington 6141, New Zealand
Tel +64 4 801 9741 Fax +64 4 801 9742
Email: info@biogro.co.nz

B11A

Application Form: Approval / Re-approval of Maintenance Compound

For maintenance compounds to be used by operators processing non-dairy animal product under the Animal Products Act regime

For NZFSA use only	
Reference	
Date	
FMD listing	
Receipt No.	

Applicant Company Name (*) Note: only asterisk fields appear in published lists

Citrox (NZ) Ltd

Postal Address (*)

PO Box 28 482
Remuera, Auckland

Company Contact Details

Telephone (*)	Fax (*)	Email	Contact Name
09 520 6161	09 5206165	sales@citrox.co.nz	Dominic Young

Third Party Company Name – supporting information Note: any information supplied by this company remains confidential to this company.

Product Trade Name (*)

Citrox SurfaceSan

Approval code (e.g. C 32)	
Requested	C43
For NZFSA use only	
Granted	

Compound description (e.g. cleaner, sanitiser, lubricant)
Note: this is based on information supplied & intended use

Requested	Surface sanitiser
For NZFSA use only	
Granted	

Disinfectants for foot-and-mouth disease responses (optional) (*)

Note: for re-approvals this information must be re-supplied to maintain this listing.

Applicants who consider that the compound above meets the requirements specified in the Approved Maintenance Compounds Manual and require listing as a disinfectant for use in foot-and-mouth disease responses please supply the following information:

Specification	acid disinfectant (tick one)	
	alkaline disinfectant (non-corrosive) (tick one)	
	alkaline disinfectant (corrosive) (tick one)	
Dilution	percent v/v (or w/v) to achieve the specified pH	
Stock Levels	stock on call (tonnes/litres)	

Product Composition[1]

Chemical Name	CAS number	Trade Name	Manufacturer	Percent
BioFlavonoid Extract	68916-04-1(72968-50-4)	Citrus aurantium L	Univar UK	< 5.0%
Citric Acid	77-92-9	Citric Acid	Univar	15%
Glycerine	56-81-5	Glycerine	Univar	< 2%
Ascorbic Acid	50-81-7	Ascorbic Acid	Univar	< 1%
Water				>77%

Have you signed the declaration?

B 11A

[1] **Product Composition notes:**

- The **full composition** is required. This includes the entire composition of any proprietary ingredients.
- Discrete values should be given. Ranges 0.2y wider than the mid range value (y) will generally not be accepted. i.e.: y=5%, 5 x 0.2 = 1→ limit of acceptable range (4-6%).
- It is the responsibility of the applicant to ensure procedures are in place with the manufacturer to guarantee this information is correct and any changes will be formally notified via application.
- Failure to provide full information at the lodgement of the application will result in the application being declined unless it is stated when, and from whom, the additional information will be supplied.

Applicant Checklist	
Product label (or copy) enclosed	Yes
Product information sheet, if required Note: a product information sheet is only required if it contains claims relating to maintenance compound use or the product label does not include directions for use.	Yes
Assessment fee included $150 (incl. GST) for **each new approval** $75 (incl. GST) for **each re-approval**	Yes
Full formulation provided	Yes
Send the completed application form together with the fee, and other appropriate documentation to: Approved Maintenance Compounds ACVM Group, New Zealand Food Safety Authority South Tower, 86 Jervois Quay PO Box 2835, Wellington, New Zealand	

Declaration

I declare that the:
a) information supplied on this application is truthful and accurate; and
b) compound, when used by operators processing non-dairy animal products who are operating under the Animal Products Act (APA) regime and in accordance with the manufacturer's instructions, will not deleteriously affect animal material or product.

Applicant Signature:

Applicant Name: Dominic Young

Applicant Title: Director

Date: 16/12?08

Collection of Personal Information on Individuals

In regard to any information being collected on this application for maintenance compound approval or re-approval, pursuant to the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004 (that is personal information identifying or being capable of identifying an individual person), notification is hereby provided in accordance with principle 3 of the Privacy Act 1993, to individuals of the following matters:

1. This information is being collected for purposes relating to maintenance compound approval and the administration of the Animal Products Act 1999.

2. The recipient of this information, which is also the agency that will collect and hold the information, is the New Zealand Food Safety Authority, PO Box 2835, Wellington.

3. The collection of this information is authorised under clause 4(1) of the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, clause 4(1) of the Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and clause 3(1) of the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004. The provision of this information is necessary in order to process this application. Failure to provide information is likely to result in the return of this application form to the applicant for completion.

4. You are reminded that under Principles 6 and 7 of the Privacy Act 1993, you have the right of access to, and correction of, any personal information, which has been provided.

B11B

Application Form: Approval / Re-approval of Maintenance Compound

For maintenance compounds to be used by operators processing non-dairy animal product under the Animal Products Act regime

For NZFSA use only	
Reference	
Date	
FMD listing	
Receipt No.	

Applicant Company Name (*) Note: only asterisk fields appear in published lists

Citrox (NZ) Ltd

Postal Address (*)

PO Box 28 482
Remuera, Auckland

Company Contact Details

Telephone (*)	Fax (*)	Email	Contact Name
09 520 6161	09 5206165	sales@citrox.co.nz	Dominic Young

Third Party Company Name – supporting information Note: any information supplied by this company remains confidential to this company.

Product Trade Name (*)

Citrox EnviroFoam (Citrox 14X)

Approval code (e.g. C 32)	
Requested	C104, C32 and C39
For NZFSA use only	
Granted	

Compound description (e.g. cleaner, sanitiser, lubricant)
Note: this is based on information supplied & intended use

Requested	Heavy Duty Cleaner
For NZFSA use only	
Granted	

Disinfectants for foot-and-mouth disease responses (optional) (*)

Note: for re-approvals this information must be re-supplied to maintain this listing.

Applicants who consider that the compound above meets the requirements specified in the Approved Maintenance Compounds Manual and require listing as a disinfectant for use in foot-and-mouth disease responses please supply the following information:

Specification	acid disinfectant (tick one)	
	alkaline disinfectant (non-corrosive) (tick one)	
	alkaline disinfectant (corrosive) (tick one)	
Dilution	percent v/v (or w/v) to achieve the specified pH	
Stock Levels	stock on call (tonnes/litres)	

Product Composition[1]

Chemical Name	CAS number	Trade Name	Manufacturer	Percent
BioFlavonoid Extract	68916-04-1(72968-50-4)	Citrus aurantium L	Univar UK	<7%
Citric Acid	77-92-9	Citric Acid	Univar	<15%
Glycerine	56-81-5	Glycerine	Univar	< 10%
Ascorbic Acid	50-81-7	Ascorbic Acid	Univar	< 1%
Malic acid	97-67-6	Malic Acid	Fuso	< 8%
Glycolic Acid	79-41-1	Glycolic Acid	Univar	<10%
LFG 61	68439-46-3	Surfactants	Univar	< 1%
	26468-80-0	Surfactants	Univar	< 1%
	54549-24-5	Surfactants	Univar	< 1%
A030	70592-80-2	Surfactant	Univar	<1%
Water				> 45%

Have you signed the declaration?

B 11B

[1] **Product Composition notes:**

- The **full composition** is required. This includes the entire composition of any proprietary ingredients.
- Discrete values should be given. Ranges 0.2y wider than the mid range value (y) will generally not be accepted. i.e.: y=5%, 5 x 0.2 = 1→ limit of acceptable range (4-6%).
- It is the responsibility of the applicant to ensure procedures are in place with the manufacturer to guarantee this information is correct and any changes will be formally notified via application.
- Failure to provide full information at the lodgement of the application will result in the application being declined unless it is stated when, and from whom, the additional information will be supplied.

Applicant Checklist	
Product label (or copy) enclosed	Yes
Product information sheet, if required Note: a product information sheet is only required if it contains claims relating to maintenance compound use or the product label does not include directions for use.	Yes
Assessment fee included $150 (incl. GST) for **each new approval** $75 (incl. GST) for **each re-approval**	Yes
Full formulation provided	Yes
Send the completed application form together with the fee, and other appropriate documentation to: Approved Maintenance Compounds ACVM Group, New Zealand Food Safety Authority South Tower, 86 Jervois Quay PO Box 2835, Wellington, New Zealand	

Declaration

I declare that the:
a) information supplied on this application is truthful and accurate; and
b) compound, when used by operators processing non-dairy animal products who are operating under the Animal Products Act (APA) regime and in accordance with the manufacturer's instructions, will not deleteriously affect animal material or product.

Applicant Signature:

Applicant Name: Dominic Young

Applicant Title: Director

Date: 16/12?08

Collection of Personal Information on Individuals

In regard to any information being collected on this application for maintenance compound approval or re-approval, pursuant to the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004 (that is personal information identifying or being capable of identifying an individual person), notification is hereby provided in accordance with principle 3 of the Privacy Act 1993, to individuals of the following matters:

1. This information is being collected for purposes relating to maintenance compound approval and the administration of the Animal Products Act 1999.

2. The recipient of this information, which is also the agency that will collect and hold the information, is the New Zealand Food Safety Authority, PO Box 2835, Wellington.

3. The collection of this information is authorised under clause 4(1) of the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, clause 4(1) of the Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and clause 3(1) of the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004. The provision of this information is necessary in order to process this application. Failure to provide information is likely to result in the return of this application form to the applicant for completion.

4. You are reminded that under Principles 6 and 7 of the Privacy Act 1993, you have the right of access to, and correction of, any personal information, which has been provided.

B 11 C

Application Form: Approval / Re-approval of Maintenance Compound

For maintenance compounds to be used by operators processing non-dairy animal product under the Animal Products Act regime

For NZFSA use only	
Reference	
Date	
FMD listing	
Receipt No.	

Applicant Company Name (*) Note: only asterisk fields appear in published lists

Citrox (NZ) Ltd

Postal Address (*)

PO Box 28 482
Remuera, Auckland

Company Contact Details

Telephone (*)	Fax (*)	Email	Contact Name
09 520 6161	09 5206165	sales@citrox.co.nz	Dominic Young

Third Party Company Name – supporting information Note: any information supplied by this company remains confidential to this company.

Product Trade Name (*)

Citrox – PWT

Approval code (e.g. C 32)	
Requested	Potable water
For NZFSA use only	
Granted	

Compound description (e.g. cleaner, sanitiser, lubricant)
Note: this is based on information supplied & intended use

Requested	Potable Water Treatment
For NZFSA use only	
Granted	

Disinfectants for foot-and-mouth disease responses (optional) (*)

Note: for re-approvals this information must be re-supplied to maintain this listing.

Applicants who consider that the compound above meets the requirements specified in the Approved Maintenance Compounds Manual and require listing as a disinfectant for use in foot-and-mouth disease responses please supply the following information:

Specification	acid disinfectant (tick one)	Yes	
	alkaline disinfectant (non-corrosive) (tick one)	☐	
	alkaline disinfectant (corrosive) (tick one)	☐	
Dilution	percent v/v (or w/v) to achieve the specified pH	0.2%	
Stock Levels	stock on call (tonnes/litres)	5000 ltrs	stock at 48 hours (tonnes/litres) 5000ltrs

Product Composition[1]

Chemical Name	CAS number	Trade Name	Manufacturer	Percent
BioFlavonoid Extract	68916-04-1(72968-50-4)	Citrus aurantium L	Univar UK	< 7.0%
Citric Acid	77-92-9	Citric Acid	Univar	15%
Glycerine	56-81-5	Glycerine	Univar	< 2%
Ascorbic Acid	50-81-7	Ascorbic Acid	Univar	< 1%
Water				>75%

Have you signed the declaration?

B 110

[1] **Product Composition notes:**

- The **full composition** is required. This includes the entire composition of any proprietary ingredients.
- Discrete values should be given. Ranges 0.2y wider than the mid range value (y) will generally not be accepted. i.e.: y=5%, 5 x 0.2 = 1→ limit of acceptable range (4-6%).
- It is the responsibility of the applicant to ensure procedures are in place with the manufacturer to guarantee this information is correct and any changes will be formally notified via application.
- Failure to provide full information at the lodgement of the application will result in the application being declined unless it is stated when, and from whom, the additional information will be supplied.

Applicant Checklist	
Product label (or copy) enclosed	
Product information sheet, if required Note: a product information sheet is only required if it contains claims relating to maintenance compound use or the product label does not include directions for use.	
Assessment fee included $150 (incl. GST) for **each new approval** $75 (incl. GST) for **each re-approval**	
Full formulation provided	
Send the completed application form together with the fee, and other appropriate documentation to: Approved Maintenance Compounds ACVM Group, New Zealand Food Safety Authority South Tower, 86 Jervois Quay PO Box 2835, Wellington, New Zealand	

Declaration

I declare that the:
a) information supplied on this application is truthful and accurate; and
b) compound, when used by operators processing non-dairy animal products who are operating under the Animal Products Act (APA) regime and in accordance with the manufacturer's instructions, will not deleteriously affect animal material or product.

Applicant Signature:

Applicant Name:

Applicant Title:

Date:

Collection of Personal Information on Individuals

In regard to any information being collected on this application for maintenance compound approval or re-approval, pursuant to the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004 (that is personal information identifying or being capable of identifying an individual person), notification is hereby provided in accordance with principle 3 of the Privacy Act 1993, to individuals of the following matters:

1. This information is being collected for purposes relating to maintenance compound approval and the administration of the Animal Products Act 1999.

2. The recipient of this information, which is also the agency that will collect and hold the information, is the New Zealand Food Safety Authority, PO Box 2835, Wellington.

3. The collection of this information is authorised under clause 4(1) of the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, clause 4(1) of the Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and clause 3(1) of the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004. The provision of this information is necessary in order to process this application. Failure to provide information is likely to result in the return of this application form to the applicant for completion.

4. You are reminded that under Principles 6 and 7 of the Privacy Act 1993, you have the right of access to, and correction of, any personal information, which has been provided.

B 11 D

Application Form: Approval / Re-approval of Maintenance Compound

For maintenance compounds to be used by operators processing non-dairy animal product under the Animal Products Act regime

For NZFSA use only	
Reference	
Date	
FMD listing	
Receipt No.	

Applicant Company Name (*) Note: only asterisk fields appear in published lists

Citrox (NZ) Ltd

Postal Address (*)

PO Box 28 482
Remuera, Auckland

Company Contact Details

Telephone (*)	Fax (*)	Email	Contact Name
09 520 6161	09 5206165	sales@citrox.co.nz	Dominic Young

Third Party Company Name – supporting information Note: any information supplied by this company remains confidential to this company.

Product Trade Name (*)

Citrox BC

Approval code (e.g. C 32)	
Requested	Processing Aid
For NZFSA use only	
Granted	

Compound description (e.g. cleaner, sanitiser, lubricant)
Note: this is based on information supplied & intended use

Requested	Processing Aid
For NZFSA use only	
Granted	

Disinfectants for foot-and-mouth disease responses (optional) (*)

Note: for re-approvals this information must be re-supplied to maintain this listing.

Applicants who consider that the compound above meets the requirements specified in the Approved Maintenance Compounds Manual and require listing as a disinfectant for use in foot-and-mouth disease responses please supply the following information:

Specification	acid disinfectant (tick one)	
	alkaline disinfectant (non-corrosive) (tick one)	☐
	alkaline disinfectant (corrosive) (tick one)	☐
Dilution	percent v/v (or w/v) to achieve the specified pH	
Stock Levels	stock on call (tonnes/litres)	

Product Composition[1]

Chemical Name	CAS number	Trade Name	Manufacturer	Percent
BioFlavonoid Extract	68916-04-1(72968-50-4) Citrus aurantium L		Univar UK	7.0%
Citric Acid	77-92-9	Citric Acid	Univar	15%
Glycerine	56-81-5	Glycerine	Univar	< 2%
Ascorbic Acid	50-81-7	Ascorbic Acid	Univar	< 1%
Water				>75%

Have you signed the declaration?

B 1 1 D

[1] **Product Composition notes:**

- The **full composition** is required. This includes the entire composition of any proprietary ingredients.
- Discrete values should be given. Ranges 0.2y wider than the mid range value (y) will generally not be accepted. i.e.: y=5%, 5 x 0.2 = 1→ limit of acceptable range (4-6%).
- It is the responsibility of the applicant to ensure procedures are in place with the manufacturer to guarantee this information is correct and any changes will be formally notified via application.
- Failure to provide full information at the lodgement of the application will result in the application being declined unless it is stated when, and from whom, the additional information will be supplied.

Applicant Checklist	
Product label (or copy) enclosed	
Product information sheet, if required Note: a product information sheet is only required if it contains claims relating to maintenance compound use or the product label does not include directions for use.	
Assessment fee included $150 (incl. GST) for each new approval $75 (incl. GST) for each re-approval	
Full formulation provided	
Send the completed application form together with the fee, and other appropriate documentation to: Approved Maintenance Compounds ACVM Group, New Zealand Food Safety Authority South Tower, 86 Jervois Quay PO Box 2835, Wellington, New Zealand	

Declaration

I declare that the:
a) information supplied on this application is truthful and accurate; and
b) compound, when used by operators processing non-dairy animal products who are operating under the Animal Products Act (APA) regime and in accordance with the manufacturer's instructions, will not deleteriously affect animal material or product.

Applicant Signature:

Applicant Name:

Applicant Title:

Date:

Collection of Personal Information on Individuals

In regard to any information being collected on this application for maintenance compound approval or re-approval, pursuant to the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004 (that is personal information identifying or being capable of identifying an individual person), notification is hereby provided in accordance with principle 3 of the Privacy Act 1993, to individuals of the following matters:

1. This information is being collected for purposes relating to maintenance compound approval and the administration of the Animal Products Act 1999.

2. The recipient of this information, which is also the agency that will collect and hold the information, is the New Zealand Food Safety Authority, PO Box 2835, Wellington.

3. The collection of this information is authorised under clause 4(1) of the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, clause 4(1) of the Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and clause 3(1) of the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004. The provision of this information is necessary in order to process this application. Failure to provide information is likely to result in the return of this application form to the applicant for completion.

4. You are reminded that under Principles 6 and 7 of the Privacy Act 1993, you have the right of access to, and correction of, any personal information, which has been provided.

B11E

Application Form: Approval / Re-approval of Maintenance Compound

For maintenance compounds to be used by operators processing non-dairy animal product under the Animal Products Act regime

For NZFSA use only	
Reference	
Date	
FMD listing	
Receipt No.	

Applicant Company Name (*) Note: only asterisk fields appear in published lists

Citrox (NZ) Ltd

Postal Address (*)

PO Box 28 482
Remuera, Auckland

Company Contact Details

Telephone (*)	Fax (*)	Email	Contact Name
09 520 6161	09 5206165	sales@citrox.co.nz	Dominic Young

Third Party Company Name – supporting information Note: any information supplied by this company remains confidential to this company.

Product Trade Name (*)

Citrox BioAlexin (Was BioAlexin Plus)

Compound description (e.g. cleaner, sanitiser, lubricant)
Note: this is based on information supplied & intended use

Requested	Fertiliser Additive
For NZFSA use only	
Granted	

Approval code (e.g. C 32)	
Requested	Fertiliser Additive
For NZFSA use only	
Granted	

Disinfectants for foot-and-mouth disease responses (optional) (*)

Note: for re-approvals this information must be re-supplied to maintain this listing.

Applicants who consider that the compound above meets the requirements specified in the Approved Maintenance Compounds Manual and require listing as a disinfectant for use in foot-and-mouth disease responses please supply the following information:

Specification	acid disinfectant (tick one)
	alkaline disinfectant (non-corrosive) (tick one) ☐
	alkaline disinfectant (corrosive) (tick one) ☐
Dilution	percent v/v (or w/v) to achieve the specified pH
Stock Levels	stock on call (tonnes/litres)

Product Composition[1]

Chemical Name	CAS number	Trade Name	Manufacturer	Percent
BioFlavonoid Extract	68916-04-1(72968-50-4)	Citrus aurantium L	Univar UK	<12%
Citric Acid	77-92-9	Citric Acid	Univar	<22%
Glycerine	56-81-5	Glycerine	Univar	< 2%
Ascorbic Acid	50-81-7	Ascorbic Acid	Univar	< 1%
Palm Oil Extract	8002-75-3	Palm oil extract	Kerfoot UK	<17%
Water				>46%

Have you signed the declaration?

B 11 E

[1] **Product Composition notes:**

- The **full composition** is required. This includes the entire composition of any proprietary ingredients.
- Discrete values should be given. Ranges 0.2y wider than the mid range value (y) will generally not be accepted. i.e.: y=5%, 5 x 0.2 = 1→ limit of acceptable range (4-6%).
- It is the responsibility of the applicant to ensure procedures are in place with the manufacturer to guarantee this information is correct and any changes will be formally notified via application.
- Failure to provide full information at the lodgement of the application will result in the application being declined unless it is stated when, and from whom, the additional information will be supplied.

Applicant Checklist	
Product label (or copy) enclosed	
Product information sheet, if required Note: a product information sheet is only required if it contains claims relating to maintenance compound use or the product label does not include directions for use.	
Assessment fee included $150 (incl. GST) for **each new approval** $75 (incl. GST) for **each re-approval**	
Full formulation provided	
Send the completed application form together with the fee, and other appropriate documentation to: Approved Maintenance Compounds ACVM Group, New Zealand Food Safety Authority South Tower, 86 Jervois Quay PO Box 2835, Wellington, New Zealand	

Declaration

I declare that the:
a) information supplied on this application is truthful and accurate; and
b) compound, when used by operators processing non-dairy animal products who are operating under the Animal Products Act (APA) regime and in accordance with the manufacturer's instructions, will not deleteriously affect animal material or product.

Applicant Signature:

Applicant Name:

Applicant Title:

Date:

Collection of Personal Information on Individuals

In regard to any information being collected on this application for maintenance compound approval or re-approval, pursuant to the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004 (that is personal information identifying or being capable of identifying an individual person), notification is hereby provided in accordance with principle 3 of the Privacy Act 1993, to individuals of the following matters:

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2. The recipient of this information, which is also the agency that will collect and hold the information, is the New Zealand Food Safety Authority, PO Box 2835, Wellington.

3. The collection of this information is authorised under clause 4(1) of the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, clause 4(1) of the Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and clause 3(1) of the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004. The provision of this information is necessary in order to process this application. Failure to provide information is likely to result in the return of this application form to the applicant for completion.

4. You are reminded that under Principles 6 and 7 of the Privacy Act 1993, you have the right of access to, and correction of, any personal information, which has been provided.

B 11 F

Application Form: Approval / Re-approval of Maintenance Compound

For maintenance compounds to be used by operators processing non-dairy animal product under the Animal Products Act regime

For NZFSA use only	
Reference	
Date	
FMD listing	
Receipt No.	

Applicant Company Name (*) Note: only asterisk fields appear in published lists

Citrox (NZ) Ltd

Postal Address (*)

PO Box 28 482
Remuera, Auckland

Company Contact Details

Telephone (*)	Fax (*)	Email	Contact Name
09 520 6161	09 5206165	sales@citrox.co.nz	Dominic Young

Third Party Company Name – supporting information Note: any information supplied by this company remains confidential to this company.

Product Trade Name (*)

Citrox - BioKlenz

Approval code (e.g. C 32)	
Requested	C43 + Processing Aid
For NZFSA use only	
Granted	

Compound description (e.g. cleaner, sanitiser, lubricant)
Note: this is based on information supplied & intended use

Requested	Processing Aid
For NZFSA use only	
Granted	

Disinfectants for foot-and-mouth disease responses (optional) (*)

Note: for re-approvals this information must be re-supplied to maintain this listing.

Applicants who consider that the compound above meets the requirements specified in the Approved Maintenance Compounds Manual and require listing as a disinfectant for use in foot-and-mouth disease responses please supply the following information:

Specification	acid disinfectant (tick one)	Yes	
	alkaline disinfectant (non-corrosive) (tick one)	☐	
	alkaline disinfectant (corrosive) (tick one)	☐	
Dilution	percent v/v (or w/v) to achieve the specified pH	1%	
Stock Levels	stock on call (tonnes/litres)	5000 ltrs	stock at 48 hours (tonnes/litres) 5000ltrs

Product Composition[1]

Chemical Name	CAS number	Trade Name	Manufacturer	Percent
BioFlavonoid Extract	68916-04-1(72968-50-4)	Citrus aurantium L	Univar UK	< 7.0%
Citric Acid	77-92-9	Citric Acid	Univar	15%
Glycerine	56-81-5	Glycerine	Univar	< 2%
Ascorbic Acid	50-81-7	Ascorbic Acid	Univar	< 1%
Water				>75%

Have you signed the declaration?

B 1 1 F

[1] **Product Composition notes:**

- The **full composition** is required. This includes the entire composition of any proprietary ingredients.
- Discrete values should be given. Ranges 0.2y wider than the mid range value (y) will generally not be accepted. i.e.: y=5%, 5 x 0.2 = 1→ limit of acceptable range (4-6%).
- It is the responsibility of the applicant to ensure procedures are in place with the manufacturer to guarantee this information is correct and any changes will be formally notified via application.
- Failure to provide full information at the lodgement of the application will result in the application being declined unless it is stated when, and from whom, the additional information will be supplied.

Applicant Checklist	
Product label (or copy) enclosed	
Product information sheet, if required Note: a product information sheet is only required if it contains claims relating to maintenance compound use or the product label does not include directions for use.	
Assessment fee included $150 (incl. GST) for each new approval $75 (incl. GST) for each re-approval	
Full formulation provided	
Send the completed application form together with the fee, and other appropriate documentation to: Approved Maintenance Compounds ACVM Group, New Zealand Food Safety Authority South Tower, 86 Jervois Quay PO Box 2835, Wellington, New Zealand	

Declaration

I declare that the:
a) information supplied on this application is truthful and accurate; and
b) compound, when used by operators processing non-dairy animal products who are operating under the Animal Products Act (APA) regime and in accordance with the manufacturer's instructions, will not deleteriously affect animal material or product.

Applicant Signature:

Applicant Name:

Applicant Title:

Date:

Collection of Personal Information on Individuals

In regard to any information being collected on this application for maintenance compound approval or re-approval, pursuant to the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004 (that is personal information identifying or being capable of identifying an individual person), notification is hereby provided in accordance with principle 3 of the Privacy Act 1993, to individuals of the following matters:

1. This information is being collected for purposes relating to maintenance compound approval and the administration of the Animal Products Act 1999.

2. The recipient of this information, which is also the agency that will collect and hold the information, is the New Zealand Food Safety Authority, PO Box 2835, Wellington.

3. The collection of this information is authorised under clause 4(1) of the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, clause 4(1) of the Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and clause 3(1) of the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004. The provision of this information is necessary in order to process this application. Failure to provide information is likely to result in the return of this application form to the applicant for completion.

4. You are reminded that under Principles 6 and 7 of the Privacy Act 1993, you have the right of access to, and correction of, any personal information, which has been provided.

B 11 G

Application Form: Approval / Re-approval of Maintenance Compound

For maintenance compounds to be used by operators processing non-dairy animal product under the Animal Products Act regime

For NZFSA use only	
Reference	
Date	
FMD listing	
Receipt No.	

Applicant Company Name (*) Note: only asterisk fields appear in published lists

Citrox (NZ) Ltd

Postal Address (*)

PO Box 28 482
Remuera, Auckland

Company Contact Details

Telephone (*)	Fax (*)	Email	Contact Name
09 520 6161	09 5206165	sales@citrox.co.nz	Dominic Young

Third Party Company Name – supporting information Note: any information supplied by this company remains confidential to this company.

Product Trade Name (*)

Citrox ProAlexin – Plant Nutrient Synergist

Compound description (e.g. cleaner, sanitiser, lubricant)
Note: this is based on information supplied & intended use

Requested	Fertiliser Additive
For NZFSA use only	
Granted	

Approval code (e.g. C 32)	
Requested	Fertiliser Additive
For NZFSA use only	
Granted	

Disinfectants for foot-and-mouth disease responses (optional) (*)

Note: for re-approvals this information must be re-supplied to maintain this listing.

Applicants who consider that the compound above meets the requirements specified in the Approved Maintenance Compounds Manual and require listing as a disinfectant for use in foot-and-mouth disease responses please supply the following information:

Specification	acid disinfectant (tick one)	
	alkaline disinfectant (non-corrosive) (tick one)	☐
	alkaline disinfectant (corrosive) (tick one)	☐
Dilution	percent v/v (or w/v) to achieve the specified pH	
Stock Levels	stock on call (tonnes/litres)	

Product Composition[1]

Chemical Name	CAS number	Trade Name	Manufacturer	Percent
BioFlavonoid Extract	68916-04-1(72968-50-4)	Citrus aurantium L	Univar UK	<12%
Citric Acid	77-92-9	Citric Acid	Univar	<22%
Glycerine	56-81-5	Glycerine	Univar	< 2%
Ascorbic Acid	50-81-7	Ascorbic Acid	Univar	< 1%
Palm Oil Extract	8002-75-3	Palm oil extract	Kerfoot UK	17%
Water				>46%

Have you signed the declaration?

B 1 1 G

[1] **Product Composition notes:**

- The **full composition** is required. This includes the entire composition of any proprietary ingredients.
- Discrete values should be given. Ranges 0.2y wider than the mid range value (y) will generally not be accepted. i.e.: y=5%, 5 x 0.2 = 1→ limit of acceptable range (4-6%).
- It is the responsibility of the applicant to ensure procedures are in place with the manufacturer to guarantee this information is correct and any changes will be formally notified via application.
- Failure to provide full information at the lodgement of the application will result in the application being declined unless it is stated when, and from whom, the additional information will be supplied.

Applicant Checklist	
Product label (or copy) enclosed	
Product information sheet, if required Note: a product information sheet is only required if it contains claims relating to maintenance compound use or the product label does not include directions for use.	
Assessment fee included $150 (incl. GST) for each new approval $75 (incl. GST) for each re-approval	
Full formulation provided	
Send the completed application form together with the fee, and other appropriate documentation to: Approved Maintenance Compounds ACVM Group, New Zealand Food Safety Authority South Tower, 86 Jervois Quay PO Box 2835, Wellington, New Zealand	

Declaration

I declare that the:
a) information supplied on this application is truthful and accurate; and
b) compound, when used by operators processing non-dairy animal products who are operating under the Animal Products Act (APA) regime and in accordance with the manufacturer's instructions, will not deleteriously affect animal material or product.

Applicant Signature:

Applicant Name:

Applicant Title:

Date:

Collection of Personal Information on Individuals

In regard to any information being collected on this application for maintenance compound approval or re-approval, pursuant to the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004 (that is personal information identifying or being capable of identifying an individual person), notification is hereby provided in accordance with principle 3 of the Privacy Act 1993, to individuals of the following matters:

1. This information is being collected for purposes relating to maintenance compound approval and the administration of the Animal Products Act 1999.

2. The recipient of this information, which is also the agency that will collect and hold the information, is the New Zealand Food Safety Authority, PO Box 2835, Wellington.

3. The collection of this information is authorised under clause 4(1) of the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, clause 4(1) of the Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and clause 3(1) of the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004. The provision of this information is necessary in order to process this application. Failure to provide information is likely to result in the return of this application form to the applicant for completion.

4. You are reminded that under Principles 6 and 7 of the Privacy Act 1993, you have the right of access to, and correction of, any personal information, which has been provided.

B 11 H

Application Form: Approval / Re-approval of Maintenance Compound

For maintenance compounds to be used by operators processing non-dairy animal product under the Animal Products Act regime

For NZFSA use only	
Reference	
Date	
FMD listing	
Receipt No.	

Applicant Company Name (*) Note: only asterisk fields appear in published lists

Citrox (NZ) Ltd

Postal Address (*)

PO Box 28 482
Remuera, Auckland

Company Contact Details

Telephone (*)	Fax (*)	Email	Contact Name
09 520 6161	09 5206165	sales@citrox.co.nz	Dominic Young

Third Party Company Name – supporting information Note: any information supplied by this company remains confidential to this company.

Product Trade Name (*)

Citrox SaniWash (Citrox 14T)

Approval code (e.g. C 32)	
Requested	C31 and C39
For NZFSA use only	
Granted	

Compound description (e.g. cleaner, sanitiser, lubricant)
Note: this is based on information supplied & intended use

Requested	Heavy Duty Cleaner
For NZFSA use only	
Granted	

Disinfectants for foot-and-mouth disease responses (optional) (*)

Note: for re-approvals this information must be re-supplied to maintain this listing.

Applicants who consider that the compound above meets the requirements specified in the Approved Maintenance Compounds Manual and require listing as a disinfectant for use in foot-and-mouth disease responses please supply the following information:

Specification	acid disinfectant (tick one)	
	alkaline disinfectant (non-corrosive) (tick one)	
	alkaline disinfectant (corrosive) (tick one)	
Dilution	percent v/v (or w/v) to achieve the specified pH	
Stock Levels	stock on call (tonnes/litres)	

Product Composition[1]

Chemical Name	CAS number	Trade Name	Manufacturer	Percent
BioFlavonoid Extract	68916-04-1(72968-50-4)	Citrus aurantium L	Univar UK	<7%
Citric Acid	77-92-9	Citric Acid	Univar	<15%
Glycerine	56-81-5	Glycerine	Univar	< 10%
Ascorbic Acid	50-81-7	Ascorbic Acid	Univar	< 1%
Malic acid	97-67-6	Malic Acid	Fuso	< 8%
Glycolic Acid	79-41-1	Glycolic Acid	Univar	<10%
LFG 61	68439-46-3	Surfactants	Univar	< 1%
	26468-80-0	Surfactants	Univar	< 1%
	54549-24-5	Surfactants	Univar	< 1%
Water				> 46%

Have you signed the declaration?

B 1 1H

[1] **Product Composition notes:**

- The full composition is required. This includes the entire composition of any proprietary ingredients.
- Discrete values should be given. Ranges 0.2y wider than the mid range value (y) will generally not be accepted. i.e.: y=5%, 5 x 0.2 = 1→ limit of acceptable range (4-6%).
- It is the responsibility of the applicant to ensure procedures are in place with the manufacturer to guarantee this information is correct and any changes will be formally notified via application.
- Failure to provide full information at the lodgement of the application will result in the application being declined unless it is stated when, and from whom, the additional information will be supplied.

Applicant Checklist	
Product label (or copy) enclosed	Yes
Product information sheet, if required Note: a product information sheet is only required if it contains claims relating to maintenance compound use or the product label does not include directions for use.	Yes
Assessment fee included $150 (incl. GST) for each new approval $75 (incl. GST) for each re-approval	Yes
Full formulation provided	Yes
Send the completed application form together with the fee, and other appropriate documentation to: Approved Maintenance Compounds ACVM Group, New Zealand Food Safety Authority South Tower, 86 Jervois Quay PO Box 2835, Wellington, New Zealand	

Declaration

I declare that the:
a) information supplied on this application is truthful and accurate; and
b) compound, when used by operators processing non-dairy animal products who are operating under the Animal Products Act (APA) regime and in accordance with the manufacturer's instructions, will not deleteriously affect animal material or product.

Applicant Signature:

Applicant Name: Dominic Young

Applicant Title: Director

Date: 16/12?08

Collection of Personal Information on Individuals

In regard to any information being collected on this application for maintenance compound approval or re-approval, pursuant to the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004 (that is personal information identifying or being capable of identifying an individual person), notification is hereby provided in accordance with principle 3 of the Privacy Act 1993, to individuals of the following matters:

1. This information is being collected for purposes relating to maintenance compound approval and the administration of the Animal Products Act 1999.

2. The recipient of this information, which is also the agency that will collect and hold the information, is the New Zealand Food Safety Authority, PO Box 2835, Wellington.

3. The collection of this information is authorised under clause 4(1) of the Animal Products (Specifications for Limited Processing Fishing Vessels) Notice 2005, clause 4(1) of the Animal Products (Specifications for Products Intended for Animal Consumption) Notice 2006 and clause 3(1) of the Animal Products (Specifications for Products Intended for Human Consumption) Notice 2004. The provision of this information is necessary in order to process this application. Failure to provide information is likely to result in the return of this application form to the applicant for completion.

4. You are reminded that under Principles 6 and 7 of the Privacy Act 1993, you have the right of access to, and correction of, any personal information, which has been provided.

Animal Movements and Exotic Diseases Division
Location: 607, 1A PAGE STREET, LONDON, SW1P 4PQ
Tel: 020 7904 6135 GTN : 3290 6135
Fax: 3290 6128
E-Mail: Karen.E.Skelton@defra.gsi.gov.uk

defra
Department for Environment Food and Rural Affairs

Our Ref: TADP 46
Date: 18/02/2005

Mr I S Ripley
Unit 1 River Court, Brighouse Business Village
Brighouse Road, Riverside Park,
Middlesbrough
TS2 1RT

Dear Mr Ripley,

DEFRA APPROVAL OF DISINFECTANTS:
Citrox BC

I have now received the test results for your product Citrox BC that was tested for efficacy for purposes of the following Orders:

Test	Result	Dilution Rate
Foot And Mouth	Test Passed	One Part To 20 Parts

If you wish to proceed with the formal approval process for Citrox BC, please let me have an undertaking that the disinfectant in every container of Citrox BC that is sold, offered or exposed for sale shall be of the same quality and composition as the sample submitted for approval. I will then write to you with further details.

Please note that Citrox BC must not be advertised, sold or exposed for sale as an approved disinfectant until this Department has advised you that it has been included in an Order amending the Diseases of Animals (Approved Disinfectants) Order 1978.

Yours sincerely

Karen Skelton

Karen Skelton
Disinfectant Approvals Team

B12A

Exotic Disease Prevention and Control Division
Location: 607, 1A Page Street, London SW1P 4PQ
Tel: 020 7904 6000 Direct: 020 7904 6135
Fax: 020 7904 6128
E-Mail: karen.e.skelton@defra.gsi.gov.uk
Website: www.defra.gov.uk

defra
Department for Environment Food and Rural Affairs

Our ref: TADP 0046
Date: 01 June 2005

Mr I Ripley
Unit 1 River Court
Brighouse Business Village
Brighouse Road
Riverside Park
Middlesbrough
TS2 1RT

Dear Mr Ripley

APPROVAL OF DISINFECTANTS: Citrox BC

I have now received the Disease of Poultry test results for your product Citrox BC.

Order	Result	Dilution rate
Diseases of Poultry Disease	Passed	1:0.7
Diseases of Poultry Disease	Passed	1:1.5
Diseases of Poultry Disease	Passed	1:2.5

If you wish to proceed with the formal approval process for Citrox BC, please let me have an undertaking that the disinfectant in every container of Citrox BC that is sold, offered or exposed for sale shall be of the same quality and composition as the sample submitted for approval. I will then write to you with further details.

Please note that Citrox BC must not be advertised, sold or exposed for sale as an approved disinfectant until this Department has advised you that it has been included in an Order amending the Diseases of Animals (Approved Disinfectants) Order 1978.

Yours sincerely

Karen Skelton

Karen Skelton
Disinfectant Approvals Team

B 12 B

Exotic Disease Prevention and Control Division
Location: 607, 1A Page Street, London SW1P 4PQ
Tel: 020 7904 6000 Direct: 020 7904 6135
Fax: 020 7904 6128
E-Mail: karen.e.skelton@defra.gsi.gov.uk
Website: www.defra.gov.uk

defra
Department for Environment Food and Rural Affairs

Our ref: TADP 0046
Date: 06 June 2005

Mr I Ripley
Unit 1 River Court
Brighouse Business Village
Brighouse Road
Riverside Park
Middlesbrough
TS2 1RT

Dear Mr Ripley

APPROVAL OF DISINFECTANTS: Citrox BC

I have now received the TB and General Purpose test results for your product Citrox BC.

Order	Result	Dilution rate
TB Orders	Passed	1 part to 1.5 parts of water
General Purpose	Passed	1 part to 4 parts of water

If you wish to proceed with the formal approval process for Citrox BC, please let me have an undertaking that the disinfectant in every container of Citrox BC that is sold, offered or exposed for sale shall be of the same quality and composition as the sample submitted for approval. I will then write to you with further details.

Please note that Citrox BC must not be advertised, sold or exposed for sale as an approved disinfectant until this Department has advised you that it has been included in an Order amending the Diseases of Animals (Approved Disinfectants) Order 1978.

Yours sincerely

Karen Skelton

Karen Skelton
Disinfectant Approvals Team

B 12 C

7. B12D

Exotic Disease Prevention and Control Division
Location: 607, 1A Page Street, London SW1P 4PQ
Tel: 020 7904 6000 Direct: 020 7904 6127
Fax: 020 7904 6128
E-Mail: disinfectants@defra.gsi.gov.uk
Website: www.defra.gov.uk

Ian Stanley Ripley
Unit 1 River Court, Brighouse Business Village
Brighouse Road, Riverside Park,
Middlesbrough
TS2 1RT

Our ref: TADP 46
09 June 2006

Dear Ian

APPROVED DISINFECTANTS ORDER 2006: LABELLING OF PRODUCTS NEWLY APPROVED FOR SALE IN ENGLAND AND SCOTLAND

As you may be aware an amendment Order will shortly be made updating the list of disinfectants approved by the Secretary of State for Environment, Food and Rural Affairs and by the Scottish Ministers for use under the Animal Health Act 1981 (as amended) and will come into force on the 16 June. Your product Citrox BC is new to the list and I am writing to inform you of the labelling requirements following the amendment Orders coming into force.

Every container containing a disinfectant which is sold/offered for sale as a disinfectant approved by Defra must have a label securely fixed to the container which includes the following information or be clearly and legibly marked with that information:

This disinfectant has been approved for use in England by the Secretary of State for Environment, Food and Rural Affairs and in Scotland by the Scottish Ministers as a disinfectant for the purposes of

the Foot and Mouth Disease Order 1983 and the Foot and Mouth Disease (Scotland) Order 2006 at the dilution rate of one part of this preparation to (20) parts of water;

the Diseases of Poultry (England) Order 2003 and the Diseases of Poultry (Scotland) Order 2003 at a dilution rate of one part of this preparation to (2.5) parts of water;

the Tuberculosis Order (England & Wales) 1984 (as amended) and the Tuberculosis (Scotland) Order 2005 at a dilution rate of one part of this preparation to (1.5) parts of water;

General orders made under the Animal Health Act 1981 at a dilution rate of one part of this preparation to (4) parts of water.

The contents of this container are guaranteed to be of the same quality and composition as the sample submitted for approval testing.

B12D

defra
Department for Environment
Food and Rural Affairs

TO WHOM IT MAY CONCERN

The disinfectant, Citrox BC, manufactured by Citrox has been officially tested on behalf of the Secretary of State for the Department for Environment, Food and Rural Affairs to determine its suitability for use in England as an approved disinfectant under the Animal Health Act 1981.

Citrox BC passed the test for efficacy for purposes of:

Foot and Mouth Disease Orders	at a dilution rate of 1 part of disinfectant to 20 parts of water
Tuberculosis Orders	at a dilution rate of 1 part of disinfectant to 1.5 parts of water
The Diseases of Poultry Order 1994 under the Animal Health Act 1981	at a dilution rate of 1 part of disinfectant to 2.5 parts water
General Orders as defined in the Diseases of Animals (Approved Disinfectant) Order 1978	at a dilution rate of 1 part of disinfectant to 4 parts of water.

Formal approval of this disinfectant under the Animal Health Act is pending. This will be accorded when Citrox BC is incorporated into Schedule 1 to the Diseases of Animals (Approved Disinfectants) Order 1978.

.....

Karen Skelton
An officer of the Department for Environment, Food and Rural Affairs

Attachment C

Product-Manufactures Certifications

1. MSDS sheet. Ferrer. EXQUIM, SA. Citrus Bioflavonoids Complex 45% HPLC.
2. Certificate of Analysis. Ferrer.
3. Ferrer. Letter confirming that CBC 45% HPLC SPD product code 1301693 come from immature oranges. The product is derived from non GM source. Origin of the oranges is the EU. No pesticides and or fertilizers have been added to CBC 45% HPLC SPD. No GM materials have been used as processing aids.
4. Non Irradiation certificate. Ferrer
5. Sewage certificate. No sewage/sludge has been used. Ferrer
6. Pesticide certificate-residues. Ferrer.
7. HPLC flow sheet for citrus bioflavonoid complex 45% HPLC.
8. Citrox Ltd United Kingdom statement. No D-Limonene.
9. Flow chart from Univar United Kingdom detailing manufacture of finished formulas.

C 1

SAFETY DATA SHEET

1. IDENTIFICATION OF THE SUBSTANCE AND THE COMPANY

Identification of Substance
Denomination: ***Citrus Bioflavonoids Complex 45% HPLC***
Identification of the company

Name: EXQUIM, S.A.
Address: Edifici L'Illa.
Av. Diagonal, 549, 5ª planta 08029 Barcelona
e-mail: exquim@ferrergrupo.com
Telephone: 34 93 504 44 00
Telefax: 34 93 589 45 02
Emergency phone: 34 93 504 44 00

2. COMPOSITION/INFORMATION ON INGREDIENTS

This product is a natural extract from bitter oranges. The main components are the citrus flavanones naringin and neohesperidin.

CAS Nº: 72968-50-4
EINECS Nº: 277-143-2

3.HAZARDS INFORMATION

According to EC criteria (67/548/EC) this product is not classified as a dangerous substance neither for environment nor for human health.
In solid form (dust) possible eye and respiratory irritant.

4. FIRST AID MEASURES

After inhalation: Hazardous effects are not foreseen. Dust can cause irritation. Avoid exposure to dust and provide fresh air.

After ingestion: Hazardous effects are not foreseen. Wash out mouth with water. This product has a slightly bitter taste. Do not attempt to give anything orally to an unconscious person.

After skin contact: Remove contaminated clothing. Wash off with plenty of water.

After eye contact: Rinse with plenty of water.

5. FIRE-FIGHTING MEASURES

Suitable extinguishing media:	Sprayed water, CO_2 dry chemicals.
Not to be used:	Not known
Special hazards:	Incomplete combustion can generate CO.
Protective equipment:	Self-contained breathing apparatus. Full protective clothing.

6. ACCIDENTAL RELEASE MEASURES

Personal:	Avoid contact with dust
Environmental:	Not applicable
Cleaning up:	Avoid dust dispersion. Eliminate dust by aspiration or any other adequate method, and store in drums. Clean up affected area with water.

7. HANDLING AND STORAGE

Handling:	Handle in ventilated areas. Avoid contact with eyes and skin Do not eat, drink or smoke during manipulation.
Storage:	Protect from humidity, the product is hygroscopic. Protect from heating sources. Maintain well ventilated areas. Store at room temperature.

8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

Respiratory:	Use dust mask to avoid dust inhalation.
Hand:	Rubber gloves.
Eye:	Safety goggles or face shield.
Skin:	Normal working clothing.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Light brown hygroscopic powder having a characteristic flavour and bitter taste.
Odour:	Odourless
pH:	2.5 - 5.5 (1% w/v)
Boiling point/range:	Not applicable (solid).
Melting point/range:	Not applicable.
Flash point:	Not applicable
Flammability:	Not applicable
Explosive properties:	Not available
Oxidising properties:	Not available
Vapour pressure:	Not applicable
Relative density:	0.85 - 0.95 g/cc
Solubility:	Soluble in water, glycerol/water (80:20), propylene glycol and aqueous alkali. Partially soluble in ethanol.

Partition coefficient n-octanol/water Not available

10. STABILITY AND REACTIVITY

Conditions to avoid:	Not known
Materials to avoid:	Not known
Hazardous decomposition products:	Not known

11. TOXICOLOGICAL INFORMATION

Citrus Bioflavonoids Complex 45% HPLC is an extract from bitter oranges obtained only by physical means (natural extract). Main components are the citrus flavanones naringin and neohesperidin of no know toxic properties.

References:

1."Evaluation of the health aspects of hesperidin, naringin and citrus bioflavonoid extracts as food ingredients". Federation of American Societies for Experimental Biology, Bethesda, MD. Prepared for FDA, Washington DC. PB 82-192931. 1982.

2."Monograph on bioflavonoids". Informatic, Inc., Rockville, MD. Prepared for FDA, PB 289 600. 1978.

12. ECOLOGICAL INFORMATION

Not available

13. DISPOSAL CONSIDERATIONS

Substance: If removal is technologically not possible, disposal according to the local legislation.

Containers: Drums must only be removed after elimination of any residual material adhered to the drum walls and labels have been properly eliminated.

14. TRANSPORT INFORMATION

This product is not subject to special regulation concerning transport.

15. REGULATORY INFORMATION

This product is not regulated by R or S phrases.

Naringin and bitter orange (flower and peel) are Generally Recognised As safe (GRAS) substances under 21 CFR 182.20.

16. OTHER INFORMATION

Product must be stored, handled and used according with good manufacturing practices. The information contained in this data sheet is, to the best of our knowledge, true and accurate, but any recommendations or suggestions which may be made are without guarantee, since the conditions of use are beyond our control.

Exquim, S.A.
Edifici L'Illa
Av. Diagonal, 549
08029 Barcelona - Spain
Tel.: +34 93 504 44 00
Fax: + 34 93 589 45 02
E-mail: exquim@ferrergrupo.com

ferrer

Manufactured by:
Zoster, S.A.
Murcia -España/Spain

ISO 9001

Certificate of analysis

Product Details:

NAME:	CBC 45% HPLC - SPD -	CODE:	1301693
BATCH:	047E001	MANUFACTURING DATE:	
D.OF ANALYSIS	06-02-2008	RETESTING DATE:	02-2011

Quality Control Results:

Appearance	Light brownish hygroscopic powder having a characteristic flavour and bitter taste.
Solubility	Soluble in water, propylene glycol, glycerol:water (80: 20), aqueous alkali and DMSO.
Identification	1. Identification Fehling Assay: Positive 2. Identification Anthocyanin Assay: Positive.

Determination	Specifications	Unit	Results
Heavy metals	20 max.	ppm	Complies
Loss on drying	5 max.	%	3.9
Residue by calcination	6 max.	%	4.3
HPLC assay (Total citrus flavonoids)	45 min.	%	45.1
Microbiological analysis			
Total plate count	< 1.000	cfu / g	Complies
Yeast and Mould	< 100	cfu / g	Complies
E. Coli	Absence / g		Complies
Salmonella	Absence / 25 g		Complies

Output of computer protected data.
Valid without signature.

boletinanalisis_zoster_exq.rep / 1.2

09-10-2008

C 2

Exquim, S.A.
Av. Diagonal, 549 5º Planta
08029 Barcelona
España/SpainTel.: +34 93 504 44 00
Fax: + 34 93 589 45 02

E-mail: exquim@ferrergrupo.com

ferrer

Manufactured by:
Zoster, S.A.
Murcia -España/Spain
ISO 9001

Specifications

Product code:	1010168
Description:	CBC 45% HPLC
Approved date:	03/01/2005

Appearance	Light brownish hygroscopic powder having a characteristic flavour and bitter taste.
Solubility	Soluble in water, propylene glycol, glycerol:water (80: 20), aqueous alkali and DMSO.
Identification	1. Identification Fehling Assay: Positive 2. Identification Anthocyanin Assay: Positive.

Determination	Specifications	Unit
Heavy metals	20 max.	ppm
Loss on drying	5 max.	%
Residue by calcination	3 max.	%
HPLC assay (Total citrus flavonoids)	45 min.	%
Microbiological analysis		
Total plate count	< 1.000	cfu / g
Yeast and Mould	< 100	cfu / g
E. Coli	Absence / g	N/A
Salmonella	Absence / 25 g	N/A

C 2

Exquim, S.A.
Diagonal 549 5ª planta
E-08029 Barcelona - España
Tel. +34 93 504 44 00 - Fax +34 93 589 45 02
www.exquim.com

CITROX LTD.
9 RIVER COURT,BRIGHOUSE BUSINESS VI
BRIGHOUSE RD, RIVERSIDE PARK,
TS2 1RT MIDDLESBROUGH, CLEVELAND
Reino Unido

Barcelona, May 13th 2009

TO WHOM IT MAY CONCERN

We hereby confirm that C.B.C. 45% HPLC- SPD- (product code 1301693) is supplied by Exquim S.A., and comes from immature oranges. The product is derived from a non GM source.

The origin of the CBC 45% HPLC- SPD- is the European Union. We also confirm that no pesticides and/or fertilizers have been added to CBC 45% HPLC- SPD-

We also confirm that no GM materials have been used as processing aids. This product does not fall into the scope of EU regulation 1829/2003 and 1830/2003. Therefore GM labeling is not required

Sònia Hurtado
QA Manager
EXQUIM, S.A.

EXQUIM S.A. Diagonal, 549 5ª planta Tel. 93 504 44 00 08029 BARCELONA

C 3

R.M.B. 09.02.93 Tomo 26.016 Folio 187 Hoja nº 47.702 Inscripción 31ª N.I.F. A-08425142

Exquim, S.A.
Diagonal 549, 5ª planta
E-08029 Barcelona - España
Tel. +34 93 504 44 00 - Fax +34 93 589 45 02
www.exquim.com

Barcelona, July 2009

NON IRRADIATION CERTIFICATE

We hereby certify that the product C.B.C 45% HPLC-SPD- (code 1301693) has not been treated by Irradiation.

Quality Department
Exquim,S.A

EXQUIM, S.A.
Diagonal, 549
5ª planta
Tel. 93 504 44 00
08029 BARCELONA

C 4

R.M.B. 09.02.93, Tomo 25.016, Folio 147, Hoja nº B-81863,
Inscripción 31ª, N.I.F. A-08425142

Exquim, S.A.
Diagonal 549, 5ª planta
E-08029 Barcelona - España
Tel. +34 93 504 44 00 - Fax +34 93 589 45 02
www.exquim.com

Barcelona, July 2009

SEWAGE CERTIFICATE

We hereby certify that in the manufacturing process of the product C.B.C 45% HPLC-SPD- (code 1301693) any sewage has been used.

Quality Department
Exquim,S.A

EXQUIM, S.A.
Diagonal, 549
5ª planta
Tel. 93.504 44 00
08029 BAR

C 5

R.M.B. 09.02.93, Tomo 25.016, Folio 147, Hoja nº B-81863,
Inscripción 31ª, N.I.F. A-08425142

Exquim, S.A.
Diagonal 549, 5ª planta
E-08029 Barcelona - España
Tel. +34 93 504 44 00 - Fax +34 93 589 45 02
www.exquim.com

PESTICIDES CERTIFICATE

Barcelona, May 2009

For pesticides residues, the production batches of the product C.B. C 45% HPLC –SPD- (1301693) are submitted to periodical controls. These test are carried by an independent laboratory, and until now any of the tested batches showed significant levels of the following compounds.

Alachlor
Aldrin and Dieldrin
Azinphos-methyl
Bromopropylate
Chlordane
Chlorfenvinphos
Chlorpyrifos
Chlorpyrifos- methyl
Cypermethrin
DDT (sumo of p,p'-DDT, o,p'-DDT, p,p'-DDE and p,p'-TDE)
Deltamethrin
Diazinon
Dichlorvos
Endosulfan
Endrin
Ethion
Fenitrothion
Fenveralate
Fonofos
Heptachlor
Hexachlorobenzene
Hexachlorocyclohexane
Lindane
Malathion
Methidation
Parathion
Parathion- methyl
Permethrin
Phosalone
Piperonyl butoxide
Pirimiphos- methyl
Quintozene (sum of quintozene, pentachloroaniline and methyl pentachlorophenyl sulphide).

Quality Department,
Exquim,S.A

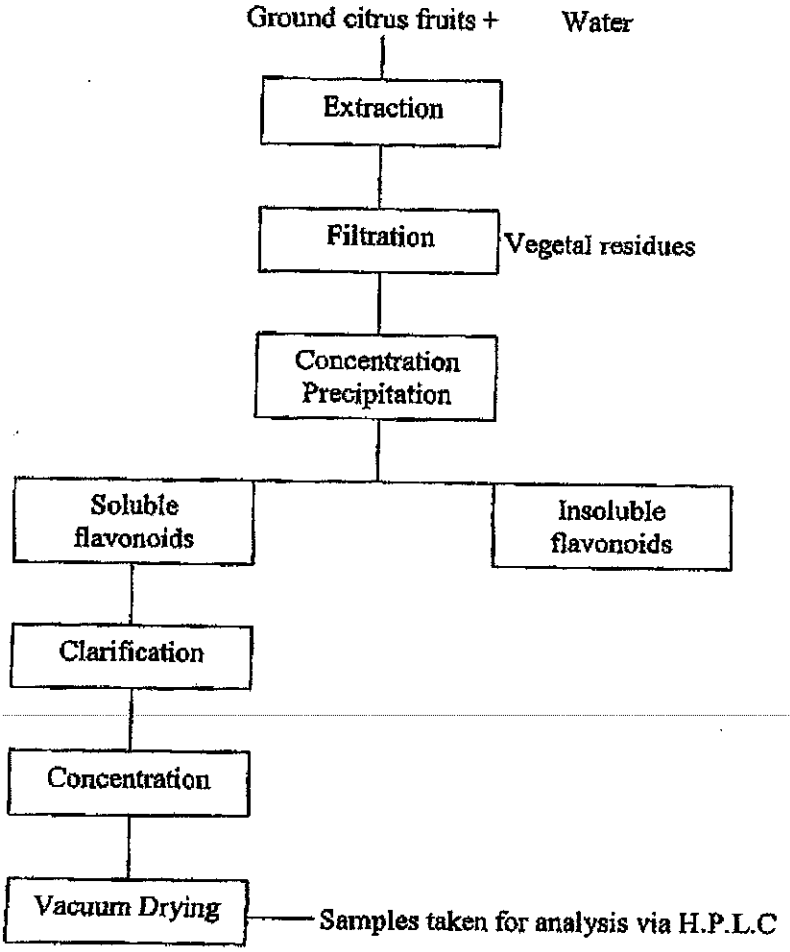
Diagonal, 549
5ª planta
Tel. 93 504 44 00
08029 BARCELONA

C 6

R.M.B. 09.02.93, Tomo 25.016, Folio 147, Hoja nº B-81863, inscripción 31ª, N.I.F. A-08425142

CITRUS BIOFLAVONOID COMPLEX 45% HPLC-FLOW SHEET

CODE N°: 01325
DRUG: *Citrus Aurentium*



C 7

To Whom It May Concern:

I can confirm that there is no D-Limonene present in the Citrus bioflavonoids that are used in Citrox ltd products. The bioflavonoids originate from immature oranges. At that stage in growth where the oranges are picked, crushed and processed D-Limonene is not present in the feedstock.

Yours sincerely

Ian Ripley
Managing Director
Citrox Ltd

C 8

Citrox Limited. Unit 9 River Court, Brighouse Road, Riverside Park, Middlesbrough, TS2 1RT United Kingdom
Tel. +44 (0)1642 241777 Fax. +44 (0)1642 219217 E-mail. info@citrox.net Website. www.citrox.net
Company Registration Number : 4857685 VAT Registration Number : 828 4006 36

C 9

FLOW CHART DETAILING MANUFACTURE

LIQUIDS

De-Min Water (Generated internally by Univar Ltd)

Glycerine (Supplied with unique batch number from supplier)

↓

SOLIDS

Bioflavonoids (Supplied with unique batch number from supplier)

Citric Acid (Supplied with unique batch number from supplier)

Malic Acid (Supplied with unique batch number from supplier)

Ascorbic Acid (Supplied with unique batch number from supplier)

↓

BLENDING

Batch sheet completed with unique batch number assigned.
Record Batch numbers and quantities for all raw materials used.

↓

SAMPLE

Physical properties measured in Univar QC laboratory (on-site) to ensure product complies with manufacturing pre-set manufacturing specifications.

Samples sent for Microbiological Analysis and GC Analysis.

↓

TRANSFER TO BULK STORAGE

Product is transferred to bulk storage awaiting results from Microbiological Analysis and Gas Chromatography Mass Spectrometry Analysis.

Bulk manufacture batch number assigned at this stage.

↓

FILLING

After all analysis is complete product is positively released.

Product filled into selected containers and assigned a new unique batch number.

All containers used are new and suitable for use with food contact products.

Product is now ready for despatch to customers.

Non conforming products

Any product which does not meet current specification is quarantined and kept in a separate storage area.

Any product that does not meet specifications detailed in the flow chart is disposed of in the appropriate manner.

This method of separation and disposal ensures that any non-conforming product is removed from the supply chain.

Attachment D

Toxicity studies

1. Reading Scientific Services Limited. RSSL Pharma. United Kingdom. Report on the determination of Acute dermal irritancy by skin patch testing. 48 hour patch test. Citrox products. Level of irritation is considered acceptable. This test aids in support of the claim Dermatologically tested.
2. Retro Screen final report. "Virucidal assay of two test articles against NIBRG-14 (H5N1) at four concentrations for four incubation time points." GLP lab report. Citrox MD Batch# MD21 and Citrox MDC Batch# MDC10. September 19 2005.
3. Retro Screen Final report. " Evaluation of the toxicity and virucidal activity of a Flavonoid formulated compound against Urbani SARS virus, Influenza A virus, Human Rhinovirus and Human Immunodeficiency Virus." Citrox BC Human cell toxicity studies. Citrox BC. Acute Toxicity assay found that the Citrox BC is non toxic to both cell lines.
4. See Attachment A 7. "Evaluation of the aspects of hesperidin, naringin, and other citrus bioflavonoid extracts as food ingredients." 1982 PB82-192931. Prepared for Bureau of Foods. Food and Drug Administration. Department of Health and Human Services. Washington DC. Contract No. FDA 223-78-2100

Residue Information

5. Residue Information. Estimation of the Value (ppm) of Citrox ProGarda Decontaminant 14WP remaining on produce during the use of a0.5% water solution of ProGarda Decontaminant 14WP.

Fish and Sea Food Industry with Citrox products

See Attachment F. 3
Toxicity Data.
The objective of this study is to assess the Acute toxicity of ProGarda 14FP on fish (Brachydanio rerio). In the present toxicity tests the fish were exposed to various concentrations of the test substance under defined conditions according to OECD 203 (1992). Not Toxic to fish.

Taints and Sensory Trial Baby milk bottles

6. Campden & Chorleywood Food. ReserachAssociation Group. CCFRA Technology Limited.
   Confidential Report. Department of consumer and sensory science.
   Report number. S/REP/102153/1
   Sensory evaluation of milk after storage in direct contact with Citrox sterilizing solution. June 29 2007.

RSSL PHARMA
science with service

REPORT
ON THE DETERMINATION OF ACUTE DERMAL IRRITANCY BY SKIN PATCH TESTING 48 HOUR PATCH TEST

RSSL Study No: P7-06864
Version: Final
Date: 8 January 2008

TEST ARTICLE IDENTIFICATION

Biosan
Sterihands
Mediwipes
Hand Sanitiser/Foam
14W Plus
14T
14X
Sodium Lauryl Sulphate
Sterile Water Control

SPONSORING COMPANY
Citrox Limited
Unit 9 River Court
Brighouse Road
Riverside Park
Middlesborough
TS2 1RT
United Kingdom

Testing Facility
Reading Scientific Services Limited
The Science & Technology Centre
The University of Reading
Earley Gate, Whiteknights Road
Reading
RG6 6BZ
United Kingdom

D 1

REPORT
ON THE DETERMINATION OF
ACUTE DERMAL IRRITANCY
BY SKIN PATCH TESTING
48 HOUR PATCH TEST

RSSL Study No: P7-06864

This was a single-blind study in healthy adult volunteers. The trial was conducted for the Sponsor by Reading Scientific Services Limited (RSSL), at their premises within The Science and Technology Centre on Reading University campus, during the period 22 - 24 August 2007, inclusive. Prior to initiation, the study protocol, associated CRF and the subject information sheet and informed consent form were approved in writing by the Chairman of the RSSL Independent Ethics Committee.

KEY PERSONNEL AND RESPONSIBILITIES

INVESTIGATOR/REPORT AUTHOR

V. A. Hart
Tel: 0118 935 7319; Fax: 0118 935 7345

The investigator assumes overall responsibility for conduct of the study.

QUALIFIED PHYSICIAN

Medical expertise and support for the study was provided by:

Dr. S. Louth

REPORT AUTHOR:

N. A. Viner

CONTACT DETAILS

Sponsor:
Contact Name: Christopher Ripley
Tel: 01642 241777
Fax: 01642 219217
E-mail: info@citrox-products.co.uk

RSSL:
Contact Name: Valerie Hart
Tel: 0118 935 7319
Fax: 0118 935 7345
E-mail: valerie.hart@rssl.com

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1 STUDY SYNOPSIS

1.1 Objective

To determine the primary skin irritation potential of seven antibacterial products versus two controls, following sequential 23-hour applications to intact skin under exaggerated conditions of exposure and to support the claim 'Dermatologically Tested'.

1.2 Summary of Method

The test was conducted in a panel of 28 healthy adult volunteer subjects (20 female and 8 male), 27 subjects completed the study. Occlusive patches bearing the test and control materials were applied to a marked area of intact skin on the upper outer arm for two periods of 23 hours. At the end of each exposure period, volunteers removed and discarded the patch, cleansed the skin as instructed, and returned for assessment of skin reactions approximately 1 hour later. The resulting skin irritation scores were compared in terms of the relative irritancy of the test samples. The two skin assessments were examined independently.

1.3 Summary of Results

Summary of results showing the mean and standard deviation

Product Code	Description	Assessment	
		24 Hours	48 Hours
1	Biosan	**0.56**	**1.22**
		1.19	1.85
2	Sterihands	**0.22**	**0.15**
		0.80	0.36
3	Mediwipes	**0.00**	**0.37**
		0.00	0.84
4	Hand Sanitiser/Foam	**0.67**	**1.89**
		1.52	3.18
5	14W Plus	**0.22**	**0.22**
		0.80	0.80
6	14T	**0.11**	**0.37**
		0.58	1.08
7	14X	**0.00**	**0.15**
		0.00	0.60
8	Sodium Lauryl Sulphate	**1.48**	**2.41**
		1.58	2.19
9	Sterile Water Contol	**0.11**	**0.15**
		0.58	0.60

Key
Mean
Standard Deviation

1.4 Conclusions

Under the conditions of this test, all the test products produced very mild or mild irritation. This level of irritation is considered acceptable for this type of product when tested under occlusion. This test aids in support of the claim 'Dermatologically tested'.

2 STUDY DESIGN

2.1 Test Materials

The materials tested were as detailed below:

Product Code	Product Name	Test Product Concentration %w/w
1	Biosan	100
2	Sterihands	100
3	Mediwipes	100
4	Hand Sanitiser/Foam	100
5	14W Plus	0.1
6	14T	0.1
7	14X	0.1
8	Sodium Lauryl Sulphate	0.3
9	Sterile Water	100
10	Blank Chamber	N/A

2.1.1 Storage and Preparation

Test materials were stored under ambient conditions prior to testing. Study products 5-8 required dilution with sterile water to the concentrations given in the table. The test samples were tested at the concentration as detailed in the table above. Dilutions were prepared prior to each study day. Accountability records were kept for amounts of sample used.

2.1.2 Application

Samples were tested under 8mm occlusive aluminium Finn chambers backed with Scanpor non-occlusive surgical tape. Maximum sample volume was 25µl and filter paper discs were supplied for absorption of liquid samples.

The products were applied by immersing filter paper discs into the product and applying the saturated disc directly to the Finn chamber. Sufficient product was applied to fill the Finn chamber without overloading the chamber, such that excess product was expressed on application of the patch to the skin.

Sample application was allocated according to a repeating block design (Appendix 1) and patches were inverted laterally for application such that the sample array on the skin was a mirror image of that on the patch. Application patterns were coded and recorded for each subject and each skin site was exposed to the same product throughout the test.

2.2 Test Panel

Twenty-eight healthy adult volunteers were recruited into the study from the RSSL volunteer panel, 28 whom were eligible and 27 of whom provided a complete set of data. Each volunteer had previously been assessed to ensure that their health status was compatible with patch test participation. Specific selection criteria were checked as follows prior to the test, using a screening questionnaire.

2.2.1 Inclusion Criteria

i) Male or female
ii) 16 years of age or over
iii) Written informed consent obtained and witnessed

2.2.2 Exclusion Criteria

i) Any significant concurrent illness or skin disease
ii) History of skin disease or allergy relevant to the study
iii) Subjects who have allergies to personal cleansing products, detergents, perfume, cosmetics, and/or toiletries relevant to the study
iv) Use of any topical or systemic medication or drug likely to affect skin response
v) Any significant visible skin abnormality at the test site
vi) Participation in an irritation test, on the same skin site, in the past month, or a sensitisation test, on any skin site, during the past 3 months
vii) Females who are lactating or may be pregnant or if of childbearing potential are not taking adequate contraceptive precautions
viii) Concurrent participation in any other safety study

2.2.3 Subject Information and Informed Consent

At the time of recruitment all volunteers were given a subject information sheet, fully describing the purpose of the test, the procedures involved and possible consequences of taking part. They were invited to ask for further information on any aspects of the study they did not fully understand and, when satisfied that they were adequately informed, were asked to sign a consent form confirming their agreement to take part. The member of staff responsible for providing study information witnessed the consent signature. Each subject received a copy of their consent form to keep.

2.3 Test Procedure

Patches were applied to the upper outer aspect of one arm and marked at the corners to facilitate location of test sites for assessment following patch removal, and to ensure accurate relocation of the next patch. Subjects were instructed to remove and discard the patch after 23 hours, cleanse the skin as detailed in their information sheet, to remove residual product, and return to the test centre approximately 1 hour later for assessment of the skin at treated sites.

In the absence of strong skin reactions, identical patches were applied to the same skin site for a further 23 hours. These patches were removed by the volunteer after 23 hours, as before, and skin sites were assessed 1 hour later.

Subjects were asked to keep patches dry while in position. They were advised that, if they experienced irritation or intense itching at treated skin sites, they should remove the patch immediately, note the time, and contact the test centre as soon as possible.

Following a significant reaction at any skin site at 24 hours, the test sample responsible was not reapplied for subsequent exposures, at the discretion of the Assessor. Significant reaction(s) at the final assessment were monitored at intervals until resolved.

2.4 Skin Assessment Procedure

The skin was assessed visually for reaction by a trained Assessor and any apparent oedema was confirmed by palpation. Assessments were made under standard conditions of illumination, using a hand-held lamp fitted with an incandescent blue daylight bulb.

Since several different visual indicators are symptomatic of skin irritation, the system of assessment used accounts for and grades each condition separately then sums them to provide a composite irritation score. In addition, since the various symptoms differ in terms of clinical significance, each is multiplied by a clinical weighting factor as illustrated below.

SYMPTOM	LETTER DESCRIPTOR	CLINICAL WEIGHTING FACTOR
Vesiculation	V	5
Erosion	E	5
Oedema	O	4
Papules	P	4
Erythema	R	3
Fissuring	F	2
Scaling	S	2
Wrinkling	W	1
Glazing	G	1

The grades used to describe the severity of each symptom observed were as follows:

0 No visible relevant reaction
1 Minimal reaction, inconclusive
2 Slight but definite reaction
3 Moderate reaction
4 Severe reaction

For example, a reaction consisting of slight oedema with moderate erythema and minimal surface glazing would be assessed as O2 R3 G1 and would be scored 4x2 + 3x3 + 1x1 = 18.

As a general rule, a score of 9 or greater at 24 hours indicated that a sample should not be reapplied to that subject but application was at the discretion of the Assessor at all times. When a sample was not reapplied at 24 hours, the site was graded again at 48 hours. The 48-hour irritation score was recorded with the resolving score in parenthesis and the higher value of the stopping score and the resolving score was used in processing the 48-hour assessment data.

The 24 and 48 hour assessments were recorded on separate score sheets and the Assessor was not aware which sample had been applied to each skin site while grading the skin.

2.5 Analysis of Results

The results of the 24 and 48-hour assessments are treated separately. No formal statistical analysis has been performed but the mean skin irritation scores and associated standard deviations are summarised and assessed for each of the test samples.

2.6 Interpretation of Results

A Test Product is characterised in the following manner using the mean total irritation scores:

Very Mild	=	Less than 1.0
Mild	=	1.0 – 2.9
Mild – Moderate	=	3.0 – 4.9
Moderate	=	5.0 – 6.9
Moderate – Severe	=	7.0 – 9.9
Severe	=	Greater than 10.0

The negative control (sterile water) will normally be classed as very mild.

The positive control (0.3% SLS) will normally be classed as moderate.

The claim 'Dermatologically tested' may be claimed on completion of this test, however, this may be dependant upon the nature of the product tested. Generally, up to the mild – moderate category may be considered acceptable, however, the number and nature of the individual reaction should be taken into consideration.

3 RESULTS

3.1 Presentation of Data

Of the 28 subjects eligible for the study, 27 subjects provided complete data sets. There were no adverse events.

Tables 1 - 9 present, by subject and assessment, the irritation readings and scores recorded for each of the test products, as well as the total and mean scores and the associated standard deviations (SD) for each sample, calculated over the panel as a whole.

A summary of mean skin irritation scores and standard deviations are presented in the table below:

Product Code	Description	Assessment	
		24 Hours	48 Hours
1	Biosan	**0.56**	**1.22**
		1.19	1.85
2	Sterihands	**0.22**	**0.15**
		0.80	0.36
3	Mediwipes	**0.00**	**0.37**
		0.00	0.84
4	Hand Sanitiser/Foam	**0.67**	**1.89**
		1.52	3.18
5	14W Plus	**0.22**	**0.22**
		0.80	0.80
6	14T	**0.11**	**0.37**
		0.58	1.08
7	14X	**0.00**	**0.15**
		0.00	0.60
8	Sodium Lauryl Sulphate	**1.48**	**2.41**
		1.58	2.19
9	Sterile Water Contol	**0.11**	**0.15**
		0.58	0.60

Key
Mean
Standard Deviation

3.2 Discussion and Overall Conclusions

At assessment 1 all test products produced very mild irritation.

At assessment 2 the test products (Biosan and Hand Sanitiser/Foam) produced mild irritation. The other test products produced very mild irritation (Sterihands, Mediwipes, 14W Plus, 14T and 14X).

The positive control produced mild irritation, which was lower than the expected moderate irritation and the negative control produced very mild irritation as expected.

Under the conditions of this test, the test products (Sterihands, Mediwipes, 14W Plus, 14T and 14X) produced very mild irritation. The test products (Biosan and Hand Sanitiser/Foam) produced mild irritation. This level of irritation is considered acceptable for this type of product when tested under occlusion. This test aids in support of the claim 'Dermatologically tested'.

4 ACCOUNTABILITY

4.1 Adverse Events

An adverse event may be defined as any unexpected, adverse change in a subject's health status while the test is in progress, regardless of whether or not that change is related to treatment with a test material.

An adverse event is classified as SERIOUS if it is one of the following:

i) Fatal or life-threatening
ii) Permanently disabling or so incapacitating that normal activity is not possible
iii) Responsible for in-patient hospitalisation
iv) A congenital abnormality, birth defect, or cancer
v) An overdose (accidental or deliberate)

All other adverse events are classified as NON-SERIOUS.

Adverse events were recorded on a specific form (Appendix 2), which recorded severity as:

i) Mild – subject may experience slight discomfort but normal activity is not limited
ii) Moderate – subject may experience significant discomfort and some limitation to normal activity
iii) Severe – subject may experience intolerable discomfort and is unable to perform normal activity

The form also recorded the probability that the adverse event was related to treatment with a test material as: definite, probable, possible, unlikely, or none, and notes the outcome.

No adverse events were reported in this study. Tape reactions are not defined as adverse events since they form part of the expected outcome of treatment encompassed by the scoring system.

4.2 Withdrawal

Any volunteer wishing to withdraw from the study was entitled to do so without being obliged to give a reason. Every attempt was made to obtain and record the reason for voluntary withdrawal.

In addition, the investigator may withdraw a subject from the study for reasons including, but not limited to the following:

i) adverse event
ii) protocol violation
iii) alteration to concomitant medication
iv) change in symptoms of concurrent illness

Of the 28 eligible subjects, 27 successfully completed the study. One subject (#003) withdrew from the study, as they were unable to attend visit two. A Subject Accountability form (Appendix 3) was completed for the subject who did not complete the study.

5 ARCHIVING

On completion of the study, the protocol, report and all raw data will be archived by RSSL for a period of 6 years, after which time they will be either destroyed or returned to the Sponsor by prior arrangement. Data identifying subjects by name will be retained by RSSL.

6 REFERENCES

ICH Harmonised Tripartite Guideline for Good Clinical Practice approved on 17th July 1996 by the Committee for Proprietary Medicinal Products (CPMP/ICH/135/95).

World Medical Association (WMA) Declaration of Helsinki
Ethical Principles for Medical Research Involving Human Subjects
Adopted by: the 18th WMA General Assembly, Helsinki, Finland, June 1964.
Amended by: the 29th WMA General Assembly, Tokyo, Japan, October 1975;
the 35th WMA General Assembly, Venice, Italy, October 1983;
the 41st WMA General Assembly, Hong Kong, September 1989;
the 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996;
the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000.
Note of Clarification on Paragraph 29 added by the WMA General Assembly, Washington 2002.

7 STUDY VALIDATION

REPORT
ON THE DETERMINATION OF
ACUTE DERMAL IRRITANCY
BY SKIN PATCH TESTING
48 HOUR PATCH TEST

RSSL Study No: P7-06864

This trial was conducted according to the principles and aims of the ICH Guidelines on Good Clinical Practice, but no formal compliance is claimed. In the interest of concise reporting, administrative information contained in the protocol or study file is not necessarily repeated in the report but is archived with it in the Study Master File for a period of 6 years. This information includes CV's of key study personnel, Ethics Committee documentation, Consent Form and Information Sheet. All routine activities conducted during the course of this study were performed in accordance with RSSL's GCP Standard Operating Procedures.

7.1 Quality Assurance

As far as can be reasonably established, this final report is an accurate reflection of the raw data generated in conducting the above study. It has been audited by the RSSL Quality Assurance Unit (Audit Report No:SENGCP3607).

QA Signature: _____ Date: 01/Feb/08.
(A Kirk/ S Bowles)

7.2 RSSL Responsible Personnel

This report is an accurate account of the procedures involved in performing the above study and the results so obtained.

Investigator: _____ Date: 31 Jan 2008
(V. A. Hart)

RSSL Principal Scientist: E. Brennan Date: 1st Feb 2008
(E Brennan)

Table 1: Individual Irritation Readings and Scores – Biosan

		Assessment 1		Assessment 2	
Subject	Code	Reading	Score	Reading	Score
01	1	R1	3	R1	3
02	1	0	0	R1	3
03	1	-	-	-	-
04	1	0	0	G1	1
05	1	0	0	0	0
06	1	0	0	0	0
07	1	0	0	G1	1
08	1	0	0	G1	1
09	1	0	0	0	0
10	1	0	0	0	0
11	1	0	0	G1	1
12	1	0	0	G1	1
13	1	0	0	R2G1	7
14	1	0	0	G1	1
15	1	R1	3	R1	3
16	1	0	0	0	0
17	1	0	0	0	0
18	1	R1	3	R1	3
19	1	R1	3	R2	6
20	1	0	0	0	0
21	1	0	0	G1	1
22	1	0	0	0	0
23	1	0	0	G1	1
24	1	0	0	0	0
25	1	0	0	0	0
26	1	R1	3	0	0
27	1	0	0	0	0
28	1	0	0	0	0
		n	27		27
		Total	15		33
		Mean	**0.56**		**1.22**
		SD	1.19		1.85

Table 2: Individual Irritation Readings and Scores – Sterihands

		Assessment 1		Assessment 2	
Subject	Code	Reading	Score	Reading	Score
01	2	0	0	0	0
02	2	0	0	0	0
03	2	-	-	-	-
04	2	0	0	G1	1
05	2	R1	3	G1	1
06	2	R1	3	G1	1
07	2	0	0	0	0
08	2	0	0	0	0
09	2	0	0	0	0
10	2	0	0	0	0
11	2	0	0	0	0
12	2	0	0	0	0
13	2	0	0	G1	1
14	2	0	0	0	0
15	2	0	0	0	0
16	2	0	0	0	0
17	2	0	0	0	0
18	2	0	0	0	0
19	2	0	0	0	0
20	2	0	0	0	0
21	2	0	0	0	0
22	2	0	0	0	0
23	2	0	0	0	0
24	2	0	0	0	0
25	2	0	0	0	0
26	2	0	0	0	0
27	2	0	0	0	0
28	2	0	0	0	0
		n	27		27
		Total	6		4
		Mean	**0.22**		**0.15**
		SD	0.80		0.36

Table 3: Individual Irritation Readings and Scores – Mediwipes

		Assessment 1		Assessment 2	
Subject	Code	Reading	Score	Reading	Score
01	3	0	0	0	0
02	3	0	0	0	0
03	3	-	-	-	-
04	3	0	0	G1	1
05	3	0	0	0	0
06	3	0	0	0	0
07	3	0	0	0	0
08	3	0	0	R1	3
09	3	0	0	0	0
10	3	0	0	0	0
11	3	0	0	0	0
12	3	0	0	0	0
13	3	0	0	G1	1
14	3	0	0	G1	1
15	3	0	0	R1	3
16	3	0	0	0	0
17	3	0	0	0	0
18	3	0	0	0	0
19	3	0	0	0	0
20	3	0	0	0	0
21	3	0	0	0	0
22	3	0	0	0	0
23	3	0	0	G1	1
24	3	0	0	0	0
25	3	0	0	0	0
26	3	0	0	0	0
27	3	0	0	0	0
28	3	0	0	0	0
		n	27		27
		Total	0		10
		Mean	**0.00**		**0.37**
		SD	0.00		0.84

Table 4: Individual Irritation Readings and Scores – Hand Sanitiser/Foam

		Assessment 1		Assessment 2	
Subject	Code	Reading	Score	Reading	Score
01	4	0	0	0	0
02	4	R1	3	R2	6
03	4	-	-	-	-
04	4	0	0	G1	1
05	4	0	0	O2R2	14
06	4	0	0	0	0
07	4	0	0	0	0
08	4	0	0	R1G1	4
09	4	R1	3	R2	6
10	4	0	0	0	0
11	4	0	0	G1	1
12	4	0	0	0	0
13	4	0	0	G1	1
14	4	0	0	0	0
15	4	R1	3	G1	1
16	4	0	0	0	0
17	4	0	0	0	0
18	4	0	0	0	0
19	4	R1	3	R1	3
20	4	0	0	0	0
21	4	0	0	R1G1	4
22	4	0	0	0	0
23	4	0	0	G1	1
24	4	0	0	0	0
25	4	0	0	R1	3
26	4	R2	6	R2	6
27	4	0	0	0	0
28	4	0	0	0	0
		n	27		27
		Total	18		51
		Mean	**0.67**		**1.89**
		SD	1.52		3.18

Table 5: Individual Irritation Readings and Scores – 14W Plus

		Assessment 1		Assessment 2	
Subject	Code	Reading	Score	Reading	Score
01	5	0	0	0	0
02	5	0	0	0	0
03	5	-	-	-	-
04	5	0	0	0	0
05	5	0	0	0	0
06	5	0	0	0	0
07	5	0	0	0	0
08	5	R1	3	R1	3
09	5	0	0	0	0
10	5	0	0	0	0
11	5	0	0	0	0
12	5	0	0	0	0
13	5	0	0	0	0
14	5	0	0	0	0
15	5	0	0	0	0
16	5	0	0	0	0
17	5	0	0	0	0
18	5	0	0	0	0
19	5	0	0	0	0
20	5	0	0	0	0
21	5	0	0	0	0
22	5	0	0	0	0
23	5	0	0	0	0
24	5	0	0	0	0
25	5	0	0	0	0
26	5	R1	3	R1	3
27	5	0	0	0	0
28	5	0	0	0	0
		n	27		27
		Total	6		6
		Mean	**0.22**		**0.22**
		SD	0.80		0.80

Table 6: Individual Irritation Readings and Scores – 14T

		Assessment 1		Assessment 2	
Subject	Code	Reading	Score	Reading	Score
01	6	0	0	0	0
02	6	0	0	0	0
03	6	-	-	-	-
04	6	0	0	0	0
05	6	0	0	0	0
06	6	0	0	0	0
07	6	0	0	0	0
08	6	R1	3	R1G1	4
09	6	0	0	0	0
10	6	0	0	0	0
11	6	0	0	0	0
12	6	0	0	0	0
13	6	0	0	0	0
14	6	0	0	0	0
15	6	0	0	0	0
16	6	0	0	0	0
17	6	0	0	0	0
18	6	0	0	0	0
19	6	0	0	0	0
20	6	0	0	0	0
21	6	0	0	R1	3
22	6	0	0	0	0
23	6	0	0	0	0
24	6	0	0	0	0
25	6	0	0	0	0
26	6	0	0	R1	3
27	6	0	0	0	0
28	6	0	0	0	0
		n	27		27
		Total	3		10
		Mean	**0.11**		**0.37**
		SD	0.58		1.08

Table 7: Individual Irritation Readings and Scores – 14X

		Assessment 1		Assessment 2	
Subject	Code	Reading	Score	Reading	Score
01	7	0	0	0	0
02	7	0	0	0	0
03	7	-	-	-	-
04	7	0	0	0	0
05	7	0	0	0	0
06	7	0	0	0	0
07	7	0	0	0	0
08	7	0	0	0	0
09	7	0	0	0	0
10	7	0	0	0	0
11	7	0	0	0	0
12	7	0	0	0	0
13	7	0	0	0	0
14	7	0	0	0	0
15	7	0	0	0	0
16	7	0	0	0	0
17	7	0	0	G1	1
18	7	0	0	0	0
19	7	0	0	0	0
20	7	0	0	0	0
21	7	0	0	R1	3
22	7	0	0	0	0
23	7	0	0	0	0
24	7	0	0	0	0
25	7	0	0	0	0
26	7	0	0	0	0
27	7	0	0	0	0
28	7	0	0	0	0
		n	27		27
		Total	0		4
		Mean	**0.00**		**0.15**
		SD	0.00		0.60

Table 8: Individual Irritation Readings and Scores – 0.3% Sodium Lauryl Sulphate

		Assessment 1		Assessment 2	
Subject	Code	Reading	Score	Reading	Score
01	8	0	0	R1	3
02	8	R1	3	R2G1	7
03	8	-	-	-	-
04	8	0	0	0	0
05	8	R1	3	R2	6
06	8	0	0	0	0
07	8	0	0	0	0
08	8	R1	3	R2	6
09	8	0	0	R1	3
10	8	0	0	R1	3
11	8	0	0	0	0
12	8	R1	3	R1	3
13	8	R1	3	R1	3
14	8	R1	3	0	0
15	8	0	0	0	0
16	8	R1	3	R1	3
17	8	R1	3	R1G1	4
18	8	R1	3	G1	1
19	8	R1	3	R1G1	4
20	8	0	0	R1	3
21	8	0	0	R1	3
22	8	0	0	0	0
23	8	0	0	G1	1
24	8	R1G1	4	0	0
25	8	0	0	R1	3
26	8	R1	3	R2	6
27	8	0	0	0	0
28	8	R1	3	R1	3
		n	27		27
		Total	40		65
		Mean	**1.48**		**2.41**
		SD	1.58		2.19

Table 9: Individual Irritation Readings and Scores –Sterile Water

		Assessment 1		Assessment 2	
Subject	Code	Reading	Score	Reading	Score
01	9	0	0	0	0
02	9	0	0	0	0
03	9	-	-	-	-
04	9	0	0	0	0
05	9	0	0	0	0
06	9	0	0	0	0
07	9	0	0	0	0
08	9	0	0	0	0
09	9	0	0	0	0
10	9	0	0	0	0
11	9	0	0	G1	1
12	9	0	0	0	0
13	9	0	0	0	0
14	9	0	0	0	0
15	9	0	0	0	0
16	9	0	0	0	0
17	9	0	0	0	0
18	9	0	0	0	0
19	9	R1	3	0	0
20	9	0	0	0	0
21	9	0	0	0	0
22	9	0	0	0	0
23	9	0	0	0	0
24	9	0	0	0	0
25	9	0	0	0	0
26	9	0	0	R1	3
27	9	0	0	0	0
28	9	0	0	0	0
		n	27		27
		Total	3		4
		Mean	**0.11**		**0.15**
		SD	0.58		0.60

APPENDIX 1 RANDOMISATION CODE

STUDY NUMBER: P7-06864

Sample position as viewed on PATCHES

A		B		C		D		E	
1	2	10	1	9	10	8	9	7	8
10	3	9	2	8	1	7	10	6	9
9	4	8	3	7	2	6	1	5	10
8	5	7	4	6	3	5	2	4	1
7	6	6	5	5	4	4	3	3	2

F		G		H		I		J	
6	7	5	6	4	5	3	4	2	3
5	8	4	7	3	6	2	5	1	4
4	9	3	8	2	7	1	6	10	5
3	10	2	9	1	8	10	7	9	6
2	1	1	10	10	9	9	8	8	7

Sample position as viewed on SKIN

A		B		C		D		E	
2	1	1	10	10	9	9	8	8	7
3	10	2	9	1	8	10	7	9	6
4	9	3	8	2	7	1	6	10	5
5	8	4	7	3	6	2	5	1	4
6	7	5	6	4	5	3	4	2	3

F		G		H		I		J	
7	6	6	5	5	4	4	3	3	2
8	5	7	4	6	3	5	2	4	1
9	4	8	3	7	2	6	1	5	10
10	3	9	2	8	1	7	10	6	9
1	2	10	1	9	10	8	9	7	8

Numbers are RSSL sample numbers and may be related to products by reference to section 2.1 of the protocol, Test Materials.

N.B. Sample 10 = Blank untreated chamber added for ease of application

RETROSCREEN VIROLOGY

Final Report

CONFIDENTIAL

Virucidal assay of two test articles against NIBRG-14 [H5N1] at four concentrations for four incubation time points

Report Author(s): Ms. Shobana Balasingam
Retroscreen Virology Ltd.
Centre for Infectious Diseases
Barts & The London
Queen Mary's School of Medicine & Dentistry
Medical Sciences Building
327 Mile End Road
London E1 4NS

Sponsor: Dr. Gail Darlington
Citrox Ltd.
8-10 Howard Street
Lorne Howard Industrial Estate
Middlesborough
TS1 5RA

Study No: TXL-PCF-002
Date Issued: 19 Sep 2005
Total number of pages: 13

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1 Contents

2 Summary of tables and figures

3 Summary

Toxicity was observed against the MDCK cells with the 0.5 (v/v) concentration of both test articles. This toxicity prevented assessment of virucidal activity at this concentration.

The test compound, Citrox MD was found to be virucidal at all the concentrations tested and time points except for the 0.001 (v/v) concentration at the 10 minute time point.

The test compound, Citrox MDC was found to be virucidal at all the concentrations tested and time points except for the 0.001 (v/v) concentration at the 10 minute time point.

4 Statement of compliance with Good Laboratory Practice regulations

The study described in this report was conducted in compliance with the following Good Laboratory Practice standards and I consider the data to be valid.

The United Kingdom Good Laboratory Practice Regulations 1999 Statutory Instrument No. 3106.

OECD Principles of Good Laboratory Practice, (Revised 1997).

	Signed	Date
Ms. Shobana Balasingam Study Director Retroscreen Virology Ltd.	.....	...../...../.....

5 Quality Assurance statement

Quality Assurance have audited this report. The methods, practices and procedures reported herein are an accurate description of those employed at Retroscreen Virology Ltd. during the course of the study. Observations and results presented in this Final Report form a true and accurate representation of the raw data generated during the conduct of the study at Retroscreen Virology Ltd.

	Signed	Date
Mr. Jonathan Riley Retroscreen Virology Ltd.	.....	...../...../.....

6 Aims and objectives of the study

To determine the virucidal activity of two test compounds against NIBRG-14 [H5N1] Influenza virus at four concentrations and at four time points.

7 Materials and methods

7.1 The test article(s)

- Citrox MD Batch# MD21
- Citrox MDC Batch# MDC10

The character of the test article(s) was the responsibility of the sponsor.

7.2 The negative control article

The negative control article was MDCK infection media only.

7.3 The positive control article

The negative control article was citrate buffer at pH 3.5

7.4 Cells and Virus

The cells used were MDCK cells.

The virus used was NIBRG-14 (AL: 870) with a stock titre of 1 x $10^{7.5}$ $TCID_{50}$/ml. The virus was diluted 1/10 before use in the assay.

7.5 The Virucidal Assay

The two test compounds were tested at the following concentrations (v/v); neat, 0.2, 0.02 and 0.002 at 0.5, 2, 5 and 10 minutes.

200µl of the test compound at each concentration was incubated with 200µl of the virus at room temperature. The reaction was terminated by adding 3.6mls of MDCK infection media.

The resulting samples were then titrated on 96 well plates containing a confluent layer of MDCK cells.

The plates were CPE scored 3 days after infection and an HAI assay was performed.

7.6 The HA assay

The HA assay was carried out in accordance with the current Retroscreen Virology Ltd. SOP #VA018-02. The plate layout used is detailed in the laboratory notebooks.

7.7 Results

The tables below show the titres obtained together with the percentage reduction after incubation with the two test compound. The concentrations noted are the final concentrations of the compound after addition of the virus.

7.7.1 Citrox-MD

Table 1: Percentage reduction in titre after incubation with 0.5 (v/v) Citrox-MD

Incubation time (mins)	Viral titre (log_{10} $TCID_{50}$/ml)		Reduction in viral titre ($-log_{10}$ $TCID_{50}$/ml)	(%)
	Virus control	Test article		
10	7.5	≤3.8*	≥3.7	99.98005
5	7.5	≤3.8*	≥3.7	99.98005
2	7.5	≤3.8*	≥3.7	99.98005
0.5	7.5	≤3.8*	≥3.7	99.98005

*** Toxicity was observed in the first concentration of the samples.**

Table 2: Percentage reduction in titre after incubation with 0.1 (v/v) Citrox-MD

Incubation time (mins)	Viral titre (log_{10} $TCID_{50}$/ml)		Reduction in viral titre ($-log_{10}$ $TCID_{50}$/ml)	(%)
	Virus control	Test article		
10	6.5	≤3.8*	≥2.7	≥99.80047
5	6.5	≤3.8*	≥2.7	≥99.80047
2	6.5	≤3.8*	≥2.7	≥99.80047
0.5	6.5	≤3.8*	≥2.7	≥99.80047

*** Toxicity was observed in the first concentration of the samples.**

Table 3: Percentage reduction in titre after incubation with 0.01 (v/v) Citrox-MD

Incubation time (mins)	Viral titre (log_{10} $TCID_{50}$/ml)		Reduction in viral titre ($-log_{10}$ $TCID_{50}$/ml)	(%)
	Virus control	Test article		
10	7.5	≤2.8	4.7	99.99801
5	7.5	≤2.8	4.7	99.99801
2	7.5	≤2.8	4.7	99.99801
0.5	7.5	≤2.8	4.7	99.99801

Table 4: Percentage reduction in titre after incubation with 0.001 (v/v) Citrox-MD

Incubation time (mins)	Viral titre (log_{10} $TCID_{50}$/ml)		Reduction in viral titre	
	Virus control	Test article	(-log_{10} $TCID_{50}$/ml)	(%)
10	≥8.2	8.5	0.0@	0.0@
5	≥8.2	7.5	0.7	80.04738
2	7.7	7.5	0.2	36.90423
0.5	≥8.2	8.0	0.2	36.90423

@ Test article value was higher than that of control virus, no reduction was calculated.

7.7.2 Citrox-MDC

Table 5: Percentage reduction in titre after incubation with 0.5 (v/v) Citrox-MDC

Incubation time (mins)	Viral titre (log_{10} $TCID_{50}$/ml)		Reduction in viral titre	
	Virus control	Test article	(-log_{10} $TCID_{50}$/ml)	(%)
10	7.5	≤3.8*	≥3.7	99.98005
5	7.5	≤3.8*	≥3.7	99.98005
2	7.5	≤3.8*	≥3.7	99.98005
0.5	7.5	≤3.8*	≥3.7	99.98005

*** Toxicity was observed in the first concentration of the samples.**

Table 6: Percentage reduction in titre after incubation with 0.1 (v/v) Citrox-MDC

Incubation time (mins)	Viral titre (log_{10} $TCID_{50}$/ml)		Reduction in viral titre	
	Virus control	Test article	(-log_{10} $TCID_{50}$/ml)	(%)
10	7.5	≤2.8	≥3.7	99.98005
5	7.5	≤2.8	≥3.7	99.98005
2	7.5	≤2.8	≥3.7	99.98005
0.5	7.5	≤2.8	≥3.7	99.98005

Table 7: Percentage reduction in titre after incubation with 0.01 (v/v) Citrox-MDC

Incubation time (mins)	Viral titre (log_{10} $TCID_{50}$/ml)		Reduction in viral titre	
	Virus control	Test article	($-log_{10}$ $TCID_{50}$/ml)	(%)
10	8.5	≤2.8	≥5.7	99.99980
5	8.5	≤2.8	≥5.7	99.99980
2	8.5	≤2.8	≥5.7	99.99980
0.5	8.5	≤2.8	≥5.7	99.99980

Table 8: Percentage reduction in titre after incubation with 0.001 (v/v) Citrox-MDC

Incubation time (mins)	Viral titre (log_{10} $TCID_{50}$/ml)		Reduction in viral titre	
	Virus control	Test article	($-log_{10}$ $TCID_{50}$/ml)	(%)
10	7.5	6.8	0.7	80.04738
5	7.5	7.3	0.2	36.90427
2	7.5	7.8	0.0@	0.0@
0.5	7.5	6.3	1.2	93.69043

@ Test article value was higher than that of control virus, no reduction was calculated.

8 Conclusion

Toxicity was evident at the 0.5 (v/v) concentration of both test compounds on the MDCK cells at the 0.5, 2, 5 and 10 minute time points.

The presence of toxicity masks any observations of viral CPE where it is observed and also affects viral growth.

Citrox MD

The highest reduction in viral titre observed was a 4.7 $-\log_{10}$ reduction (99.998%) of the Influenza NIBRG-14 [H5N1] virus at the 0.01 (v/v) concentration at the 0.5, 2, 5 and 10 minute time points.

Citrox MDC

The highest reduction in viral titre observed was a ≥ 5.7 $-\log_{10}$ reduction (99.9998%) of the Influenza NIBRG-14 [H5N1] virus at the 0.01 (v/v) concentration at the 0.5, 2, 5 and 10 minute time points.

9 Archive statement

The dedicated laboratory notebooks, Study Protocol, and Final Report, together with any other relevant information, may be held in the Retroscreen Virology Ltd.'s secure archive for 12 months, from the issuing of the Final Report at no charge. After 12 months, storage may continue at the cost of £50.00 plus VAT per annum per box (W x D x H; 418mm x 710mm x 280mm) payable in advance or, the material may be returned to the Sponsor.

9.1 Sample storage

All samples provided by the Sponsor and all samples generated during the Research Project will be disposed of three months after completion of the Research Project and the issue of the Final Report, unless otherwise requested by the Sponsor. Storage costs are £10.00 plus VAT per box (9 x 9 samples) per month.

Retroscreen Virology Ltd. reserves the exclusive rights on a small proportion of any samples recovered. The use of these samples will be limited to use as control samples. None of the samples shall be used until completion and final closure of the study. Confirmation that the samples are not required will be sought from the sponsor prior to any other use.

Final Report

RETROSCREEN VIROLOGY

CONFIDENTIAL

Evaluation of the toxicity and virucidal activity of a Flavonoid formulated compound against Urbani SARS virus, Influenza A virus, Human Rhinovirus and Human Immunodeficiency Virus

Report Author : Miss Charlotte Chan
Retroscreen Virology Ltd
Barts & The London
Queen Mary's School of Medicine & Dentistry
Medical Sciences Building
327 Mile End Road
London
E1 4NS

Sponsor : Dr. Gail Darlington
Citrox Ltd
First Floor, Unit 1, River Court
Brighouse Business Village
Brighouse Road
Riverside Park
Middlesbrough
TS2 1RT

Study No : TXL-PCS-001

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D 3

Extract from the main report – re human cell toxicity evaluation

Citrox BC Human Cell Toxicity Studies

The chosen cell lines were selected on the basis of relevance to subsequent anti-pathogenic studies.

The cell lines used were; C1008
MDCK
MRC-5
C8166

Cell lines C1008, MDck and MRC-5 cells are all adherent cell lines, whereas C8166 cells are a suspension cell line.

Both types of cell line differ such that the adherent cell from monolayers by adhering to a surface, whereas suspension cells remain free and in suspension cell maintenance media.

Procedures

For the acute toxicity assay three different dilutions of each Citrox BC concentration was prepared:

- 10^{0} dilution – test concentration prepared undiluted
- 10^{-1} dilution – test concentrations diluted 10-fold
- $10^{-0.7}$ dilution – test concentrations diluted 5-fold

The concentrations resulting from each dilution factor are indicated in Table 2.

The 10^{0} and 10^{-1} dilutions were prepared for the acute toxicity assay performed on the adherent lines, whereas the $10^{-0.7}$, in addition to the 10^{0} dilution, was prepared for the acute toxicity assay performed on the suspension cell line.

Table 1: Initial Citrox BC concentrations resulting from three different dilutions prepared for the acute toxicity assay

Reference Citrox BC concentration (% v/v)	Citrox BC concentrations after each dilution factor (% v/v)		
	Dilution Factors (10^X)		
	0	^{T}-1	*-0.7
5	5	0.5	1.0
2	2	0.2	0.4
0.5	0.5	0.05	0.1
0.25	0.25	0.025	0.05

^{T} applicable only to the acute toxicity assay performed on MDCK, MRC-5 and C1008 cells
* applicable only to the acute assay performed on C8166 cells

10^0 Dilution

To determine the specificity of a compound is to determine if it is active against the various viruses chosen. If a compound is active against both the chosen viruses and cells, that that compound would be non-specific to the virus. The 10^0 dilution serves to determine the specificity of each compound concentration, by evaluating the toxicity of the same concentrations as those to be tested against the chosen pathogen.

10^{-1} Dilution

Due to the methodology of the viral assay, each compound concentration becomes diluted 10-fold before it is exposed to the cells. Therefore, it is only relevant, in terms of the pathogen assay, to assess the toxicity of each compound concentration at a dilution of 10-fold (or 10^{-1}) less.

$10^{-0.7}$ Dilution

As mentioned in previous text, two types of cell line are used; suspension cells and adherent cells.

Unlike the latter, suspension cells are not readily separable (unless centrifuged) from the cell maintenance media. There fore, the presence of this media must be accounted for, such that the addition of a compound to a suspension cell line results in a dilution of that compound.

To overcome this problem the test concentrations were prepared 5-fold (or $10^{-0.7}$) less, rather that 10-fold (or 10^{-1}) less, so that when the suspension cells were added to the compound, the concentration of that compound was halved. Therefore, in this instance, the total dilution of each test concentration, in the presence of the cells, was 10-fold (or 10^{-1}).

Compound Preparation for the chosen pathogen assay

In the pathogen assay, Citrox BC is subject to an 11% dilution after it is mixed with the virus. This dilution was accounted for by making up Citrox BC at concentrations of 11% greater than those detailed in section 8.1.1.

Table 3 details the initial concentrations of Citrox BC prepared for the chosen viruses assay for both types of cell line.

Table 2: Initial Citrox BC concentrations for the chosen pathogen assays prepared 11% greater that the final concentration

Final Citrox BC concentration % (v/v)	Initial Citrox BC concentration (% v/v)
5	5.56
2	2.22
0.5	0.56
0.25	0.28

Acute Toxicity Assay

Each Citrox BC concentration was tested for toxicity on each of the four cell lines. In addition to this, each concentration was measured for levels of pH after completion of the assay.

The procedure for the acute toxicity assay performed on adherent and suspension cell lines differ in methodology, as detailed below.

Figure 1 shows the typical plate layout of a 96-well plate used in the acute toxicity assay.

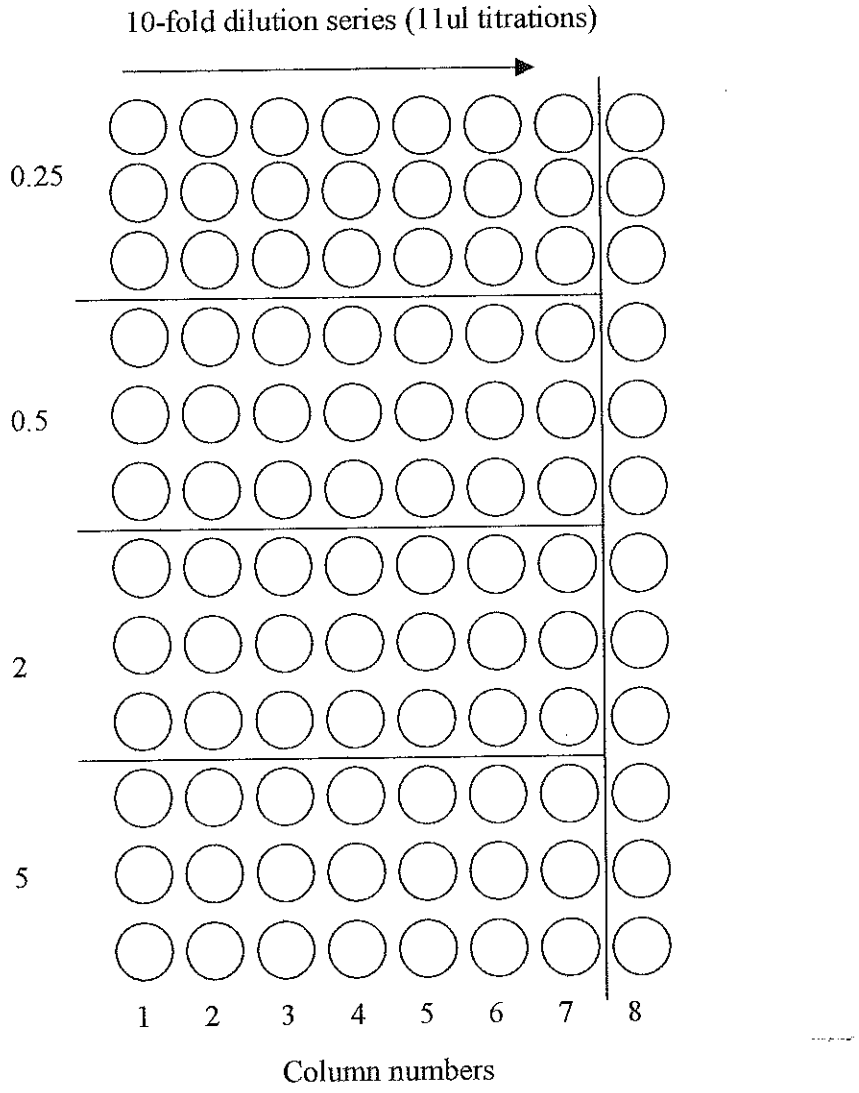

Figure 1: The typical plate layout of a 96-well plate used in the acute toxicity assay of Citrox BC

Adherent Cell Line (MDCK, MRC-5 and C1008)

Preparation of cells

1) Cells (100 ul/well) at 2 x 10^5 cells/ml were seeded onto 96-well plates and incubated at 37^0C, 5% co_2 for 24 hours (MDCK and C1008 cells) or 48 hour (MRC-5 cells).

2) The maintenance media on the plates was removed and the cell monolayers washed twice with PBS (100ul/well).

3) Infection media (100ul/well) specific to each cell line was added to the plates/

Reaction and termination

4) The 10^0 and 10^{-1} dilutions of each concentration of Citrox BC were prepared as detailed previously

5) The infection media in column 1 of the 96-well plates (prepared in steps 1-3) was removed and replaced, in triplicate, with each 10^0 compound concentration (100ul/well).

6) The reaction was incubated for 5 minutes at room temperature.

7) The reaction was terminated by removal of the 10^0 compound concentrations and replacing it with the 10^{-1} compound concentrations (111ul/well).

Compound titration and incubation

8) The 10^{-1} compound concentrations were titrated across the plate following a 10-fold dilution series.

9) The cells were incubated for 5 days at 37^0C. 5% CO_2 (MDCK and C1008 cells) or at 35^0C, 5% CO_2 (MRC-5 cells).

10) The toxicity of Citrox BC was determined by assessing cell viability using the XTT method.

Suspension Cell Line (C8166)

Compound titration

1) The 10^0 and $10^{-0.7}$ dilutions of each concentration of Citrox BC were prepared as detailed in section 8.3.1.1.

2) C8166 growth media (100ul/well) was plated onto all wells of a 96-well plate, except column 1.

3) Each $10^{-0.7}$ compound concentration (111ul/well) was plated, in triplicate, onto column 1.

4) The compounds were titrated 10-fold across the 96-well plate from column 1 to column 7.

5) 50ul of solution was removed from each well, leaving 50ul remaining.

Preparation of cells

6) Cells at 2×10^5 cells/ml were spun down (1000 rpm for 5 minutes) and the supernatant poured off.

7) The cell pellet was re-suspended in C8166 growth media, at a volume such that the resulting number of cells in suspension became 4×10^5 cells/ml.

8) The cells (50ul/well) were added to all wells of the 96-well plate, except column 1.

Reaction and termination

9) Cells (1ml/centrifuge tube) at 2 x 10^5 cells/ml were spun down (1000 rpm for 5 minutes) and the supernatant poured off.

10) The cell pellet was re-suspended in each 10^0 compound concentration (1ml/centrifuge tube), and incubated for 5 minutes at room temperature.

11) The reaction was terminated by the addition of growth media (500ul/centrifuge tube).

12) The terminated reaction was spun down (1000 rpm for 5 minutes) and the supernatant poured off.

Incubation

13) The cell pellet was re-suspended in growth media (0.5ml/centrifuge tube).

14) The terminated cell suspension (50ul/well) was added, in triplicate, to column 1 of the 96-well plates (prepared in steps 1-8).

15) The cells were incubated for 5 days at 37^0C, 5% CO_2.

16) The toxicity of each concentration was determined by assessing cell viability using the XTT method.

Results

Acute Toxicity Assay

Table 3: Percentage cell survival of four different cell lines incubated at room temperature for 5 minutes with four different concentrations of Citrox BC

Cell Line	‡Percentage cell survival (%)			
	Citrox BC Concentration (%v/v)			
	5	2	0.5	0.25
C1008	95	100	100	100
MDCK	80	100	100	100
MRC-5	55	85	85	90
C8166	45	85	90	95

‡ values are rounded to the nearest 5

The results of the acute toxicity assay for all four Citrox BC concentrations are shown in Table 4, which represent cell viability after exposure to the 10^0 dilution only

Compound toxicity is indicated by a cell viability of<80%.

Discussion

Acute Toxicity Assay

Each cell line exhibited 80% cell viability after exposure to all four concentrations of Citrox BC, with the expectation of the 5% v/v concentration on MRC-5 and C8166 cells, as shown in Table 4. The cell Viability of MRC-5 and C8166 cells after exposure to 5% v/v Citrox BC was 55% and 45%, respectively.

Despite this, the 5% v/v Citrox BC concentration was deemed appropriate, in terms of toxicity, for use in the viral assay. The reason for this deduction is that in the pathogen assay, each compound is diluted 10-fold prior to addition to the cells. Therefore, in this instance, the 5% v/v Citrox BC concentration was diluted 10-fold to 0.5% v/v, which according to Table 4 **is non-toxic** to both cell lines.

The hypothesis for the acute toxicity assay was:

- If the compound is non-toxic then the percentage cell survival will not alter with varying Citrox BC concentrations.
- If the compound is toxic then the percentage cell survival will increase with decreasing concentrations of Citrox BC.

The results displayed in Table 4 agree with this hypothesis and show a trend in cell viability from the highest (5% v/v) to the lowest (0.25% v/v) compound concentration.

This is markedly evident in the results for the MRC-5 and C8166 cell lines in which cell viability increases 64% and 111%, respectively, from the 5% v/v concentration to the 0.25% v/v concentration.

When made up in the C1008 and MDCK infection media, the test compound is highly acidic at the 2% v/v and 5% v/v concentrations, but is neutral at the two lower concentrations. For the concentrations made up in C8166 and MRC-5 infection media, however, a more or less neutral pH is exhibited by the 0.25% v/v concentration only, with a much lower pH measured at the higher concentrations.

The difference in pH between the different media used for diluting the compound is probably due to the composition of each.

Appendix

Toxicity Assay

Table 4: Percentage cell viability of C1008 cells after exposure to different concentrations of Citrox BC

Dilution (10^X)	‡Percentage cell survival (%)			
	Citrox BC Concentration (%v/v)			
	5	2	0.5	0.25
0	95	100	100	100
-1	100	100	100	100
-2	100	100	100	100
-3	100	100	100	100
-4	100	100	100	100
-5	100	100	100	100
-6	100	100	100	100

‡ values are rounded to the nearest 5

Table 5: Percentage cell viability of MDCK cells after exposure to different concentrations of Citrox BC

Dilution (10^X)	‡Percentage cell survival (%)			
	Citrox BC Concentration (%v/v)			
	5	2	0.5	0.25
0	80	100	100	95
-1	100	100	100	100
-2	100	100	100	100
-3	100	100	100	100
-4	100	100	100	100
-5	100	100	100	100
-6	100	100	100	100

‡ values are rounded to the nearest 5

Table 6: Percentage cell viability of MRC-5 cells after exposure to different concentrations of Citrox BC

Dilution (10^X)	‡Percentage cell survival (%)			
	Citrox BC Concentration (%v/v)			
	5	2	0.5	0.25
0	55	85	85	90
-1	90	100	100	100
-2	100	100	100	100
-3	100	100	100	100
- 4	100	100	100	100
-5	95	100	100	100
-6	95	100	100	100

‡ values are rounded to the nearest 5

Table 7: Percentage viability of C8166 cells after exposure to different concentrations of Citrox BC

Dilution (10^X)	‡Percentage cell survival (%)			
	Citrox BC Concentration (%v/v)			
	5	2	0.5	0.25
0	45	85	90	95
-1	90	100	90	90
-2	90	100	100	95
-3	90	100	90	95
-4	90	95	95	100
-5	100	95	100	100
-6	100	100	100	95

‡ values are rounded to the nearest 5

RESIDUE INFORMATION

Estimation of the value (ppm) of ProGarda™ Decontaminant (ref 14WP) residues remaining on produce during the use of a 0.5% water solution of ProGarda™ Decontaminant (ref 14WP)

ProGarda™ 14WP has a total concentration value of 222g / 1000g (222mg/ml).

Conventionally, 14WP is used as a 0.5% wt/wt solution in water.

Processing concentration value $\frac{222 \times 0.5}{100}$ = 1.11mg/ml.

Assume produce is lettuce, and 2% wt/wt processing water is retained on leaves.

The 14WP residue is **22.2 ppm** on a wt/wt basis $\frac{1.11 \times 2}{100}$ = 0.0222mg = 22.2 ppm.

Residual value, based on flavonoid content = 22.2 x 0.054 = 1.2 ppm

Notes

1. The daily intake of mixed flavonoids in the average Western diet is 1g (1).
2. The polyphenolic content of 1g of 2 varieties of lettuce is quoted as having a value of between 200 - 250 µg/g (2), (3).
3. **Assume iceberg lettuce has 225 µg/g polyphenols and 14WP residues are 1.2 ppm then there is 187 times more polyphenols in the lettuce than residues on the surface.**
4. The surface area of 1g iceberg lettuce was measured and the value was 64.0cm^2.

Residue value on basis of 1cm^2 of geometric/apparent surface area = $\frac{22.2}{64}$ = 0.35 ppm/g/cm^2.

Residue value, based on flavonoid content = 0.35 x 0.054 = 0.019 ppm/g/cm^2.

It has been suggested (4) that the real surface area will be at least 10^3 X the geometric surface area, leading to a residue value of bioflavonoid of 0.000019 ppm/g/cm^2 ie

20 parts per trillion

Citrox Limited. Unit 9 River Court, Brighouse Road, Riverside Park, Middlesbrough, TS2 1RT United Kingdom
Tel. +44 (0)1642 241777 Fax. +44 (0)1642 219217 E-mail. info@citrox-products.co.uk Website. www.citrox.net

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School of Agriculture, Food and Rural Development
Newcastle University NE1 7RU (24/07/07) - Private Communication.

Citrox Limited. Unit 9 River Court, Brighouse Road, Riverside Park, Middlesbrough, TS2 1RT United Kingdom
Tel. +44 (0)1642 241777 Fax. +44 (0)1642 219217 E-mail. info@citrox-products.co.uk Website. www.citrox.net

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CCFRA Technology Limited
Chipping Campden
Gloucestershire
GL55 6LD, UK

Tel: +44 (0)1386 842000
Fax: +44 (0)1386 842100
www.campden.co.uk

Campden & Chorleywood Food Research Association Group

Christopher Ripley
Citrox Ltd
Unit 9, River Court
Brighouse Business Village
Brighouse Road, Riverside Park
Middlesborough
Cleveland
TS2 1RT

Date: 29th June 2007

DEPARTMENT OF CONSUMER & SENSORY SCIENCE

CONFIDENTIAL REPORT

SENSORY EVALUATION OF MILK AFTER STORAGE IN DIRECT CONTACT WITH CITROX STERILISING FLUID SOLUTION

Report No. S/REP/102153/1

Prepared by : Anna Knight

Checked & authorised by : Susan M. Rogers

Name : Susan Rogers

Authorised Signatories:

S.M. Rogers	Taints & Quality Systems Supervisor
L. Brown	Sensory Services Manager
S.Purcell	Sensory Projects Group Manager
J. Menneer	Sensory Training Group Manager

SUMMARY

Citrox Sterilising Fluid (intended as a natural cleaning fluid for babies feeding bottles), was tested using the sensory test method, TES-S-004, Potential Taint from Direct Contact with Test Materials. The aim of the test was to determine whether the test material had the potential to taint Whole Milk. Babies bottles were cleaned with a 2% solution of Citrox and distilled water, left in contact for 5 minutes and drained, no rinse was specified by the client. Once dry the bottles were filled with milk which was stored in direct contact with the cleaned bottles for 24 hours at 5°C or less. The treated milk samples were compared to untreated (control) milk samples (the bottles were prepared in the same way but omitting the test product, and using water only to clean the bottles, then filled with milk and stored as above), using the Triangle Test Method for similarity and a panel of thirty trained (Triangle Test Method) sensory assessors.

For a triangle test for similarity, using 30 judgements, a maximum number of 11 correct responses are required to establish similarity between the two samples at 10% beta (β) and 30% Pd. The results indicate that 9 assessors correctly identified the odd sample. We can therefore conclude that the samples are similar, at the chosen levels 10% beta (β) and 30% Pd, i.e. we are 90% confident that only 30% of discriminators can detect a difference between the samples.

No taint comments were used to describe the treated milk; stored with bottles cleaned with a 2% Citrox sterilising fluid.

The test results above indicate that the test product, Citrox Sterilising Fluid, does not have the potential to taint when used to clean bottles and stored in direct contact with milk as above.

SAMPLE INFORMATION

Date samples received:	22nd June 2007
Condition on receipt:	Good
Stored:	Room 983, then filled bottles of milk stored in Refrigerator N located in Sensory Kitchen
Date samples tested:	27th June 2007

Sample Description

CCFRA Code	Company Code/Sample Description
SA/102153/1	Marks and Spencers Whole Milk Use by: 02/07/07 UK DG 001 MEC
SA/102153/2	Citrox Sterilising Fluid

METHODS AND REFERENCES

Method reference: Triangle Test No. TES-S-004
(British Standard, Sensory Analysis – Methodology – Triangle Test, BS ISO 4120: 2004)

Deviations from method: Milk stored in direct contact with babies feeding bottles, cleaned with a 2% solution of Citrox Sterilising Fluid, no rinse specified by client

AIM

The aim of the test was to determine whether the Citrox Sterilising Fluid had the potential to taint Whole Milk.

PREPARATION

Six plastic babies feeding bottles (260ml volume) were rinsed with distilled water and left to air dry (control sample). A further six bottles were cleaned with a 2% solution of Citrox sterilising fluid and distilled water (actual application rate of Citrox is 1% but this was doubled to 2% for the purposes of the taint test). The test material was in contact with the bottles for 5 minutes, then emptied and air-dried, no rinse was specified by the client (treated sample). Once bottles were dry, each bottle was filled with Whole Milk and sealed using the bottle lid. The six untreated and six treated prepared milk samples, were then stored for 24 hours at 5°C or less. After storage the treated and untreated (control) milk samples were poured into two separate coded glass jugs and mixed to homogenise each sample. The treated and untreated milk samples were then poured into coded containers (50ml maximum volume). Each assessor received approximately 20ml of milk per coded sample, presented according to he experimental design of the test.

SENSORY TESTING

The samples were evaluated using the Triangle Test Procedure (TES-S-004). In the triangle test assessors are presented with a set of three coded samples, two of which are the same and one of which is different. The sets of samples are presented equally often in each of the six possible orders; this experimental design minimises any possible order and carryover effects.
Thirty trained assessors are used for each test, fifteen receiving "test" as the different sample and fifteen receiving "control" as the different sample. After tasting the three samples in the designated order, each assessor is asked to select the different sample and to describe the difference(s) perceived.

TEST CONDITIONS

The test was carried out in a purpose-built testing room. Each assessor was required to undertake the tests in an individual booth. The room was positively pressurised to minimise the entrance of external odours. Yellow coloured lighting was used to mask any colour difference between the samples. The panel used filtered water and plain crackers as palate cleansers between the samples.

TRIANGLE TEST RESULTS

Results of the test are given in Table 1.

Table 1: Results of Triangle Test

Test Reference No.	No. of Assessors	No. Correctly Identifying the Different Sample	Significance
102153/1 Untreated (control) versus treated whole milk (Treated milk: previously stored in direct contact with bottles cleaned with a 2% solution of Citrox Sterilising Fluid and stored under refrigerated conditions for 24 hours at 5°C or less)	30	9	Similar at 10% beta and 30% Pd

Reference: Sensory Analysis Methodology – Triangle Test BS ISO 4120: 2004.

For a triangle test for similarity, using 30 judgements, a maximum number of 11 correct responses are required to establish similarity between the two samples. The results indicate that 9 assessors correctly identified the odd sample. We can therefore conclude that the samples are similar, at the chosen levels of 10% beta (β) and 30% Pd, that is we are 90% confident that only 30% of discriminators can detect a difference between the samples.

Alpha (α) - probability of concluding that a perceptible difference exists when one does not
Beta (β) – probability of concluding that no perceptible difference exists when one does
Pd – the proportion of assessments in which a perceptible difference is detected between the two products.

Descriptors given when the different sample was correctly identified can be seen in Table 2.

Table 2: Description

Test Reference No. 102153/1

SA/102153/1 Whole Milk	Untreated (Control)	More creamy (1) Tastes fattier (1) Watery taste (1)
SA/102153/2 Whole Milk (treated milk previously stored in direct contact with bottles cleaned with a 2% solution of Citrox Sterilising Fluid and stored under refrigerated conditions for 24 hours at 5°C or less)	Treated (Test)	Sweeter (3) Less harsh aftertaste, slightly less creamy in texture and taste (1) Slight watery flavour compared to other two samples (1) More creamy (1)

() Number of assessors using the descriptor

Attachment E

GLP Microbiological Laboratory Analysis and results.

1. ATS Labs Time Kill Test Assay for Antimicrobial agents. E Coli and Listeria monocytogenes. GLP June 9 2009
2. MICROBIOTEST Labs. AOAC Use dilution test, Health care. Staphylococcus aureus, Pseudomonas aeruginosa, Salmonella enteric. EPA Guidleines 810.2100 ©, (d), (e) GLP
3. BioBest Research. Phytologica Sanitizer-Citrox on Swine Influenza strain H1N1. GLP
4. MICROBEST Labs. Decosan (CitroxAV20) Norwalk virus (Norovirus). GLP

Note 5,6 and 7 were trials done to show the enhancement of the Bioflavonoids combined with Citric acid. To pass the BSEN 1276 standards which were adhered to requiring a Log 5 reduction.

Note as stand alones they do pass the BSEN 1276 standards of a Log 5 reduction. Citric Acid combined with the Bioflavonoids do PASS the BSEN 1276 Log 5 reductions

5. Spartan Nano. Report 2008-04-23 BSEN 1276 under dirty conditions. ***Bioflavonoid and Fruit acid blend***. Citrox BC concentrate. Pseudomonas aeruginosa, Escherichia coli, Staphylococcus, Bacillus subtillis. Non GLP. PASS. The next two reports show Citric acid by itself and the Bioflavonoids by themselves.
6. Spartan Nano. Report 2008-06-03. **Citric Acid** 20%. BSEN 1276 under dirty conditions. Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtillis.
7. Spartan Nano. Report 2008-04-04. **Bioflavonoids.** BSEN 1276 under dirty conditions. Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Bacillus subtillis. Non GLP

Establishing different addition rates for the Bioflavonoids complex.

8. Abbot Analytical. Certificate No: 08k.004.cit. Citrus Bioflavonoids received fron Citrox. BSEN 1276 under dirty conditions. Product test concentrations-0.5%. Log 3 reduction against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus. Does not pass BSEN 1276 Log 5 reduction.
9. Abbot Analytical. Certificate No: 08k.004.a cit. Citrus Bioflavonoids received fron Citrox. BSEN 1276 under dirty conditions. Product test concentrations-10%. Log 3 reduction against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus hirae. Pass BSEN 1276 Log 5 reduction.
10. Abbot Analytical. Certificate No. 07B.115.CIT. BSEN 1276 under dirty conditions. Hand Foam, Citrox. Pseudomonas aeruginosa, Escherichia coli, MRSA NCIMB 50143, Enterococcus hirae. PASS
11. Abbot Analytical. Certificate No. 07B.008.CIT. BSEN 1276 under dirty conditions. Clinisan, Citrox. Clostridium difficile NCTC 11209. PASS
12. Abbot Analytical. Certificate No. 03A.102. Citrox Surface Spray. Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus hirae PASS

13. Abbot Analytical. Certificate No.05D,121.GWP. Citrox Surface cleaner. BSEN 1276 under dirty conditions. Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus hirae. PASS 0.2% v/v
14. Abbot Analytical. Certificate No.04G.003V.CIT. Citrox BC concentrate. BSEN 1276 under dirty conditions. Acitvity against Vibro parahaemolyticus.ATTC 17802. PASS
15. Abbot Analytical. Certificate No. 03A.102.GWP Citrox BC. Pseudomonas aeruginosa. PASS 3%v/v
16. Abbot Analytical. Certificate No. 3m.155.CIT. Citrox BC. Challenge test against Phytophthora Spp @ 0.12%, 0,25% and 0.5%. At 0.5% Citrox BC shows significant reduction in numbers of Phytophthora Spp.
17. Abbot Analytical. Certificate No. 0.3D.314m.GWP. Citrox BC disinfectant. Activity against Mycobacterium. BSEN 1276 under dirty conditions. 15 minutes Log 3 reduction. 30 minutes Log 5 reduction.
18. Abbot Analytical. Certificate No. 02.b.136.GWP. Citrox BC. Challenge test against Moulds, Penicillium digitatum. 30 minutes exposure. PASS
19. Abbot Analytical. Certificate No. 04C.100.CIT. Citrox BC disinfectant. Activity against Listeria on surfaces using an Electrostatic sprayer giving 20 micron droplet size. 1920 cfu per sq. 2.5% (16.8% kill) 10% (100% kill) 25% (100% kill)
20. Abbot Analytical. Certificate No. 03D.3141.GWP. Citrox BC disinfectant. Legionella pneumophila. BSEN 1276 under dirty conditions. PASS
21. Abbot Analytical. Certificate No. 04G.0031.CIT. Citrox BC concentrate. Against Lactobacillus acidophilus. BSEN 1276 under dirty conditions. 0.2% product test concentrations. PASS
22. Abbot Analytical. Certificate No. 06E.184.CIT. Citrox BC. Against Enterococcous Faecalis NCTC 8213. BSEN 1276 under dirty conditions. Product concentration 0.6%v/v. PASS
23. Abbot Analytical. Certificate No. 05F.214.CIT. Citrox BC. Against Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Enterococcus hirae. Product test concentrations. 0.2% w/w BSEN 1276 under dirty conditions. PASS
24. Abbot Analytical. Certificate No. 04H.246C. Citrox BC against Clostridium perfringens. BSEN 1276 under dirty conditions. Product concentrations 0.4% w/w Log 4. Satisfies requirements.
25. Abbot Analytical. Certificate No. 04H,246cd.CIT. Citrox BC against Clostridium diccicile using EN 13704 under dirty conditions. Product concentrations 0.4% v/v Log 3 reduction.
26. Abbot Analytical. Certificate No. 04H.246C. Citrox BC against Campylobacter jejuni NCTC 11322. PASS
27. Abbot Analytical. Certificate No. 5H.099a.KAE. Citrox hand gel activity against EN 1500. EN 1500 criteria were satisfied.

FINAL STUDY REPORT

STUDY TITLE

Time Kill Test Assay for Antimicrobial Agents

Test Organisms:

Escherichia coli (ATCC 11229)
Listeria monocytogenes (ATCC 19117)

PRODUCT IDENTITY

FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750

AUTHOR

Anne Stemper, B.S.
Study Director

STUDY COMPLETION DATE

June 9, 2009

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

SPONSOR

Ahava International
19692 Black Fox Drive
Cottonwood, CA 96022

PROJECT NUMBER

A07762

GOOD LABORATORY PRACTICE STATEMENT

The study referenced in this report was conducted in compliance with U.S. Food and Drug Administration Good Laboratory Practice (GLP) regulations set forth in 21 CFR Part 58.

The studies not performed by or under the direction of ATS Labs are exempt from this Good Laboratory Practice Statement and include: characterization and stability of the compounds.

Submitter:_____ Date:_____

Sponsor:_____ Date:_____

Study Director: Anne Stemper Date: 6-9-09
Anne Stemper, B.S.

QUALITY ASSURANCE UNIT SUMMARY

Study: Time Kill Test Assay for Antimicrobial Agents

The objective of the Quality Assurance Unit is to monitor the conduct and reporting of non-clinical laboratory studies. These studies have been performed under Good Laboratory Practice regulations (21 CFR Part 58) and in accordance to standard operating procedures and standard protocols. The Quality Assurance Unit maintains copies of study protocols and standard operating procedures and has inspected this study on the dates listed below. Studies are inspected at time intervals to assure the integrity of the study.

Phase Inspected	Date	Study Director	Management
Critical Phase	May 21, 2009	May 21, 2009	June 9, 2009
Final Report	June 8, 2009	June 8, 2009	

The findings of these inspections have been reported to management and the Study Director.

Quality Assurance Auditor: Lisa Kahle Date: 6-9-09

TABLE OF CONTENTS

STUDY PERSONNEL

STUDY DIRECTOR:	Anne Stemper, B.S.
Professional personnel involved:	
David Rottjakob, M.T.	- Director, Microbiology Services
Scott R. Steinagel, B.S.	- Manager, Microbiology Laboratory Operations
Joy Salverda, B.S.	- Research Scientist I
Matthew Sathe, B.S.	- Research Assistant II
Erin Hawkinson, B.S.	- Research Assistant I
Megan Polos, B.S.	- Research Assistant I

STUDY REPORT

GENERAL STUDY INFORMATION

Protocol Title: Time Kill Test Assay for Antimicrobial Agents

Project Number: A07762

Protocol Number: AHA01050409.TK

Sponsor: Ahava International
19692 Black Fox Drive
Cottonwood, CA 96022

Test Facility: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

TEST SUBSTANCE IDENTITY

Test Substance Name: FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750

Test Substance Characterization
Test substance characterization as to content, stability, etc., (21 CFR, Part 58) is the responsibility of the Sponsor.

STUDY DATES

Date Sample Received: May 14, 2009
Study Initiation Date: May 18, 2009
Experimental Start Date: May 21, 2009
Experimental End Date: May 26, 2009
Study Completion Date: June 9, 2009

OBJECTIVE

The objective of this testing was to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

SUMMARY OF RESULTS

Test Substance: FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750

Dilution: 1:150 in filter sterilized deionized water

Test Organism: *Escherichia coli* (ATCC 11229)
Listeria monocytogenes (ATCC 19117)

Exposure Time: 30 seconds and 60 seconds

Exposure Temperature: Ambient temperature (22°C)

Organic Soil Load: 5% fetal bovine serum

Efficacy Result: FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750 demonstrated a >99.99% (>4.8 $\log_{10}$) reduction of *Escherichia coli* (ATCC 11229) following a 30 second and a 60 second exposure time at ambient temperature (22°C).

FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750 demonstrated a >99.999% (>5.1 $\log_{10}$) reduction of *Listeria monocytogenes* (ATCC 19117) following a 30 second and a 60 second exposure time at ambient temperature (22°C).

STUDY MATERIALS

Test System/Culture Media

Test Organism	ATCC #	Culture Medium
Escherichia coli	11229	Blood Agar
Listeria monocytogenes	19117	Blood Agar

Blood Agar = Tryptic Soy Agar with 5% Sheep Blood (BAP)

The microorganisms used in this study were obtained from the American Type Culture Collection, Manassas, Virginia.

Recovery Media
Neutralizer: 1.0% Tween 80 + 3.0% Saponin + 0.1% Histadine + 0.1% Cysteine
Agar Plate Medium: Tryptic Soy Agar with 5% Sheep Blood (BAP)

Reagents
Organic Soil Load Description: 5% fetal bovine serum (FBS)

TEST METHOD

Preparation of Test Substance

A 1:150 dilution was prepared using 1.00 mL of the test substance and 149.0 mL of filter sterilized deionized water. A 9.9 mL aliquot of the prepared test substance was transferred to a sterile vessel for testing procedures. The prepared test substance was homogenous as determined by visual observation and was used within three hours of preparation.

Test Organism Preparation

Using a stock culture of the test organism, a culture of each test organism was streaked onto a Tryptic Soy + 5% Sheep Blood Agar plate and incubated for 24-48 hours at 35-37°C. On the day of testing, a sterile swab was used to harvest a sufficient amount of culture from each agar plate and was added to Butterfield's Buffer to yield a culture suspension equal to a 0.5 McFarland Turbidity Standard.

Addition of Organic Soil Load

A 0.25 mL aliquot of FBS was added to 4.75 mL of each broth culture to yield a 5% fetal bovine serum soil load.

Test Exposure

An inoculum of 0.100 mL of each organism suspension was added to 9.9 mL of the test substance and vortex mixed. The test mixture was exposed for 30 seconds and 60 seconds at ambient temperature (22°C).

Subculture

At each exposure time, a 0.100 mL sample was removed from each test mixture and added to 9.9 mL of neutralizer representing a 10^0 dilution of the neutralized inoculated test mixture. A 5.0 mL aliquot of the 10^0 neutralized inoculated test mixture was added to a sterile 0.45 µm filter apparatus pre-wet with 10.0 mL of 0.85% sterile saline. The sample was filter concentrated. The filter was rinsed with ≥50 mL of 0.85% sterile saline, aseptically removed from the apparatus, and transferred to the appropriate agar plate. Additional 1:10 serial dilutions were prepared from the 10^0 neutralized inoculated test mixture in Butterfield's Buffer. Aliquots (1.00 mL) of the 10^{-1}-10^{-4} dilutions of neutralized inoculated test mixture were plated in duplicate on appropriate agar.

Incubation and Observation

The bacterial subculture plates were incubated for 48±4 hours at 35-37°C. Subcultures were refrigerated for three days at 2-8°C prior to examination. Following incubation and storage, the agar plates were observed visually for the presence of growth. The colony forming units were enumerated and the number of survivors at each exposure time was determined.

STUDY CONTROLS

Test Population Control

In a similar manner as the culture inoculum was added to the test substance, an equivalent volume (0.100 mL) of each inoculum was added to 9.9 mL of Butterfield's Buffer (same volume as the test substance). This suspension was neutralized as in the test procedure. This suspension was serially diluted and appropriate dilutions were plated using standard microbiological techniques. Following incubation, the organism plates were observed to enumerate the concentration of the test organism present in the test substance at the time of testing (time 0 analysis). The acceptance criterion for this study control is growth and the value is used for calculation purposes only.

Purity Control

A "streak plate for isolation" was performed on each organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Initial Suspension Population Control

Each prepared test organism suspension was serially diluted and plated using standard microbiological techniques. Following incubation, the organism plates were observed to enumerate the concentration of the test organism inoculated into the test substance at the time of testing. The acceptance criterion for this study control is growth at $\geq 1.0 \times 10^6$ CFU/mL.

Neutralizer Sterility Control

A representative sample of uninoculated neutralizer was incubated and observed. The acceptance criterion for this study control is lack of growth.

Organic Soil Sterility Control

A 1.00 mL aliquot of the serum used for the soil load was added to a tube of Fluid Thioglycollate Medium, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralization Control

To simulate testing conditions, 9.9 mL of the test substance was inoculated with 0.100 mL Butterfield's Buffer in place of the test organism suspension (NC Suspension).

1. Filtration Neutralization:
   A 0.100 mL aliquot of the NC Suspension was transferred to 9.9 mL neutralizing broth and mixed thoroughly. The control suspension (5.0 mL) was filter concentrated and the filter was rinsed as in the test procedure. An aliquot (1.00 mL) of an organism suspension containing approximately 100 CFU/mL was added to the filter apparatus and processed through the apparatus. An aliquot (1.00 mL) of the organism suspension was added to a second filter apparatus to be used as an inoculum population control and processed. The filters were aseptically transferred to recovery agar plates and incubated. The acceptance criteria for this study control requires the filtration neutralization control and corresponding population control results to be within 1.0 Log.

2. Chemical Neutralization:
A 0.100 mL aliquot of the NC Suspension was transferred to 9.9 mL neutralizing broth and mixed thoroughly. A 1.00 mL aliquot of the neutralized sample was then removed and discarded. To the neutralized sample, 1.00 mL of the organism suspension (containing approximately 1000 CFU/mL) was added and mixed thoroughly. An aliquot (1.00 mL) of the neutralized mixture was plated in duplicate and incubated. An inoculum population control was performed by adding 1.00 mL of the same organism suspension to 9 mL of Butterfield's Buffer and plating in duplicate and incubating. The acceptance criterion for this study control requires the chemical neutralization control and corresponding population control results to be within 1.0 Log.

STUDY ACCEPTANCE CRITERIA

Test Substance Performance Criteria
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results are expressed in percent and log reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

PROTOCOL CHANGES

Protocol Amendments:
No protocol amendments were required for this study.

Protocol Deviations:
No protocol deviations occurred during this study.

DATA ANALYSIS

Calculations
Test Data CFU/mL: $\frac{\text{(avg. \# colonies found/plate @ dilution used) (dilution factor) (volume neutralized solution)}}{\text{(volume plated)}}$

Percent Reduction: [1-(test survivors/test population control)] x 100

Log_{10} Reduction: Log_{10} (test population control) – Log_{10} (test survivors)

Statistical Analysis
None used.

STUDY RETENTION

Record Retention
All of the original raw data developed exclusively for this study shall be archived at ATS Labs, 1285 Corporate Center Drive, Suite 110, Eagan, MN 55121. The original data includes, but is not limited to, the following:

1. Certified copy of final study report.
2. Original signed protocol.
3. Any protocol amendments/deviation notifications.
4. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
5. All measured data used in formulating the final report.
6. Memoranda, specifications, and other study specific correspondence relating to interpretation and evaluation of data, other than those documents contained in the final study report.
7. Study-specific SOP deviations made during the study.

Test Substance Retention
The test substance will be discarded following study completion per Sponsor approved protocol. It is the responsibility of the Sponsor to retain a sample of the test material.

REFERENCES

1. American Society for Testing and Materials (ASTM). E2315-03, Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, Volume 11.05, Copyright 2005 ASTM International.

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

RESULTS

For Control and Neutralization Results, see Tables 1-3.
All data measurements/controls including neutralization confirmation, purity, initial suspension, test population, organic soil load sterility and neutralizer sterility controls performed within acceptance criteria listed in the study controls section of the protocol.

For Test Results, see Tables 4 and 5.

ANALYSIS AND CONCLUSION

Under the conditions of this study, FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750, demonstrated a >99.99% (>4.8 $\log_{10}$) reduction of *Escherichia coli* survivors following a 30 second and a 60 second exposure time when tested at ambient temperature (22°C).

Under the conditions of this study, FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750, demonstrated a >99.999% (>5.1 $\log_{10}$) reduction of *Listeria monocytogenes* survivors following a 30 second and a 60 second exposure time when tested at ambient temperature (22°C).

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

TABLE 1: CONTROL RESULTS

The following results from controls confirmed study validity:

Type of Control	Results	
	Escherichia coli **(ATCC 11229)**	***Listeria monocytogenes*** **(ATCC 19117)**
Purity Control	Pure	Pure
Organic Soil Load Sterility Control	No Growth	
Neutralizer Sterility Control	No Growth	

TABLE 2: INITIAL SUSPENSION POPULATION CONTROL

Test Organism	Date Performed	Result
Escherichia coli (ATCC 11229)	5-21-09	1.78×10^8 CFU/mL
Listeria monocytogenes (ATCC 19117)		3.2×10^8 CFU/mL

CFU = Colony Forming Unit

TABLE 3: NEUTRALIZATION CONTROLS

Filtration Neutralization Confirmation Control						
Test Substance	Test Organism	Date Performed	Organism Dilution	Number of Survivors Recovered		±1.0 log_{10} Pass/ Fail
				With Product	Numbers Control	
FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750	*Escherichia coli* (ATCC 11229)	5-21-09	10^{-7}	17	16	-0.03 Pass
	Listeria monocytogenes (ATCC 19117)		10^{-7}	30	45	0.17 Pass
Chemical Neutralization Confirmation Control						
Test Substance	Test Organism	Date Performed	Organism Dilution	Number of Survivors Recovered		±1.0 log_{10} Pass/ Fail
				With Product	Numbers Control	
FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750	*Escherichia coli* (ATCC 11229)	5-21-09	10^{-6}	18, 24	24, 19	0.02 Pass
	Listeria monocytogenes (ATCC 19117)		10^{-6}	50, 23	37, 27	-0.06 Pass

TABLE 4: TEST RESULTS

Test Substance: FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750

DILUTION	Test Organism: *Escherichia coli* (ATCC 11229)	
	EXPOSURE TIME	
	30 Seconds	60 Seconds
	Number of Survivors	
Filtration of 5.0 mL at 10^0 dilution	0	0
10^{-1}	0, 0	0, 0
10^{-2}	0, 0	0, 0
10^{-3}	0, 0	0, 0
10^{-4}	0, 0	0, 0
DILUTION	**Test Organism: *Listeria monocytogenes* (ATCC 19117)**	
	EXPOSURE TIME	
	30 Seconds	60 Seconds
	Number of Survivors	
Filtration of 5.0 mL at 10^0 dilution	0	0
10^{-1}	0, 0	0, 0
10^{-2}	0, 0	0, 0
10^{-3}	0, 0	0, 0
10^{-4}	0, 0	0, 0

TABLE 5: CALCULATED DATA

Test Substance: FlavoKlenz Plus Citrox BC-Liquid Concentrate # 372750

Test Organism	Exposure Time	Test Population Control CFU/mL* (Log_{10})	Number of Survivors (CFU/mL)*	Log_{10} Number of Survivors	Percent Reduction	Log_{10} Reduction
Escherichia coli (ATCC 11229)	30 Seconds	1.40×10^5 (5.146)	<2	<0.3	>99.99%	>4.8
	60 Seconds		<2	<0.3	>99.99%	>4.8
Listeria monocytogenes (ATCC 19117)	30 Seconds	2.41×10^5 (5.382)	<2	<0.3	>99.999%	>5.1
	60 Seconds		<2	<0.3	>99.999%	>5.1

* colony forming units per mL of test mixture

(For Laboratory Use Only)
ATS Labs Project # A 0 7 7 6 2

ATS LABS

PROTOCOL

Time Kill Test Assay For Antimicrobial Agents

DRAFT COPY
INITIALS AS DATE 6-9-09

Test Organism(s):
Escherichia coli (ATCC 11229)
Listeria monocytogenes (ATCC 19117)

PROTOCOL NUMBER

AHA01050409.TK

PREPARED FOR

Ahava International
19692 Black Fox Drive
Cottonwood, CA 96022

PERFORMING LABORATORY

ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PREPARED BY

Anne Stemper, B.S.
Research Scientist I

DATE

May 4, 2009

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1285 Corporate Center Drive, Suite 110 • Eagan, MN 55121 • 877.287.8378 • 651.379.5510 • Fax: 651.379.5549

Time Kill Test Assay For Antimicrobial Agents

SPONSOR: Ahava International
19692 Black Fox Drive
Cottonwood, CA 96022

TEST FACILITY: ATS Labs
1285 Corporate Center Drive, Suite 110
Eagan, MN 55121

PURPOSE
The objective of this testing is to produce data that provides basic information on rate-of-kill of antimicrobial formulations tested against single selected microorganisms.

TEST SUBSTANCE CHARACTERIZATION
Test substance characterization as to content, stability, etc. (21 CFR, Part 58) is the responsibility of the sponsor. The test substance shall be characterized by the sponsor prior to the experimental start date of this study. Pertinent information, which may affect the outcome of this study, shall be communicated in writing to the Study Director upon sample submission to ATS Labs.

SCHEDULING AND DISCLAIMER OF WARRANTY
Experimental start dates are generally scheduled on a first-come/first-serve basis once ATS Labs receives the Sponsor approved/completed protocol, signed fee schedule and corresponding test substance(s). Based on all required materials being received at this time, the proposed experimental start date is May 8, 2009. Verbal results may be given upon completion of the study with a written report to follow on the proposed completion date of May 26, 2009. To expedite scheduling, please be sure all required paperwork and test substance documentation is complete/accurate upon arrival at ATS Labs.

A "case-by-case" approach is generally taken by the regulatory authorities and cannot be over-emphasized when considering a testing regimen. While this protocol is based upon our experience in the field of germicidal testing, and the current EPA and/or FDA guidelines, each product presents a different set of issues to the regulatory authorities. We recommend that you consult with the appropriate agency (EPA or FDA) before finalizing your testing regimen, as ATS Labs cannot guarantee acceptance of this protocol by the regulating authorities.

If a test must be repeated, or a portion of it, due to failure by ATS Labs to adhere to specified procedures, it will be repeated free of charge. If a test must be repeated, or a portion of it, due to failure of internal controls, it will be repeated free of charge. "Methods Development" fees shall be assessed, however, if the test substance and/or test system require modifications due to complexity and difficulty of testing.

If the Sponsor requests a repeat test, they will be charged for an additional test. The repeat testing will be conducted following this initiated protocol.

The Sponsor is responsible for any rejection of the final report by the United States FDA or EPA concerning report format, pagination, etc. To prevent rejection, Sponsor should carefully review the ATS Labs final report and notify ATS Labs of any perceived deficiencies in these areas before submission of the report to the regulatory agency. ATS Labs will make reasonable changes deemed necessary by the Sponsor, without altering the technical data.

Neither the name of ATS Labs or any of its employees are to be used in advertising or other promotion without written consent from ATS Labs.

JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM
Analyzing the efficacy of antimicrobial agents may be performed by various suspension and susceptibility methods. This study is designed to examine the rate-of-kill of a test substance against sponsor selected pure cultures of microorganisms. This is accomplished by exposing the target microorganism(s) to the test substance and inspecting the solution for potential survivors at various time periods. The experimental design in this protocol meets these requirements.

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TEST PRINCIPLE

A suspension of bacterial cells is exposed to the test substance for specified contact times. After exposure, an aliquot of the suspension is transferred to a neutralizer and assayed for survivors. Appropriate purity, sterility, microorganism population and neutralization controls are performed. The current version of Standard Operating Procedure CGT-4130 reflects the methods which shall be used in this study.

TEST METHOD

Test Organism	ATCC #	Culture Medium	Incubation Parameters
Escherichia coli	11229	Blood Agar	35-37°C, aerobic
Listeria monocytogenes	19117	Blood Agar	35-37°C, aerobic

Blood Agar = Tryptic Soy Agar containing 5% Sterile Sheep Blood

The test organisms to be used in this study were obtained from the American Type Culture Collection (ATCC), Manassas, VA.

Test Organism Preparation

Using a stock culture of the test organism, streak a culture of each test organism onto the culture medium listed above. Incubate the bacterial cultures for 24-48 hours at 35-37°C (alternate or extended incubation may be required for certain strains).

Transfer a sufficient amount of organism growth into a sterile diluent to yield a uniform suspension of approximately $1x10^8$ CFU / mL. Most bacterial strains should approximately match a 0.5 McFarland standard. Yeast strains should approximately match a 4.0 McFarland standard. Cultures may be further adjusted as needed.

An organic soil load will be added to the test culture per Sponsor's request.

Preparation of Test Substance

The test substance to be tested is prepared according to the directions supplied by the sponsor. A 9.9 mL aliquot of the prepared test substance will be transferred to a sterile vessel (glass tube, stomacher bag, etc.) for testing procedures.

The test substance shall be used within 3 hours of preparation if additional preparation is required by ATS Labs.

Test Exposure

A 0.1 mL aliquot of the standardized inoculum will be added to the test substance representing the start of the test exposure. The inoculated test substance will be immediately mixed thoroughly using a laboratory stomacher, vortex mixer or other applicable method. The inoculated and mixed test substance will be held at the sponsor specified temperature. If the requested exposure temperature lies outside of achievable ambient conditions, the test substance may be equilibrated in a water bath (or other appropriate device) to equilibrate to the desired exposure temperature.

Subculture

At each Sponsor specified exposure sample time, a 0.1 mL aliquot of the inoculated test substance will be transferred to 9.9 mL of neutralizer broth (10^0) dilution. For additional 1:10 dilutions in Butterfield's Buffer will be prepared. Using a standard microbiological spread plate count procedure, 1.0 mL aliquots of each dilution (10^{-1} – 10^{-4}) will be plated in duplicate to the appropriate recovery media.

A 5.0 mL of the neutralized sample (10^0 dilution) will be transferred to a sterile 0.2 – 0.45 µm filter apparatus system pre-wet with 10 mL of sterile diluent. Filter concentrate the sample and rinse the filter using ≥50 mL sterile diluent. Aseptically remove the filter and place it on the surface of the recovery agar medium.

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Incubation and Observation
All bacterial subculture plates are incubated for 48±4 hours at 35-37°C (or other appropriate time/temperatures). Subculture plates may be refrigerated at 2-8°C for ≤3 days prior to examination.

Following incubation, the test and controls will be visually examined for growth. Agar plates will be enumerated and recorded. Log and percent reductions will be determined for each time point.

Representative subcultures demonstrating growth will be appropriately examined for confirmation of the test organism.

STUDY CONTROLS

Purity Control
A "streak plate for isolation" will be performed on the organism culture and following incubation examined in order to confirm the presence of a pure culture. The acceptance criterion for this study control is a pure culture demonstrating colony morphology typical of the test organism.

Organic Soil Sterility Control
If applicable, 1.0 mL of the serum used for soil load will be added to a tube of Fluid Thioglycollate, incubated, and observed for lack of growth. The acceptance criterion for this study control is lack of growth.

Neutralizer Sterility Control
A representative sample of neutralizer will be incubated and observed. The acceptance criterion for this study control is lack of growth.

Test Population Control
In a similar manner as the culture inoculum is added to the test substance, add an equivalent volume of inoculum (0.1 mL) to 9.9 mL Butterfield's buffer (same volume as the test substance). This suspension will be neutralized as in the test procedure. The suspension will be serially diluted and appropriate dilutions plated using standard microbiological techniques. Following incubation, the organism plates will be observed to enumerate the concentration of the test organism present in the test substance at the time of testing (time 0 analysis). The acceptance criteria for this study control is growth and the value is used for calculation purposes only.

Initial Suspension Population Control
The prepared test organism suspension will be serially diluted and plated using standard microbiological techniques. Following incubation, the organism plates will be observed to enumerate the concentration of the test organism inoculated into the test substance at the time of testing. The acceptance criteria for this study control is growth at $\geq 1.0 \times 10^5$ CFU/mL.

Neutralization Control
To simulate testing conditions, 9.9 mL of the test substance will be inoculated with 0.1 mL Butterfield's Buffer in place of the test organism suspension (NC Suspension). If multiple concentrations of a test substance are evaluated in the test procedure, only the most concentrated test substance(s) need to be evaluated in the neutralization control.

1. Filtration Neutralization:
   The NC suspension will be neutralized as in the test procedure. Filter concentrate 5.0 mL of the control suspension and rinse filter as in the test procedure. Add 1.0 mL of an organism suspension containing approximately 100 CFU/mL to the filter apparatus and process through the apparatus. Add 1.0 mL of the organism suspension to a second filter apparatus to be used as an inoculum population control and process. Aseptically transfer the filters to recovery agar plates and incubate. The acceptance criteria for this study control requires the filtration neutralization control and corresponding population control results to be within 1.0 Log.

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2. Chemical Neutralization:
The NC suspension will be neutralized as in the test procedure. Remove and discard 1.0 mL of the neutralized sample. To the neutralized sample, add 1.0 mL of an organism suspension containing approximately 1000 CFU/mL and mix thoroughly. Plate in duplicate 1.0 mL of neutralized mixture to appropriate recovery medium and incubate. Perform an inoculum population control by adding 1.0 mL of the same organism suspension to a volume of Butterfield's Buffer equivalent to the volume of neutralized sample and plate in duplicate and incubate. The acceptance criteria for this study control requires the chemical neutralization control and corresponding population control results to be within 1.0 Log.

TEST CRITERIA

Test Substance Performance Criteria
This study is designed to examine the rate-of-kill of a test substance after inoculation with a test organism. Results will be expressed in percent and log reduction of the test organism. Minimum percent and log reduction values do not exist to specify a "passing" or "failing" test substance.

Control Acceptance Criteria
The study controls must perform according to the criteria detailed in the study controls description section.

DATA ANALYSIS

Calculations

Test Data CFU/mL: $\frac{\text{(avg. \# colonies found/plate @ dilution used) (dilution factor) (volume of neutralized solution)}}{\text{(volume plated)}}$

Percent Reduction: [1-(test survivors/test population control)] x100

Log_{10} Reduction: Log_{10} (test population control) – Log_{10} (test survivors)

Statistical Analysis None used

PROCEDURE FOR IDENTIFICATION OF THE TEST SYSTEM

ATS Labs maintains Standard Operating Procedures (SOPs) relative to efficacy testing studies. Efficacy testing is performed in strict adherence to these SOPs which have been constructed to cover all aspects of the work including, but not limited to, receipt, log-in, and tracking of biological reagents including bacterial strains for purposes of identification, receipt and use of chemical reagents. These procedures are designed to document each step of efficacy testing studies. Appropriate references to medium batch number, etc. are documented in the raw data collected during the course of each study.

Additionally, each efficacy test is assigned a unique Project Number when the protocol for the study is initiated by the Study Director. This number is used for identification of the test subculture tubes, etc. during the course of the test. Test subculture tubes are also labeled with reference to the test organism, experimental start date, and test product. Microscopic and/or macroscopic evaluations of positive subcultures are performed in order to confirm the identity of the test organism. These measures are designed to document the identity of the test system.

METHOD FOR CONTROL OF BIAS: NA

REPORT
The report will include, but not be limited to, identification of the sample, date received, initiation and completion dates, identification of the bacterial strains used, description of media and reagents, description of the methods employed, tabulated results and conclusion as it relates to the purpose of the test, and all other items required by 21 CFR Part 58.

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PROTOCOL CHANGES

If it becomes necessary to make changes in the approved protocol, the revision and reasons for changes will be documented, reported to the Sponsor and will become a part of the permanent file for that study. Similarly, the Sponsor will be notified as soon as possible whenever an event occurs that may have an effect on the validity of the study.

Standard operating procedures used in this study will be the correct effective revision at the time of the work. Any minor changes to SOPs (for this study) or methods used will be documented in the raw data and approved by the Study Director.

PRODUCT DISPOSITION

It is the responsibility of the Sponsor to retain samples of the test substance. All unused test substance will be discarded following study completion unless otherwise requested.

RECORD RETENTION

Study Specific Documents

All of the original raw data developed exclusively for this study shall be archived at ATS Labs. These original data include, but are not limited to, the following:

1. All handwritten raw data for control and test substances including, but not limited to, notebooks, data forms and calculations.
2. Any protocol amendments/deviation notifications.
3. All measured data used in formulating the final report.
4. Memoranda, specifications, and other study specific correspondence relating to interpretation, and evaluation of data, other than those documents contained in the final study report.
5. Original signed protocol.
6. Certified copy of final study report.
7. Study-specific SOP deviations made during the study.

Facility Specific Documents

The following records shall also be archived at ATS Labs. These documents include, but are not limited to, the following:

1. SOPs which pertain to the study conducted.
2. Non study-specific SOP deviations made during the course of this study which may affect the results obtained during this study.
3. Methods which were used or referenced in the study conducted.
4. QA reports for each QA inspection with comments.
5. Facility Records: Temperature Logs (ambient, incubator, etc.), Instrument Logs, Calibration and Maintenance Records.
6. Current curriculum vitae, training records, and job descriptions for all personnel involved in the study.

REFERENCES

1. American Society for Testing and Materials (ASTM). E2315-03, Guide for Assessment of Microbiocidal Activity Using a Time-Kill Procedure, Volume 11.05, Copyright 2005 ASTM International.

2. Food and Drug Administration. Tentative Final Monograph for Healthcare Antiseptic Drug Products; Proposed rule. Code of Federal Regulations, 21 CFR parts 333 and 369. June 17, 1994.

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Study Information

(All sections must be completed prior to submitting protocol)

Sponsor (Date/Initial): 5-11-2009 JM

Test Substance (Name and Batch Number - exactly as it should appear on final report):
* CITROX BC-HYDRO CONCENTRATE FF 372750 JM 5-18-09

Expiration Date: 5-11-2011 * FlavoKlenz Plus (part of test substance name, added per Sponsor request)

Test Substance Active Concentration (upon submission to ATS Labs): 2.5% bioflavonoids and 1% Citric acid JM 5-18-09

Product Description:
- ☐ Quaternary ammonia
- ☐ Iodophor
- ☐ Sodium hypochlorite
- ☐ Peracetic acid
- ☐ Peroxide
- ☒ Other BIOFLAVONOID Complex

Neutralization/Subculture Broth:
- ☐ _____
- ☒ ATS Labs' Discretion. By checking, the Sponsor authorizes ATS Labs, at their discretion, to perform neutralization confirmation assays at the Sponsor's expense prior to testing to determine the most appropriate neutralizer. (See Fee Schedule). – NOTE: DO NOT USE ANY CHLORINE BASED PRODUCTS!! – VIP –

Storage Conditions:
- ☒ Room Temperature
- ☐ 2-8°C
- ☐ Other: _____

Hazards:
- ☒ None known: Use Standard Precautions
- ☒ Material Safety Data Sheet, Attached for each product
- ☐ As Follows: _____

Product Preparation
- ☐ No dilution required, Use as received (RTU)
- ☒ *Dilutions/Concentrations to be tested 1:150
  - ☒ Deionized Water (Filter Sterilized)
  - ☐ Tap Water (Filter Sterilized)
  - ☐ AOAC Synthetic Hard Water: _____ PPM
  - ☐ Other _____

Note: An equivalent dilution may be made unless otherwise requested by the Sponsor.

Exposure Periods: 30 & 60 seconds

Exposure Temperature
- ☒ Ambient
- ☐ 20±1°C
- ☐ Other _____

Organic Soil Load:
- ☒ Minimum 5% Organic Soil Load (Fetal Bovine Serum)
- ☐ No Organic Soil Load Required
- ☐ Other: _____

Test Organisms:
- ☑ *Escherichia coli* (ATCC 11229)
- ☑ *Listeria monocytogenes* (ATCC 19117)

* Per Sponsor clarification, use 1.0% Tween 80 + 3.0% Saponin + 0.1% Histadine + 0.1% Cysteine as the neutralizer JM 5-18-09

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TEST SUBSTANCE SHIPMENT STATUS

- ☐ Has been used in one or more previous studies at ATS Labs .
- ☐ Has been shipped to ATS Labs (but has not been used in a previous study).
  Date shipped to ATS Labs: 5-7-09 Sent via *overnight* delivery? ☐ Yes ☒ No
- ☒ Will be shipped to ATS Labs.
  Date of expected receipt at ATS Labs: 5-16-2009
- ☐ Sender (if other than Sponsor):_____

COMPLIANCE

Study to be performed under FDA Good Laboratory Practice regulations (21 CFR Part 58) and in accordance to standard operating procedures.

☑ Yes
☐ No (Non-GLP Study)

PROTOCOL MODIFICATIONS

☒ Approved without modification
☐ Approved with modification - Supplemental Information Form Attached - ☐ Yes ☐ No

APPROVAL SIGNATURES

SPONSOR:

NAME: Tim McCarley TITLE: President

SIGNATURE: Tim McCarley DATE: 5-11-2009

PHONE: 530-227-9494 FAX: 530-347-0716 EMAIL: timahava@aol.com

For confidentiality purposes, study information will be released only to the sponsor/representative signing the protocol (above) unless other individuals are specifically authorized in writing to receive study information.

Other individuals authorized to receive information regarding this study: ☐ See Attached

ATS Labs:

NAME: Anne Stemper
Study Director

SIGNATURE: Anne Stemper DATE: 5-18-09
Study Director

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Volume _____

FINAL REPORT

AOAC USE DILUTION TEST HEALTHCARE

Test Agent: Citrox AV25

Data Requirements
EPA Guidelines 810.2100 (c), (d), (e)

Author
Travis R. Farley

Study Completion Date
pending

Performing Laboratory
MICROBIOTEST
105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
661-101

Submitted to: Citrox Limited
Unit 9 River Court
Bridgehouse Road
Riverside Park
Middlesbrough TS2-1RT
England

STATEMENT OF NO DATA CONFIDENTIALITY

Title: AOAC Use Dilution Test - Healthcare

Performed by: MICROBIOTEST
105 Carpenter Drive
Sterling, Virginia 20164

No claim of confidentiality is made for any information contained in this study on the basis of its falling within the scope of FIFRA § 10(d)(1)(A), (B) or (C).

Company Agent _____ _____
Date

COMPLIANCE STATEMENT

This study meets the requirements for 40 CFR § 160 with the following exceptions:

- Information on the identity, strength, purity, stability, uniformity, and dose solution analysis of the test agent resides with the sponsor of the study.

The following technical personnel participated in this study:

Travis R. Farley, Adam A. Peters, Nadia A. Hashimee

Study Director: MICROBIOTEST

_____ _____
Travis R. Farley Date

Submitted by:

_____ _____
Name Title

_____ _____
Signature Date

Sponsor: Citrox Limited

_____ _____
Name Title

_____ _____
Signature Date

QUALITY ASSURANCE UNIT STATEMENT

Title of Study: AOAC Use Dilution Test - Healthcare

The Quality Assurance Unit of MICROBIOTEST has inspected Project Number 661-101 in compliance with current Good Laboratory Practice regulations, (40 CFR § 160).

The dates that inspections were made and the dates that findings were reported to management and to the study director are listed below.

PHASE INSPECTED	DATE OF INSPECTION	DATE REPORTED TO STUDY DIRECTOR	DATE REPORTED TO MANAGEMENT
Protocol	08/14/08	08/15/08	10/10/08
In Process	09/18/08	09/18/08	pending
Final Report	10/08/08, 10/10/08	10/10/08	pending

Nathan S. Jones, RQAP-GLP
Quality Assurance Unit

Date

TABLE OF CONTENTS

TEST SUMMARY

TITLE: AOAC Use Dilution Test - Healthcare

STUDY DESIGN: This study was performed according to the signed protocol and project sheets issued by the Study Director.

See Project Sheets (Appendix I)
See signed protocol (Appendix II)

TEST MATERIALS SUPPLIED BY THE SPONSOR OF THE STUDY:

1. Citrox AV25, Lot No. 807010 (≥60 days old), received at MICROBIOTEST on 09/15/08, and assigned DS No. 9658.

2. Citrox AV25, Lot No. 809010 (≥60 days old) , received at MICROBIOTEST on 09/15/08, and assigned DS No. 9659.

3. Citrox AV25, Lot No. 809030 (≥60 days old), received at MICROBIOTEST on 09/15/08, and assigned DS No. 9660.

SPONSOR: Citrox Limited
Unit 9 River Court
Bridgehouse Road
Riverside Park
Middlesbrough TS2-1RT
England

TEST CONDITIONS

Challenge microorganisms:

Staphylococcus aureus, ATCC 6538
Pseudomonas aeruginosa, ATCC 15442
Salmonella enterica, ATCC 10708

Active ingredient in test product:

Bioflavonoid

Neutralizer:

DE Neutralizing Broth (double strength)

Contact time:

10 minutes

Contact temperature:

Ambient Room Temperature (20C)

Carriers:

Stainless steel penicylinders

Dilution:

Ready to Use

Media and reagents:

Nutrient Broth
Asparagine solution, 0.1%
Sodium hydroxide solution, 1N
DE Neutralizing Broth (double strength)
Phosphate Buffered Saline + 1% Polysorbate 80
Phosphate Buffered Saline
Nutrient Agar
Tryptic Soy Agar
Gram Stain Reagents

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164, from 09/18/08 to 09/21/08. Testing initiated on 08/14/08 was invalidated due to insufficient growth in the controls. The study director signed the protocol 08/11/08. The study completion date is the date the study director signed the final report.

All changes or revisions of the protocol were documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modifications, test material records, the final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

RESULTS

Results are presented in Tables 1 and 2. The challenge microorganisms were confirmed by colony morphology and gram stain to be consistent with *S. aureus*, *P. aeruginosa* and *S. enterica*. The sterility control exhibited no growth. The viability and neutralizer effectiveness controls exhibited growth. An average of 70 colony-forming units (CFU) of *S. aureus*, 70 CFU of *P. aeruginosa*, and 51 CFU of *S. enterica* were added to the neutralizer effectiveness controls. Bacteriostasis streaks exhibited no growth for *S. aureus* and *S. enterica*. Due to the opacity of the neutralizer, all *P. aeruginosa* test and control tubes were streaked for confirmation of growth or no growth; therefore, an evaluation of bacteriostasis was not applicable.

Table 1

Test Results

Results Expressed as Number of Tubes Exhibiting Growth / Total Number of Tubes

Microorganism	Lot No. 807010	Lot No. 809010	Lot No. 809030
S. aureus	0/60	0/60	0/60
P. aeruginosa	0/60	0/60	0/60
S. enterica	0/60	0/60	0/60

Table 2

Carrier Counts
Results Expressed as Average Colony Forming Units (CFU) per carrier

Microorganism	Average CFU/carrier
S. aureus	7.7×10^5
P. aeruginosa	7.1×10^4
S. enterica	8.4×10^4

CONCLUSIONS

When tested as described, Citrox AV25 passed the AOAC Use Dilution Test - Healthcare when *S. aureus*, *P. aeruginosa* and *S. enterica* were exposed to the test agent for 10 minutes at 20C. All of the controls met the criteria established for a valid test. These conclusions are based on observed data.

E 3

biobest research

Certificate of Analysis

The effect of Phytologica Sanitiser (Citrox) on Swine Influenza Strain H1N1.

Time	Control $10^X TCID_{50}$/ml	Neat $10^X TCID_{50}$/ml	Log reduction observed
5min	5.00	≤2.5	≥2.5
15min	4.75	≤2.5	≥2.25
30min	4.25	≤2.5	≥1.75
60min	4.75	≤2.5	≥2.25

No virus was detected in the Phytologica Sanitiser solution consistent with the solution being effective at inactivating swine influenza H1N1.

Results Entered by..... Date...04 Dec 09.....

Results Verified by..... Date...04Dec09.....

Biobest Laboratories Ltd is a GLP and UKAS accredited laboratory.

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FINAL REPORT

CONFIRMATORY VIRUCIDAL EFFECTIVENESS TEST
Using Feline Calicivirus
(Surrogate for Norwalk virus (Norovirus))

TEST AGENT:
Decosan (also known as Citrox AV20)

Data Requirements
EPA Guidelines 810.2100 (g)

Author
Peggy R. Cherwoo

Study Completion Date
May 15, 2006

Performing Laboratory
MICROBIOTEST
105 Carpenter Drive
Sterling, Virginia 20164

Laboratory Project Identification Number
557-106

Submitted to: BIOLOGICAL SOLUTIONS LLC
900 East Liberty Street
Sumter, SC 29153
(Citrox Limited exclusive agent in USA)

TEST SUMMARY

TITLE:	Confirmatory Virucidal Effectiveness Test Using Feline calicivirus (Surrogate for Norwalk virus)
STUDY DESIGN:	This study was performed according to the signed protocol and project sheets issued by the Study Director. See Project Sheets (Appendix I) See signed protocol (Appendix 2)
TEST MATERIALS:	Decosan, Lot No. 33106D, received at MICROBIOTEST, 04/04/06 and assigned DS No. 8194.
SPONSOR:	BIOLOGICAL SOLUTIONS, LLC 900 East Liberty Street Sumter, SC 29153

TEST CODITIONS

Challenge Virus:
Deline calicivirus, University of Ottawa (CREM)

Host:
CrFK cells, American BioResearch Laboratories

Active ingredient in test product:
Citric acid / Bioflavonoid complex

Neutraliser used:
Fluid thioglycollate medium +20% Newborn calf serum
+1% Polysorbate 80

Dilution:
Ready to use

Spray distance:
6 inches

Contact time:
1 minute

Contact temperature:
22°C

Organic load:
Viral stock contained at least 5% organic load

Media and reagents:
Newborn calf serum (NCS)
RPMI 1640 containing 10% NCS
Fluid thioglycollate medium
Polysorbate 80

STUDY DATES AND FACILITIES

The laboratory phase of this test was performed at MICROBIOTEST, 105 Carpenter Drive, Sterling, VA 20164, from 04/26/06 to 05/02/06. The study director signed the protocol 04/25/06. The study completion date is the date the study director signed the final report.

All changes or revisions of the protocol we documented, signed by the study director, dated and maintained with the protocol.

RECORDS TO BE MAINTAINED

All testing data, protocol, protocol modification, test material records, the final report, and correspondence between MICROBIOTEST and the sponsor will be stored in the archives at MICROBIOTEST, 105, Carpenter Drive, Sterling, VA 20164, or at a controlled facility off site.

RESULTS

Results are presented in Tables 1 – 4. A titration was performed to determine the titer of the viral stock. The log_{10} reduction (LR) of infectious virus observed as cytopathic effects of Feline calicivirus eas determined using the Most Probable Number (MPN) method as described in EPA-Statistic Primer (EPA-SP). The cell viability control demonstrated CrFK cell viability and media sterility. Virus was not recovered in the cell viability control. The log_{10} reduction was calculated in the following manner:

Log_{10} reduction = Infectious virus titer from plate recovery control – Infectious virus titer recovered from test.

RESULTS (Continued)

Table 1
Test Results

Dilution	Decosan	
	Lot No. 33106D	
	Replicate 1	Replicate 2
10^{-2}	CCCC	CCCC
10^{-3}	CCCC	CCCC
10^{-4}	0000	0000
10^{-5}	0000	0000
10^{-6}	0000	0000
10^{-7}	0000	0000
Log_{10}MPN/mL	3.37985	3.37985
Mean (Log_{10}MPN/mL)	3.37985	

Table 2
Neutraliser Effectiveness and Cytotoxicity Related Controls
Lot No. 33106D

Dilution	Neutraliser Effectiveness Control	Cytotoxicity Control	Cytotoxicity-related Viral Interference Control
10^{-2}	CCCC	CCCC	CCCC
10^{-3}	CCCC	CCCC	CCCC
10^{-4}	++++	0000	++++

Key: + = Feline calicivirus infected cells were detected, cytopathic effects observed
0 = Feline calicivirus infected cells were not detected, no cytopathic effects observed, no cytotoxicity observed.
C = Cytotoxicity observed

RESULTS (Continued)

Table 3
Control Results

Dilution	Feline calicivirus Plate Recovery Control	
	Replicate 1	Replicate 2
10^{-2}	++++	++++
10^{-3}	++++	++++
10^{-4}	++++	++++
10^{-5}	++++	++++
10^{-6}	++++	++++
10^{-7}	++++	++++
Log_{10}MPN/mL	$\geq$ 7.37983	$\geq$ 7.37983
Mean (log_{10}MPN/mL)	$\geq$ 7.37983	

Key: + = Feline calicivirus infected cells were detected, cytopathic effects observed.
0 = Feline calicivirus infected cells were not detected, no cytopathic effects observed, no cytotoxicity observed.
C = Cytotoxicity.

Table 4
Log_{10} Reduction

Decosan
Lot No. 33106D
3.99998

CONCLUSION

According to the regulatory agencies, the test agent passes the test if there is complete inactivation of the virus at all dilutions. When cytotoxicity is evident, at least a three-log reduction in titer must be demonstrated beyond the cytotoxic level. When tested as described, Decosan (also known as Citrox AV20) passed the Virucidal Effectiveness Test when Feline calicivirus was exposed to the test agent for 1 minute at 22°C. All of the controls met the criteria for a valid test. These conclusions are based on observed data.

Certificate of Analysis

Samples: BA-01

Analysis Required: BS EN 1276 under dirty conditions

Samples Tested: 23/04/2008

Product stored at room temperature.

Active substance: Bioflavonoid and Fruit Acid blend

Experimental conditions:

Product diluent used during test	- Sterile hard water 300 mg/kg $CaCO_3$
Product test concentrations	- 0.5 % v/v
Contact time	- 5 min
Test temperature	- 20 °C ± 0.5 °C
Interfering substance	- 3.0 g/l Bovine albumin
Neutralising solution	- 3 % Tween 80, 3 % Saponin, 0.1 % Histidine, 0.1 % Cysteine
Temperature of incubation	- 37 °C ± 1 °C
Identification of bacterial strains used	- ***Pseudomonas aeruginosa ATCC 15442***
	- ***Escherichia coli 11867***
	- ***Staphylococcus aureus NCTC 6571***
	- ***Bacillus subtillis 10262***

Test Results: BA-01. 0.5 % 5 minutes

Validation test	Gram Negative		Gram Positive	
	E. coli	*P. Aeruginosa*	*S. Aureus*	*B. Subtillis*
Bacterial suspension	Vc 398, 413 Nv 4.06 x 10^3	Vc 710, 735 Nv 7.23 x 10^3	Vc 382, 356 Nv 3.69 x 10^3	Vc 537, 546 Nv 5.42 x 10^3
Experimental conditions	Vc 599, 571 A 5.85 x 10^2	Vc 421, 477 A 4.49 x 10^2	Vc 315, 297 A 3.06 x 10^2	Vc 562, 598 A 5.80 x 10^2
Neutraliser Control	Vc 641, 678 B 6.60 x 10^2	Vc 344, 365 B 3.55 x 10^2	Vc 467, 418 B 4.43 x 10^2	Vc 544, 612 B 5.78 x 10^2
Dilution – Neutralisation Control	Vc 449, 504 C 4.77 x 10^2	Vc 529, 546 C 5.38 x 10^2	Vc 524, 500 C 5.12 x 10^2	Vc 416, 378 C 3.97 x 10^2
Bacterial suspension	N 1.51 x 10^8	N 1.69 x 10^8	N 1.74 x 10^8	N 1.58 x 10^8
Test Results				
Na R	< 100 9.62 x 10^5	< 100 3.75 x 10^6	< 100 4.14 x 10^5	< 100 3.84 x 10^5

Vc = Viable count.

N = Number of cfu/ml of the bacterial test suspension.

Nv = Number of cfu in bacterial suspension.

R = Reduction in viability.

Na = Number of cfu/ml in the test mixture.

BA-01. 0.5 % 5 minutes Conclusion:

E. Coli - Satisfactory

P. Aeruginosa.- Satisfactory

S. Aureus - Satisfactory

B. Subtillis - Satisfactory

Certificate of Analysis

Samples: Citric Acid 20%

Analysis Required: BS EN 1276 under dirty conditions

Samples Tested: 03/06/2008

Product stored at room temperature.

Active substance: Citric Acid.

Experimental conditions:

Product diluent used during test	- Sterile hard water 300 mg/kg $CaCO_3$
Product test concentrations	- 0.5 % v/v
Contact time	- 5 min
Test temperature	- 20 °C ± 0.5 °C
Interfering substance	- 3.0 g/l Bovine albumin
Neutralising solution	- 3 % Tween 80, 3 % Saponin, 0.1 % Histidine, 0.1 % Cysteine
Temperature of incubation	- 37 °C ± 1 °C
Identification of bacterial strains used	- ***Pseudomonas aeruginosa ATCC 15442***
	- ***Escherichia coli 11867***
	- ***Staphylococcus aureus NCTC 6571***
	- ***Bacillus subtillis 10262***

Test Results: Citric Acid 0.5 % 5 minutes

Validation test	Gram Negative		Gram Positive	
	E. coli	*P. Aeruginosa*	*S. Aureus*	*B. Subtillis*
Bacterial suspension	Vc 425, 395 Nv 4.10×10^3	Vc 613, 586 Nv 6.00×10^3	Vc 526, 543 Nv 5.35×10^3	Vc 499, 480 Nv 4.90×10^3
Experimental conditions	Vc 544, 539 A 5.42×10^2	Vc 462, 498 A 4.80×10^2	Vc 481, 444 A 4.63×10^2	Vc 386, 429 A 4.08×10^2
Neutraliser Control	Vc 510, 534 B 5.22×10^2	Vc 355, 370 B 3.63×10^2	Vc 516, 502 B 5.09×10^2	Vc 558, 543 B 5.51×10^2
Dilution – Neutralisation Control	Vc 402, 418 C 4.11×10^2	Vc 569, 582 C 5.76×10^2	Vc 565, 522 C 5.44×10^2	Vc 469, 507 C 4.88×10^2
Bacterial suspension	N 1.67×10^8	N 1.53×10^8	N 1.50×10^8	N 1.78×10^8
Test Results				
Na R	> 100 6.42×10^2	> 100 2.67×10^3	> 100 3.01×10^2	> 100 2.29×10^2

Vc = Viable count.

N = Number of cfu/ml of the bacterial test suspension.

Nv = Number of cfu in bacterial suspension.

R = Reduction in viability.

Na = Number of cfu/ml in the test mixture.

Citric Acid 0.5 % 5 minutes Conclusion:

E. Coli - Unsatisfactory

P. Aeruginosa.- Unsatisfactory

S. Aureus - Unsatisfactory

B. Subtillis - Unsatisfactory

Certificate of Analysis

Samples: Bioflavonoids

Analysis Required: BS EN 1276 under dirty conditions

Samples Tested: 04/04/2008

Product stored at room temperature.

Active substance: Bioflavonoids

Experimental conditions:

Product diluent used during test	- Sterile hard water 300 mg/kg $CaCO_3$
Product test concentrations	- 0.5 % v/v
Contact time	- 5 min
Test temperature	- 20 °C ± 0.5 °C
Interfering substance	- 3.0 g/l Bovine albumin
Neutralising solution	- 3 % Tween 80, 3 % Saponin, 0.1 % Histidine, 0.1 % Cysteine
Temperature of incubation	- 37 °C ± 1 °C
Identification of bacterial strains used	- ***Pseudomonas aeruginosa ATCC 15442***
	- ***Escherichia coli 11867***
	- ***Staphylococcus aureus NCTC 6571***
	- ***Bacillus subtillis 10262***

Test Results: GRO#04 0.5 % 5 minutes

Validation test	Gram Negative		Gram Positive	
	E. coli	*P. Aeruginosa*	*S. Aureus*	*B. Subtillis*
Bacterial suspension	Vc 491, 534 Nv 5.13 x 10^3	Vc 652, 603 Nv 6.28 x 10^3	Vc 423, 405 Nv 4.14 x 10^3	Vc 512, 560 Nv 5.36 x 10^3
Experimental conditions	Vc 522, 568 A 5.45 x 10^2	Vc 520, 567 A 5.44 x 10^2	Vc 562, 537 A 5.50 x 10^2	Vc 399, 446 A 4.23 x 10^2
Neutraliser Control	Vc 489, 430 B 4.60 x 10^2	Vc 516, 534 B 5.25 x 10^2	Vc 580, 612 B 5.96 x 10^2	Vc 552, 577 B 5.65 x 10^2
Dilution – Neutralisation Control	Vc 412, 466 C 4.39 x 10^2	Vc 424, 445 C 4.35 x 10^2	Vc 375, 362 C 3.69 x 10^2	Vc 526, 501 C 5.14 x 10^2
Bacterial suspension	N 1.66 x 10^8	N 1.54 x 10^8	N 1.80 x 10^8	N 1.52 x 10^8
Test Results				
Na R	> 100 7.11 x 10^3	> 100 1.94 x 10^4	> 100 2.05 x 10^2	> 100 6.53 x 10^3

Vc = Viable count.

N = Number of cfu/ml of the bacterial test suspension.

Nv = Number of cfu in bacterial suspension.

R = Reduction in viability.

Na = Number of cfu/ml in the test mixture.

Bioflavonoids. 0.5 % 5 minutes Conclusion:

E. Coli - Unsatisfactory

P. Aeruginosa.- Unsatisfactory

S. Aureus - Unsatisfactory

B. Subtillis - Unsatisfactory

E 8

Abbott Analytical

Consulting Scientists to the Disinfectant Industry

7th July 2008

Certificate of Analysis

Samples: One sample of Citrus Bioflavonoids received from Citrox Ltd, Unit 9 River Court, Brighouse Business Village, Brighouse Road, Riverside Park, Middlesborough. TS2 1RT 1st July 2008.

Certificate No: 08K.004.CIT

Page: 1 of 2

Sample Ref: 8k / 004

Analysis Required: BS EN 1276 under dirty conditions

Samples Tested: 3rd July 2008

Product stored at room temperature.
Active substance: Not declared
Batch Number: CIT/07/10
Eperimental conditions:

Product test concentrations	-	0.5%
Contact time	-	5 min
Test Temperature	-	$20^{0}C \pm 0.5^{0}C$
Interferring substance	-	3.0g/l Bovine albumin
Neutralising solution	-	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	-	$37^{0}C \pm 1^{0}C$
Identification of bacterial strains used	-	Pseudomonas aeruginosa ATCC 15442 Escherichia coli NCTC 10418 Staphylococcus aureus NCTC 10788 Enterococcus hirae ATCC 8043

D C Watson

D C Watson BSc, C Biol, MIBiol, MIFST, ACIEHO
PO Box 95, New Ferry, Wirral, CH62 6HA. Tel: 07767 871275 Fax: 0151 630 6540
email: dcwatson@abbottanalytical.fsbusiness.co.uk

E 8

Abbott Analytical

Consulting Scientists to the Disinfectant Industry

7th July 2008

Certificate No 08k.004.CIT

Test Results

Validation test	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Enterococcus hirae
Bacterial suspension	Vc 136, 147 Nv 1.41×10^3	Vc 212, 185 Nv 1.98×10^3	Vc 136, 147 Nv 1.41×10^3	Vc 212, 185 Nv 1.98×10^3
Experimental conditions	Vc 151, 142 A 1.46×10^2	Vc 210, 232 A 2.21×10^2	Vc 151, 142 A 1.46×10^2	Vc 210, 232 A 2.21×10^2
Neutraliser control	Vc 160, 146 B 1.53×10^2	Vc 208, 182 B 1.95×10^2	Vc 160, 146 B 1.53×10^2	Vc 208, 182 B 1.95×10^2
Dilution-neutralisation control	Vc 155, 136 C 1.45×10^2	Vc 230, 216 C 2.23×10^2	Vc 155, 136 C 1.45×10^2	Vc 230, 216 C 2.23×10^2
Bacterial Test Suspension	10^{-5} 392 256 10^{-6} 45 61 N 4.27×10^7	10^{-6} 138, 164 10^{-7} 13 10 N 1.33×10^8	10^{-5} 392 256 10^{-6} 45 61 N 4.27×10^7	10^{-6} 138, 164 10^{-7} 13 10 N 1.33×10^8
Test results				
Neat Vc Na R	7020 702000 6.08×10^2	2740 274000 4.85×10^2	4050 405000 1.05×10^3	1260 126000 1.06×10^3

Vc = Viable Count.
N = Number of cfu/ml of the bacterial test suspension.
Nv = Number of cfu in bacterial suspension.
R = Reduction in viability.
Na = Number of cfu/ml in the test mixture

Conclusion: According to EN1276 this batch of Citrus Bioflavonoids when used at 0.5% dilution does not possess satisfactory bactericidal activity in 5 minutes at 20°C under dirty conditions (3.0g/l bovine albumin) for the reference organisms detailed.

D C Watson

D C Watson BSc, C Biol, MIBiol, MIFST, ACIEHO
PO Box 95, New Ferry, Wirral, CH62 6HA. Tel: 07767 871275 Fax: 0151 630 6540
email: dcwatson@abbottanalytical.fsbusiness.co.uk

E 9

Abbott Analytical

Consulting Scientists to the Disinfectant Industry

7th July 2008

Certificate of Analysis

Samples: One sample of Citrus Bioflavonoids received from Citrox Ltd, Unit 9 River Court, Brighouse Business Village, Brighouse Road, Riverside Park, Middlesborough. TS2 1RT 1st July 2008.

Certificate No: 08K.004a.CIT

Page: 1 of 2

Sample Ref: 8j / 009

Analysis Required: BS EN 1276 under dirty conditions

Samples Tested: 3rd July 2008

Product stored at room temperature.
Active substance: Not declared
Batch Number: CIT/07/10
Eperimental conditions:

Product test concentrations	-	10%
Contact time	-	5 min
Test Temperature	-	20°C ± 0.5°C
Interferring substance	-	3.0g/l Bovine albumin
Neutralising solution	-	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	-	37°C ± 1°C

Identification of bacterial strains used - Pseudomonas aeruginosa ATCC 15442
Escherichia coli NCTC 10418
Staphylococcus aureus NCTC 10788
Enterococcus hirae ATCC 8043

D C Watson

D C Watson BSc, C Biol, MIBiol, MIFST, ACIEHO
PO Box 95, New Ferry, Wirral. CH62 6HA. Tel: 07767 871275 Fax: 0151 630 6540
email: dcwatson@abbottanalytical.fsbusiness.co.uk

E 9

Abbott Analytical

Consulting Scientists to the Disinfectant Industry

7th July 2008

Certificate No 08K.004a.CIT

Test Results

Validation test	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Enterococcus hirae
Bacterial suspension	Vc 534, 570 Nv 5.52×10^3	Vc 354, 418 Nv 3.86×10^3	Vc 277, 322 Nv 2.99×10^3	Vc 574, 492 Nv 5.33×10^3
Experimental conditions	Vc 555, 523 A 5.34×10^2	Vc 372, 348 A 3.60×10^2	Vc 366, 408 A 3.87×10^2	Vc 514, 540 A 5.27×10^2
Neutraliser control	Vc 568, 530 B 5.49×10^2	Vc 394, 346 B 3.70×10^2	Vc 388, 454 B 4.21×10^2	Vc 522, 476 B 4.99×10^2
Dilution-neutralisation control	10^{-6} 760 620 10^{-7} 84 68 N 7.25×10^8	10^{-6} 314 422 10^{-7} 41 36 N 3.76×10^8	10^{-6} 600 528 10^{-7} 43 48 N 5.09×10^8	10^{-6} 418 472 10^{-7} 54 61 N 5.10×10^8
Bacterial Test Suspension				
Test results	0 <100 $>7.25 \times 10^6$	0 <100 $>3.76 \times 10^6$	0 <100 $>5.09 \times 10^6$	0 <100 $>5.10 \times 10^6$

Vc = Viable Count.
N = Number of cfu/ml of the bacterial test suspension.
Nv = Number of cfu in bacterial suspension.
R = Reduction in viability.
Na = Number of cfu/ml in the test mixture

Conclusion: According to EN1276 this batch of Citrus Bioflavonoids when used at 10% dilution possesses satisfactory bactericidal activity in 5 minutes at 20°C under dirty conditions (3.0g/l bovine albumin) for the reference organisms detailed.

D C Watson

D C Watson BSc, C Biol, MIBiol, MIFST, ACIEHO
PO Box 95, New Ferry, Wirral, CH62 6HA. Tel: 07767 871275 Fax: 0151 630 6540
email: dcwatson@abbottanalytical.fsbusiness.co.uk

E 10

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Hand Foam received from Citrox Limited, Unit 1 River Court, Brighouse Road, Riverside Park, Middlesbrough TS2 1RT 26th February 2007
Certificate Number: 07B.115.CIT
Analysis Required: BSEN 12054
Samples Tested: 28th February 2007

Product stored at room temperature in the dark.
Active substance: Not declared.

Experimental conditions:

Product test concentrations	Neat as received
Contact time	1 min
Test Temperature	$20^0C \pm 0.5^0C$
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	$30^0C \pm 1^0C$
Identification of bacterial strains used	Pseudomonas aeruginosa ATCC 15442 Escherichia coli NCTC 10418 MRSA NCIMB 50143 Enterococcus hirae ATCC 8043

Test Results

Validation test	Pseudomonas aeruginosa	Escherichia coli	MRSA NCIMB 50143	Enterococcus hirae
Bacterial suspension	Vc 536, 447 Nv 4.91×10^3	Vc 372, 338 Nv 3.55×10^3	Vc 392, 438 Nv 4.15×10^3	Vc 432, 455 Nv 4.43×10^3
Experimental conditions	Vc 416, 442 A 4.29×10^2	Vc 300, 354 A 3.27×10^2	Vc 372, 414 A 3.93×10^2	Vc 440, 394 A 4.17×10^2
Neutraliser control	Vc 488, 496 B 4.92×10^2	Vc 344, 282 B 3.13×10^2	Vc 358, 446 B 4.02×10^2	Vc 456, 424 B 4.40×10^2
Dilution-neutralisation control	Vc 520, 486 C 5.03×10^2	Vc 290, 336 C 3.13×10^2	Vc 375, 436 C 4.05×10^2	Vc 422, 356 C 3.89×10^2
Bacterial Test Suspension	10^{-6} 944, 638 10^{-7} 43, 59 N 6.50×10^8	10^{-6} 330, 312 10^{-7} 20, 28 N 2.80×10^8	10^{-6} 334, 416 10^{-7} 48, 52 N 4.37×10^8	10^{-6} 448, 512 10^{-7} 64, 47 N 5.17×10^8
Test results				
Neat Vc Na R	0 <100 $>6.50 \times 10^6$	0 <100 $>2.80 \times 10^6$	0 <100 $>4.37 \times 10^8$	0 <100 $>5.17 \times 10^6$

Vc = Viable Count
N = Number of cfu/ml of the bacterial test suspension
Nv = Number of cfu in bacterial suspension
R = Reduction in viability
Na = Number of cfu/ml in the test mixture

Conclusion:

According to EN12054 this batch of Hand Foam when used neat as received possesses satisfactory bactericidal activity in 1 minute at 20^0C

Independent Laboratory Verification by Abbott Analytical

citrox

E11

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Clinisan received from Citrox Ltd, Unit 1 River Court, Brighouse Road, Riverside Park, Middlesbrough TS2 1RT 5th February 2007
Certificate Number: 07B.008.CIT
Analysis Required: BS EN 13704 using Clostridium difficile
Samples Tested: 15th February 2007

Product stored at room temperature in the dark.
Active substance: Not declared.

Experimental conditions:

Product test concentrations	Neat as recieved
Contact time	15 min
Test Temperature	$20^0C \pm 0.5^0C$
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	$30^0C \pm 1^0C$
Identification of bacterial strains used	Clostridium difficile NCTC 11209

Test Results

Validation test		Clostridium difficile
Bacterial Suspension		Vc 494, 526 Nv 5.10×10^3
Experimental Conditions		Vc 500, 467 A 4.83×10^2
Neutraliser Control		512, 480 B 4.96×10^2
Dilution neutralization Control		Vc 454, 486 C 4.70×10^2
Bacterial Test Suspension		10^{-6} 490, 556 10^{-7} 48, 52 N 5.11×10^8
Test results		
15 min	Vc	52
	Na	5200
	R	1.0×10^5

Vc = Viable Count
N = Number of cfu/ml of the bacterial test suspension
Nv = Number of cfu in bacterial suspension
R = Reduction in viability
Na = Number of cfu/ml in the test mixture

Conclusion:
According to EN13704 this sample of Clinisan when used neat as received possesses satisfactory bactericidal activity in 15 minutes at 20^0C for the reference organism detailed..

Independent Laboratory Verification by Abbott Analytical

citROX

E 12

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox Surface Spray received from Citrox Limited
Certificate Number: 03A.102
Analysis Required: BS EN 1276 under dirty conditions
Samples Tested: 13th July 2003

Product stored at 5^0C in the dark.
Active substance: Not declared.

Experimental conditions:

Product diluent used during test	Sterile hard water 300mg/kg CaCO3
Product test concentrations	Neat
Contact time	5 min
Test Temperature	20^0C $\pm$ 0.5^0C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30^0C $\pm$ 1^0C
Identification of bacterial strains used	Pseudomonas aeruginosa ATCC 15442 Escherichia coli NCTC 10418 Staphylococcus aureus ACTA 6571 Enterococcus hirae ATCC 8043

Test Results

Validation test	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Enterococcus hirae
Bacterial suspension	Vc 184, 166 Nv 1.75 x 10^3	Vc 154, 138 Nv 1.46 x 10^3	Vc 136, 147 Nv 1.41 x 10^3	Vc 212, 185 Nv 1.98 x 10^3
Experimental Conditions	Vc 210, 180 A 1.95 x 10^2	Vc 166, 192 A 1.79 x 10^2	Vc 151, 142 A 1.46 x 10^2	Vc 210, 232 A 2.21 x 10^2
Neutraliser control	Vc 188, 196 B 1.92 x 10^2	Vc 158, 142 B 1.50 x 10^2	Vc 160, 146 B 1.53 x 10^2	Vc 208, 182 B 1.95 x 10^2
Dilution-neutralisation Control	Vc 220, 186 C 2.03 x 10^2	Vc 182, 164 C 1.73 x 10^2	Vc 155, 136 C 1.45 x 10^2	Vc 230, 216 C 2.23 x 10^2
Bacterial Test Suspension	10-6 83, 94 10-7 8, 8 N 8.45 x 10^8	10-7 78, 92 10-7 6, 8 N 7.75 x 10^8	10-7 52, 66 10-7 6, 6 N 5.95 x 10^8	10-7 34, 18 10-7 4, 5 N 3.55 x 10^8
Test result				
1 : 50 Vc Na R	102 10200 1.25 x 10^5	58 5800 1.34 x 10^5	47 4700 1.26 x 10^5	29 2900 1.22 x 10^5

Vc = Viable Count
N = Number of cfu/ml of the bacterial test suspension
Nv = Number of cfu in bacterial suspension
R = Reduction in viability
Na = Number of cfu/ml in the test mixture

Conclusion:

According to EN1276 this batch of Citrox Surface Spray when tested neat possesses satisfactory bactericidal activity in 2 minutes at 20^0C under dirty conditions (3.0g/l bovine albumin) for the organisms listed.

Independent Laboratory Verification by Abbott Analytical

citROX

E13

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox Surface Cleaner received from Citrox Limited 11th April 2005
Certificate Number: 05D.121.GWP
Analysis Required: BS EN 1276 under dirty conditions
Samples Tested: 13th April 2005

Product stored at 5°C in the dark.
Active substance: Not declared.
Batch Number: 70

Experimental conditions:

Product diluent used during test	Sterile hard water 300mg/kg CaCO3
Product test concentrations	2% v/v
Contact time	5 min
Test Temperature	20°C ± 0.5°C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30°C ± 1°C
Identification of bacterial strains used	Pseudomonas aeruginosa ATCC 15442 Escherichia coli NCTC 10418 Staphylococcus aureus NCTC 6571 Enterococcus hirae ATCC 8043

Test Results

Validation test	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Enterococcus Hirae
Bacterial Suspension	Vc 184, 166 Nv 1.75 x 10^3	Vc 154, 138 Nv 1.46 x 10^3	Vc 136, 147 Nv 1.41 x 10^3	Vc 212, 185 Nv 1.98 x 10^3
Experimental Conditions	Vc 210, 180 A 1.95 x 10^2	Vc 166, 192 A 1.79 x 10^2	Vc 151, 142 A 1.46 x 10^2	Vc 210, 232 A 2.21 x 10^2
Neutraliser Control	Vc 188, 196 B 1.92 x 10^2	Vc 158, 142 B 1.50 x 10^2	Vc 160, 146 B 1.53 x 10^2	Vc 208, 182 B 1.95 x 10^2
Dilution neutralization Control	Vc 220, 186 C 2.03 x 10^2	Vc 182, 164 B 1.73 x 10^2	Vc 155, 136 C 1.45 x 10^2	Vc 230, 216 C 2.23 x 10^2
Bacterial Test Suspension	10^{-6} 856, 820 10^{-7} 87, 86 N 8.52 x 10^8	10^{-6} 344, 306 10^{-7} 34, 36 N 3.37 x 10^8	10^{-6} 416, 388 10^{-7} 44, 42 N 4.16 x 10^8	10^{-6} 472, 434 10^{-7} 48, 50 N 4.71 x 10^8
Test results				
1 : 50 Vc Na R	34 3400 2.50 x 10^5	0 <100 >3.37 x 10^6	3 300 1.38 x 10^6	14 1400 3.36 x 10^6

Vc = Viable Count
N = Number of cfu/ml of the bacterial test suspension
Nv = Number of cfu in bacterial suspension
R = Reduction in viability
Na = Number of cfu/ml in the test mixture

Conclusion:

According to EN1276 this batch of Citrox Surface Cleaner when diluted at 1:50 (v/v) in hard water possesses satisfactory bactericidal activity in 5 minutes at 20°C under dirty conditions (3.0g/1 bovine albumin) for reference organisms indicated.

Independent Laboratory Verification by Abbott Analytical

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E 14

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox BC received from Citrox Limited 1st July 2004
Certificate Number: 04G.003V.CIT
Analysis Required: Activity against Vibro parahaemolyticus using modified EN 1276 under dirty conditions.
Samples Tested: 7th & 8th July 2004

Product stored at 5°C in the dark.
Active substance: Not declared.

Experimental conditions:

Product diluent used during test	Artificial Hard Water 300mg $CaCO_3$
Product test concentrations	0.2% v/v
Contact time	5 min
Test Temperature	20°C ± 1°C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	37°C ± 1°C
Identification of bacterial strains used	Vibro parahaemolyticus ATTC 17802

Test Results

Validation Test	Vibro Parahaemolyticus
Bacterial Suspension	Vc 224, 258 Nv 2.41×10^3
Experimental Conditions	Vc 188, 162 A 1.75×10^2
Neutraliser Control	Vc 190, 214 B 2.02×10^2
Dilution neutralization Control	Vc 177, 205 C 1.91×10^2
Bacterial Test Suspension	10^{-6} 154 138 10^{-7} 12 15 N 1.40×10^8
Test results at concentrations	
1 : 500 Vc	4
Na	400
R	3.50×10^5

VC = Viable Count
Nv = Number of cfu/ml in bacterial suspension
A,B & C = Mean actual count cfu in suspension
N = Number of cfu in bacterial test suspension
Na = Number of cfu in test mixture
R = Reduction on viability

Conclusion:

According to EN1276 Citrox BC when diluted 1 : 500 in sterile hard water possesses bactericidal activity in 5 minutes at 20°C under dirty conditions for the reference strains detailed.

Independent Laboratory Verification by Abbott Analytical

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E 15

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox BC received from Citrox Limited 13th January 2003
Certificate Number: 03A.102.GWP
Analysis Required: BSEN 1276 under dirty conditions
Samples Tested: 17th January 2003

Product stored at 5°C in the dark.
Active substance: Not declared.
Batch number: BC Aus / 14W

Experimental conditions:

Product diluent used during test	Sterile Hard Water 300mg/kg $CaCO_3$
Product test concentrations	3% v/v
Contact time	5 min
Test Temperature	20°C ± 0.5°C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30°C ± 1°C
Identification of bacterial strains used	Pseudomonas aeruginosa ATCC 15442

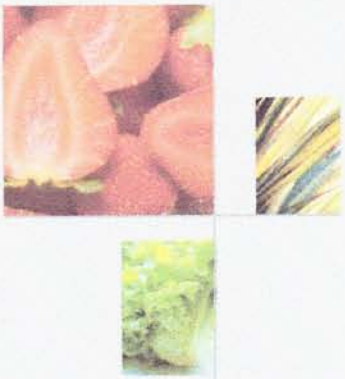
Test Results

Validation Test		Pseudomonas aeruginosa
Bacterial Suspension		Vc 184, 166 Nv 1.75 x 10^3
Experimental Conditions		Vc 210, 180 A 1.95 x 10^2
Neutraliser Control		Vc 188, 196 B 1.92 x 10^2
Dilution neutralization Control		Vc 220, 186 C 2.03 x 10^2
Bacterial Test Suspension		10^{-6} 54, 76 10^{-7} 5, 5 N 5.57 x 10^8
Test results at concentrations		
1 : 33	Vc	37
	Na	3700
	R	1.55 x 10^5

VC = Viable Count
Nv = Number of cfu/ml in bacterial suspension
A,B & C = Mean actual count cfu in suspension
N = Number of cfu in bacterial test suspension
Na = Number of cfu in test mixture
R = Reduction on viability

Conclusion:

According to EN 1276 this batch of Citrox BC when diluted at 1 : 33 (v/v) in hard water possesses satisfactory bactericidal activity in 5 minutes at 20°C under dirty conditions (3.0g/l bovine albumin) for Pseudomonas.



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E 16

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox BC received from Citrox Limited 10th December 2003
Certificate Number: 3m.155.CIT
Analysis Required: Challenge test against Phytophthora Spp as indicated using adaptation of EN 1650
Samples Tested: 15th January 2004

Product stored at 5°C in the dark.
Active substance: Not declared.

Experimental conditions:

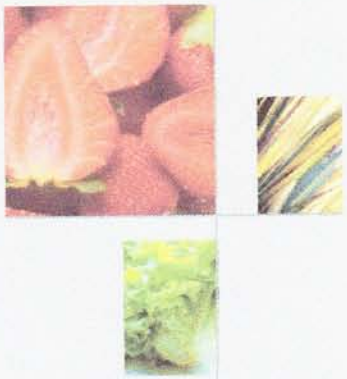
Product diluent used during test	Sterile hard water 300mg/kg $CaCO_3$
Product test concentrations	0.12%, 0.25% & 0.5% v/v
Contact time	30 min
Test Temperature	20°C ± 0.5°C
Interfering substance	5% w/v yeast suspension
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30°C ± 1°C
Identification of bacterial strains used	Phytophthora ramorum

Test Results

Sample challenged with spore suspension of the mold under test. After 30 minutes exposure the following reduction in numbers was observed:

0.12%	0.25%	0.5%
3.72×10^3	8.46×10^3	2.76×10^4

At 0.5% v/v Citrox BC shows significant reduction in numbers of Phytophthora under the test conditions described.



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E 17

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
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Samples: One sample of Citrox BC disinfectant received from Citrox Limited 23rd April 2003
Certificate Number: 0.3D.314m.GWP.
Analysis Required: Activity against Mycobacterium fortuitum at 0.6% v/v under simulated EN 1276 test conditions
Samples Tested: 16th May 2003

Test Results

Mycobacterium fortuitum is considered a suitable organism disinfectant materials for tuberculocidal activity. It forms the organism of choice for testing for tuberculocidal activity of a product for registration under MAFF Bovine TB Orders (BS 6734)

Mycobacterium fortuitum NCTC 8573

Reduction in numbers after 15 minutes 9.15×10^3

Reduction in numbers after 30 minutes 7.25×10^5

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E 18

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
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Samples: One sample of Citrox BC received from Citrox Limited 13th February 2002
Certificate Number: 02b.136.GWP. Interim report
Analysis Required: Challenge tested against Moulds as indicated using adaptation of EN 1650
Samples Tested: 13th February 2002

Product stored at 5^0C in the dark.
Active substance: Not declared.

Experimental conditions:

Product diluent used during test	Sterile hard water 300mg/kg $CaCO_3$
Product test concentrations	1.5% w/w
Contact time	30 min
Test Temperature	20^0C $\pm$ 0.5^0C
Interfering substance	5% w/v yeast suspension
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30^0C $\pm$ 1^0C
Identification of mould strains used	penicillium digitatum

Test Results

Sample challenged with spore suspension of the mould under test. After 30 minutes exposure the following reduction in numbers was observed.

Penicillium digitatum 2.16×10^6

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E 19

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
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Samples: One sample of Citrox BC disinfectant received from Citrox Limited 5th March 2004
Certificate Number: 04C.100.CIT
Analysis Required: Activity against Listeria on surfaces using an Electrostatic sprayer giving 20 micron droplet size.
Samples Tested: 5th March 2004

An overnight broth culture of *Listeria monocytogenes* was sprayed on to a food grade plastic clad wall using an atomizer and allowed to dry for approximately 2 hours. The wall was then divided in squares for approximately 500mm x 500mm with masking tape. A swab was taken from one of the squares before any treatment, placed in 10ml of recovery medium shaken vigorously to disperse the organisms and plated on to Listeria selective agar to establish the base level of contamination. Subsequent squares were treated with 2.5%v/v, 10%v/v and 25%v/v respectively of Citrox BC in sterile tap water by spraying from the electrostatic sprayer for a measured time of 2 minutes. After each square had been treated it was allowed to be in contact with the contaminated area for 5 minutes before the area was swabbed with a fresh sterile swab which was then placed in 10ml of a sterile neutralizing solution before examining for surviving organisms as with the control. Only after the swabbing was done was then next square sprayed. The results obtained for survival of Listeria after treatment are recorded below.

Test Results

Control	2.5%	10%	25%
1920 cfu per sq.	465 (16.8% kill)	0 (100% kill)	0 (100% kill)

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E 20

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
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Samples: One sample of Citrox BC disinfectant received from Citrox Limited 23rd April 2003
Certificate Number: 03D.3141.GWP
Analysis Required: Activity against Legionella pneumophila at 0.6% v/v under simulated EN 1276 test conditions
Samples Tested: 14th May 2003

Test Results

Legionella pneumophila NCTC 11192

Reduction in numbers after 15 minutes	1.90×10^4
Reduction in numbers after 30 minutes	1.20×10^6

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E 21

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox BC received from Citrox Limited 1st July 2004
Certificate Number: 04G.0031.CIT
Analysis Required: Activity against Lactobacillus acidophilus using modified EN 1276 under dirty conditions.
Samples Tested: 7th & 8th July 2004

Product stored at 5°C in the dark.
Active substance: Not declared.

Experimental conditions:

Product diluent used during test	Sterile hard water 300mg/kg $CaCo_3$
Product test concentrations	0.2% v/v
Contact time	5 min
Test Temperature	$20^0C \pm 1^0C$
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	$37^0C \pm 1^0C$
Identification of bacterial strains used	Lactobacillus acidophilus ATCC 4356

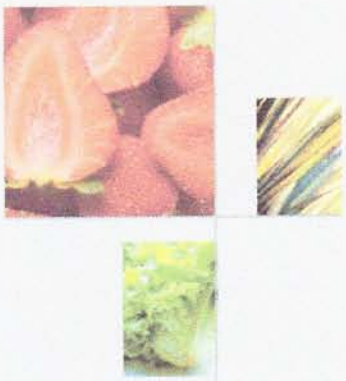
Test Results

Validation Test	Lactobacillus Acidophilus
Bacterial Suspension	Vc 166, 132 Nv 1.49×10^3
Experimental Conditions	Vc 155, 111 A 1.33×10^2
Neutraliser Control	Vc 128, 102 B 1.15×10^2
Dilution neutralization Control	Vc 152, 169 C 1.60×10^2
Bacterial Test Suspension	10^{-6} 252 206 10^{-7} 26 27 N 2.47×10^8
Test results at concentrations	
1 : 500 Vc	21
Na	2100
R	1.17×10^5

Vc = Viable Count
Nv = Number of cfu/ml in bacterial suspension
A.B & C = Mean actual count cfu in suspension
N = Number of cfu in bacterial test suspension
Na = Number of cfu in test mixture
R = Reduction on viability

Conclusion:

According to EN 1276 Citrox BC when diluted 1 : 500 in sterile hard water possesses bacterial activity in 5 minutes at 20^0C under dirty conditions for the referenced strains detailed.



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E 22

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox BC received from Citrox Limited 31st May 2006
Certificate Number: 06E.184.CIT
Analysis Required: Activity against Enterococcus Faecalis under dirty conditions
Samples Tested: 1st June 2006

Product stored at 5^0C in the dark.
Active substance: Not declared.
Batch number :366362

Experimental conditions:

Product diluent used during test	Artificial Hard Water 300mg $CaCO_3$
Product test concentrations	0.6% v/v
Contact time	5 min
Test Temperature	20^0C ± 0.5^0C
Interfering substance	a) 3.0g/l Bovine Albumin b) 3.0g/l Bovine Albumin/ 3.0ml/l sheep erythrocytes c) 10.0g/l Bovine Albium/ 10.0g/l Yeast Extract
Temperature of incubation	30^0C ± 1^0C
Identification of bacterial strains used	Enterococcus Faecalis NCTC 8213

Test Results

Validation Test		Enterococcus Faecalis
Bacterial Suspension		Vc 156, 172 Nv 1.64×10^3
Experimental Conditions		Vc 138, 166 A 1.52×10^2
Neutraliser Control		Vc 128, 154 B 1.41×10^2
Dilution neutralization Control		Vc 133, 115 C 1.24×10^2
Bacterial Test Suspension		10^{-6} 124, 144 10^{-7} 11, 15 N 1.32×10^8
Test results		
A.	Vc Na R	0 <100 $>1.32 \times 10^6$
B.	Vc Na R	8 800 1.65×10^5
C.	Vc Na R	11 1100 1.20×10^5

VC = Viable Count
Nv = Number of cfu/ml in bacterial suspension
A,B & C = Mean actual count cfu in suspension
N = Number of cfu in bacterial test suspension
Na = Number of cfu in test mixture
R = Reduction on viability

Conclusion:

According to EN1276 Citrox BC when diluted 1 : 166 (v/v) in hard water possesses satisfactory bactericidal activity in 5 minutes at 20^0C under dirty conditions (10g/l bovine serum, 10g/l yeast extract) for Enterococcus Faecalis, under the conditions of Norwich Hospital protocol there is a possibility of visible growth of organisms in broth culture after 7 days.

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E 2 3

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox BC received from Citrox Limited 23rd June 2005
Certificate Number: 05F.214.CIT
Analysis Required: BS EN 1276 under dirty conditions
Samples Tested: 24th June 2005

Product stored at 5°C in the dark.
Active substance: Not declared.
Batch Number: 363016 / 363017

Experimental conditions:

Product diluent used during test	Sterile hard water 300mg/kg CaCO3
Product test concentrations	0.2% w/w
Contact time	5 min
Test Temperature	20°C ± 0.5°C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30°C ± 1°C
Identification of bacterial strains used	Pseudomonas aeruginosa ATCC 15442 Escherichia coli NCTC 10418 Staphylococcus aureus NCTC 6571 Enterococcus hirae ATCC 8043

Test Results

Validation test	Pseudomonas aeruginosa	Escherichia coli	Staphylococcus aureus	Enterococcus hirae
Bacterial suspension	Vc 184, 166 Nv 1.75 x 10^3	Vc 154, 138 Nv 1.46 x 10^3	Vc 136, 147 Nv 1.41 x 10^3	Vc 212, 185 Nv 1.98 x 10^3
Experimental conditions	Vc 210, 180 A 1.95 x 10^2	Vc 166, 192 A 1.79 x 10^2	Vc 151, 142 A 1.46 x 10^2	Vc 210, 232 A 2.21 x 10^2
Neutraliser control	Vc 188, 196 B 1.92 x 10^2	Vc 158, 142 B 1.50 x 10^2	Vc 160, 146 B 1.53 x 10^2	Vc 208, 182 B 1.95 x 10^2
Dilution-neutralisation control	Vc 220, 186 C 2.03 x 10^2	Vc 182, 164 C 1.73 x 10^2	Vc 155, 136 C 1.45 x 10^2	Vc 230, 216 C 2.23 x 10^2
Bacterial Test Suspension	10^{-6} 354, 416 10^{-7} 38, 37 N 3.80 x 10^8	10^{-6} 408, 372 10-7 44, 40 N 4.05 x 10^8	10^{-6} 127, 139 10^{-7} 12, 15 N 1.34 x 10^8	10^{-6} 224, 272 10^{-7} 22 20 N 2.29 x 10^8
Test results				
0.2% w/w Vc Na R	2 200 1.9 x 10^6	0 <100 >4.05 x 10^6	0 <100 >1.34 x 10^6	0 <100 >2.29 x 10^6

Vc = Viable Count.
N = Number of cfu/ml of the bacterial test suspension.
Nv = Number of cfu in bacterial suspension.
R = Reduction in viability.
Na = Number of cfu/ml in the test mixture

Conclusion:
According to BS EN1276 this batch of Citrox BC when diluted at 1:250 (v/v) in hard water possess satisfactory bactericidal activity in 5 minutes at 20°C under dirty conditions (3.0g/l bovine albumin) for the organisms detailed.

Independent Laboratory Verification by Abbott Analytical

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E 24

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox BC received from Citrox Limited 20th August 2004
Certificate Number: 04H.246C.
Analysis Required: Activity against Clostridium perfringens using EN 13704 under dirty conditions
Samples Tested: 7th & 8th October 2004

Product stored at 5°C in the dark.
Active substance: Not declared.
Batch Number: 033567

Experimental conditions:

Product diluent used during test	Sterile hard water 300mg/kg CaCO3
Product test concentrations	0.4% w/w
Contact time	30 min
Test Temperature	20°C ± 0.5°C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30°C ± 1°C
Identification of bacterial strains used	Clostridium perfringens

Test Results

Sample challenged at 0.4% w/w. After 30 minutes exposure a 1.08 x 10^4 reduction in numbers was observed. This would satisfy the requirements of BS and EN test protocols for efficacy against this organism.

Conclusion:

Conclusion: According to EN13704 this batch of Citrox BC disinfectant when diluted at 0.4% w/w in hard water possesses satisfactory bactericidal activity in 30 minutes at 20°C under dirty conditions (3.0g/l bovine albumin) for the reference strain detailed.

Independent Laboratory Verification by Abbott Analytical

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E 2 5

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox BC received from Citrox Limited 20th August 2004
Certificate Number: 04H.246cd.CIT
Analysis Required: Activity against Clostridium difficile using EN 13704 under dirty conditions:
Samples Tested: 8th - 9th December 2004

Product stored at 5°C in the dark.
Active substance: Not declared.

Experimental conditions:

Product diluent used during test	Sterile Hard Water 300mg/kg $CaCO_3$
Product test concentrations	0.4% v/v
Contact time	30 min
Test Temperature	20°C ± 1°C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	37°C ± 1°C
Identification of bacterial strains used	Clostridium difficile ATCC 11437

Test Results

Validation Test		Clostridium difficile
Bacterial Suspension		Vc 254, 206 Nv 2.30 x 10^3
Experimental Conditions		Vc 210, 228 A 2.19 x 10^2
Neutraliser Control		Vc 198, 224 B 2.11 x 10^2
Dilution neutralization Control		Vc 186, 214 C 2.00 x 10^2
Bacterial Test Suspension		10^{-6} 228, 200 10^{-7} 25, 23 N 2.27 x 10^6
Test results at concentrations		
1 : 33	Vc	724
	Na	72400
	R	3.93 x 10^3

VC = Viable Count
Nv = Number of cfu/ml in bacterial suspension
A,B & C = Mean actual count cfu in suspension
N = Number of cfu in bacterial test suspension
Na = Number of cfu in test mixture
R = Reduction on viability

Conclusion:

According to EN13704 Citrox BC when diluted 1 : 250 in sterile hard water possesses sporicidal activity in 30 minutes at 20°C under dirty conditions for the referenced strain detailed.

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E 26

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Samples: One sample of Citrox BC received from Citrox Limited 5th July 2004
Certificate Number: 04H.246C.
Analysis Required: BS EN 1276 under dirty conditions
Samples Tested: 9th July 2004

Product stored at 5°C in the dark.
Active substance: Not declared.
Batch Number: 033567

Experimental conditions:

Product diluent used during test	Sterile hard water 300mg/kg CaCO3
Product test concentrations	0.2% w/w
Contact time	5 min
Test Temperature	20°C ± 0.5°C
Interfering substance	3.0g/l Bovine albumin
Neutralising solution	3% Tween 80, 3% Saponin, 0.1% Histidine, 0.1% Cysteine
Temperature of incubation	30°C ± 1°C
Identification of bacterial strains used	Campylobacter jejuni NCTC 11322

Test Results

Sample challenged with 24 hour culture of Campylobacter jejuni with 0.3% bovine serum as organic challenge. After 5 minutes exposure a 3.26 x 10^5 reduction in numbers was observed. This would satisfy the requirements of BS and EN test protocols for efficacy against this organism.

Conclusion:

Conclusion: According to EN1276 this batch of Citrox BC disinfectant when diluted at 0.2% w/w in hard water possesses satisfactory bactericidal activity in 5 minutes at 20°C under dirty conditions (3.0g/l bovine albumin) for the reference strain detailed.

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E 27

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
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Samples: One sample of Citrox Hand Gel received from Citrox Bioscience Ltd, 4th August 2005
Certificate Number: 5H.099a.KAE
Analysis Required: Activity against EN 1500
Samples Tested: 22nd August 2005

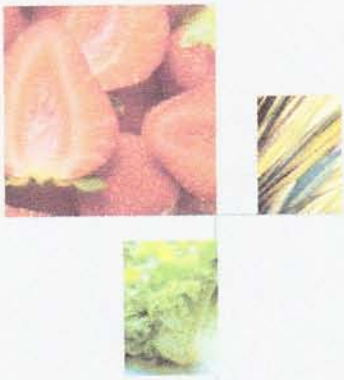
Principle of test:
The number of test organisms released from the fingertips of artificially contaminated hands is assessed before and after the hygienic handrub. The ratio of the two resulting values is called the reduction factor. It represents a measure of antimicrobial activity of the hygienic handwash product tested. In order to achieve the necessary precision a large number of subjects has to be used because of the possible variation in bacterial flora found on human skin. In this case a total of **twelve (12)** healthy adults were chosen each one carrying out the test procedure in precisely the same way as the others. To compensate for extraneous influences the test sample reduction factor (P) is compared with the reduction factor obtained with a parallel reference handwash procedure (R) which is performed with the same subjects, on the same day and under comparable environmental conditions.
Experimental procedure:

1) Application of the contamination fluid.

Each of the 12 subjects was asked to wash their hands for 1 minute in soft soap to remove natural commensal organisms and dried thoroughly on a paper towel. The hands were then contaminated with very large numbers of bacteria well in excess of that experienced in normal everyday occurrence. The hands were immersed in the contamination fluid (containing an overnight culture of the test organism in this instance E. coli at a concentration of approximately 10^8 cfu per ml) in a suitable sized container for 5 seconds. The hands were removed from the contamination fluid and surplus liquid allowed to drain back into the container. This time the hands were allowed to air dry for approximately 3 minutes holding them horizontally with fingers spread out and rotating them to and fro to avoid the formation of droplets.

2) Pre-values.

Immediately after drying, each of the 12 subjects was asked to rub their fingertips, including the thumbs for 1 minute on the base of a petri dish, using a separate petri dish for each hand, containing 10ml of maximum recovery diluent (MRD) without neutraliser, in order to assess the release of test organisms before treatment of the hands. Dilutions of these sample fluids were prepared to 10^{-3} and 10^{-4}. A 1ml aliquot of each dilution for each hand was placed in a separate petri dish 10 - 15ml of Tryptone Soy Agar sterilised and cooled to 45^0C added and mixed thoroughly. Plates were allowed to set and incubated at 37^0C for 24 hours. Each plate was then examined for growth of the test organism..



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Certificate number: 05h.099h.KAE

3) Hygienic Handrub procedure.

Each of the 12 subjects was asked to pour 3ml of propan-2-ol into the cupped hands and rub vigorously for 30s onto the skin up to the wrists in accordance with the standard handrub procedure. This comprises five strokes backwards and forwards palm to palm, right palm over left dorsum and left palm over right dorsum, palm to palm with fingers interlaced, back of fingers to opposing palms with fingers interlocked, rotational rubbing of right thumb clasped in left palm and left thumb clasped in right palm, rotational rubbing with clasped fingers of right hand in palm of left hand and clasped fingers of left hand in right palm. Repeat with a further 3ml propan-2-ol to give a total rubbing time of 60s. After 60 seconds the hands are rinsed under running tap water for 5 seconds, excess water is shaken off.

4) Handrub procedure with test product (P).

The above procedure was repeated exactly using the product Citrox Hand Gel in place of propan-2-ol.

5) Post-values.

Immediately after rinsing the 12 subjects were asked to rub the fingertips on the base of a petri dish containing 10ml of MRD with neutraliser for 1 minute using a separate petri dish for each hand. Then 1ml of each of the undiluted sample fluids was placed in a petri dish and covered with 15ml of TSA mixed thoroughly and allowed to set. Plates were then incubated overnight at 37^{0}C and examined for growth of the test organism.

6) Calculation.

The number of colony forming units (CFU) per plate for each dilution was recorded and the number of cfu's per ml of sample fluid calculated. For both reference and test procedure the log counts from right and left hands of each subject were averaged separately for pre-values and post-values.

From the difference between this individual combined log pre-value and the log post-value a log reduction factor is established for each subject. Then the two arithmetic means of all individual log reduction factors are calculated for both the reference and the test procedure. For **Citrox Hand Gel** to pass the criteria of EN 1500 the mean log reduction factor obtained must not be significantly smaller than that obtained for the alcohol rub. Test of significance of log reduction factors of P against R is carried out using the Wilcoxon matched pairs signed ranks test.

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Microbiological Activity

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Certificate number: 05h.099h.KAE

Results:

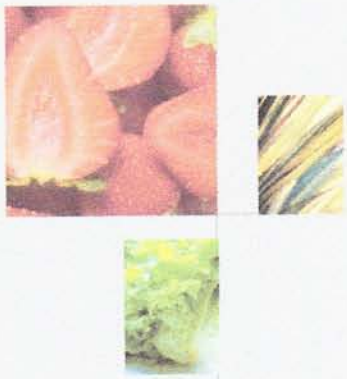
Reference Handwash Procedure (R) Handwash with Citrox Hand Gel (P)

Subject	Log x	Log y	Log z	Log x	Log y	Log z
1	6.41	0	6.41	6.56	1.70	4.86
2	6.49	0	6.49	6.46	1.90	4.56
3	6.29	1.48	4.81	6.59	1.60	4.99
4	6.42	1.30	5.12	6.63	1.48	5.15
5	6.50	0	6.50	6.38	0	6.38
6	6.27	0	6.27	6.44	1.60	4.84
7	6.22	0	6.22	6.51	1.84	4.67
8	6.38	0	6.38	6.55	2.04	4.51
9	6.44	1.70	4.74	6.29	1.30	4.99
10	6.50	0	6.50	6.39	0	6.39
11	6.40	1.48	4.92	6.44	0	6.44
12	6.51	0	6.51	6.48	1.70	4.78
X	6.40	0.50	5.90	6.48	1.26	5.22
N	12	12	12	12	12	12

Where:
Log x = log pre-value.
Log y = log post-value
Log z = log reduction factor
X = overall mean value of log x, log y and log z.
N = number of subjects in each column.

The above data table shows that all criteria of the Test Validation (ref EN 1500:1997 clause 5.8) were satisfied.

The mean log reduction factor for the test material, Citrox Hand Gel, was 5.22 compared to 5.90 for the reference material, propan-2-ol. Therefore, the Wilcoxon matched-pairs signed-ranks test was used to assess whether the mean log reduction factor for Citrox Hand Gel is significantly smaller than that obtained with propan-2-ol.



Independent
Laboratory
Verification
by Abbott Analytical

citROX

Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
Consulting Scientists to the Disinfectant Industry

Certificate number: 05h.099h.KAE

Wilcoxon matched-pairs signed-ranks test:

Log RF derived from Difference Rank of difference

Subject	R	P	R - P	Without sign	With sign
1	6.41	4.86	1.55	8.5	8.5
2	6.49	4.56	1.93	12	12
3	4.81	4.99	-0.18	4	- 4
4	5.12	5.15	-0.03	1	- 1
5	6.50	6.38	0.12	3	3
6	6.27	4.84	1.43	6	6
7	6.22	4.67	1.55	8.5	8.5
8	6.38	4.51	1.87	11	11
9	4.74	4.99	-0.25	5	- 5
10	6.50	6.39	0.11	2	2
11	4.92	6.44	-1.52	7	- 7
12	6.51	4.78	1.73	10	10
Sum of ranks (+)					61 (+)
Sum of ranks (-)					17 (-)

The results of the Wilcoxon matched-pairs signed-ranks test shows that there is no significant difference in the log reduction values at the required level of significance ($p = 0.1$) as the smaller sum of ranks for the test material is 17 compared to minimum value of 12 (ref EN 1500:1997 Table C.5) for 12 subjects.

Therefore, the conclusion is that Citrox Hand Gel, when used as received, conforms to the requirements of EN 1500 and can be considered a suitable hygienic handrub when used under the procedures described above.

D C Watson

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Microbiological Activity

Independent Laboratory Verification

Abbott Analytical
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Samples: Five samples of Handwash received from Citrox Limited 29th September 2003
Certificate Number: 03J.375.GWP
Analysis Required: Persistence on hands over working day using a contact technique by Ciba products.
Samples Tested: 7th October 2003

Protocol: Each product was tested by a different volunteer under carefully supervised conditions. Hands were washed twice with 3ml of product. Activity on the hands was assessed by pressing a finger tip for 30 seconds on agar plates seeded with the test bacteria which in this instance was Staphylococcus aureus. This was repeated at 2 hourly intervals through the working day. Volunteers were advised to go about their normal business during this time. The presence of the product on the fingers was indicated by a reduction in growth of the test organism on the agar plate. The results obtained were scored on a subjective basis with 5+ being total kill of the organism to 1+ being substantial growth of the test organism in the area tested. A count of 0 shows that there was no activity registered.

Test Results

	Time 0	2 hours	4 hours	6 hours
Citrox	+++++	++++	++++	++
Sterisol	+++++	++++	++	0
Purell	+++++	+++	++	0
Pfizer Alcohol based Sanitising gel	+++++	++	+	0

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Attachment F

Fish and Sea Food Industry with Citrox products

1. The use of ProGarda FL003 in ice production for the Decontamination of Fish
   - Storage
   - Transportation
2. Trial Protocols
3. Toxicity Data.
   The objective of this study is to assess the Acute toxicity of ProGarda 14FP on fish (Brachydanio rerio). In the present toxicity tests the fish were exposed to various concentrations of the test substance under defined conditions according to OECD 203 (1992). Not toxic to fish.

F 1

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THE USE OF PROGARDA FL003 IN ICE PRODUCTION FOR THE DECONTAMINATION OF FISH

- STORAGE
- TRANSPORTATION

Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

TECHNICAL REPORT 1947

The Use of ProGarda FL003 in Ice Water to Extend the Shelf Life of Fish Products

1. Introduction.
Whole and partly processed fish products (e.g.) fillets, are generally packed in ice, or held in ice water, to protect them from deterioration during processing, storage and transportation. There is extensive evidence from large scale field trials that the addition of ProGarda FL003 to the water from which ice and ice water is manufactured gives significant extension to the shelf life of the various fish products.

The addition rate for the ProGarda FL003 is recommended at 0.5%. The addition of ProGarda FL003 to the feed water results in a slight pale amber opalescent tint to the ice produced. This causes no discoloration whatever of the produce and no adverse organoleptic properties.

This note describes the layout and functioning of a system for the fully controlled addition of ProGarda FL003 to the feed water entering the ice making machine.

2. Ice Making Machines.
Ice making machines vary widely in their production capacity, and in the form of the ice which they produce. A typical fish processing plant will require some 20 tonnes of ice in 24 hours, and the water feed rate will therefore be of the order of 0.83 cubic meters per hour, or approximately 14 liters per minute.

Note: - ProGarda FL003 is effective on all forms of ice produced.

3. The Feed/Dosing System.
Please refer to Fig (1) appended.
Water is taken from the town mains or other suitable source, and passes through the "Dosatron" pump, P1. This pump is driven by the pressure of the feed water, and delivered at a dosing rate directly proportional to the feed water throughput. The advantage of this type of pump is its inherent simplicity and reliability, coupled with the fact that no electrical supply is required, there are no power supply costs, and no switchgear/wiring installation costs are incurred. The pump is readily and quickly disassembled for cleaning when necessary.

Experiments have shown that the electrical conductivity of the feed water after the addition of the ProGarda FL003 is proportional to the ProGarda FL003 dosage, and this can provide a convenient method of quality control where required.
Refer to Fig (3) appended.

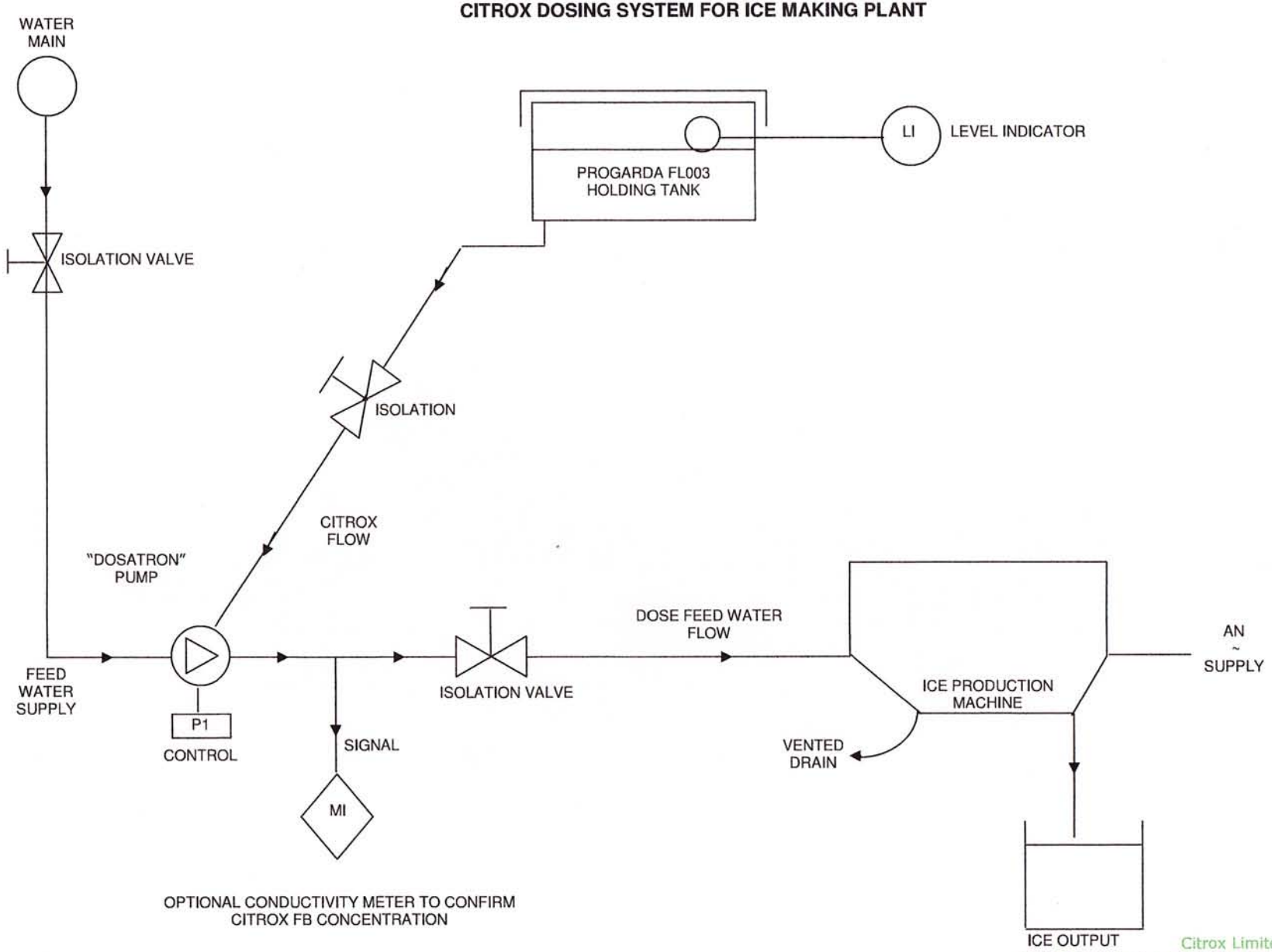
4. Citrox Usage.
At 0.5% dosage rate, and an output of 20 tonnes of ice per 24 hours, the daily usage of ProGarda 14W amounts to 40 liters.

5. Micro Analysis of Fish Produce.

Refer to Fig (2) appended.
The effectiveness of ProGarda FL002 on extending the shelf life of salmon fillets stored in flaked ice. The results shows control of Listeria levels over a 96 hour period.

Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

CITROX DOSING SYSTEM FOR ICE MAKING PLANT



OPTIONAL CONDUCTIVITY METER TO CONFIRM CITROX FB CONCENTRATION

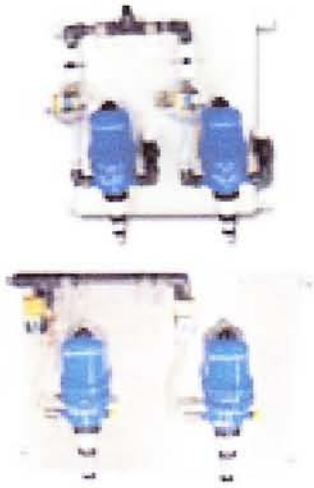
Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

Dosatron DI.1500 Specifications

Irrigation
Brewery
Livestock
Soluble Oils
Timber Treatment
Printing
Water Treatment
Vehicle Washing
Food, Beverage & Hygiene
Odour Control

Dosage Rate	0.07% - 0.2%
Flow Rate	10 - 2500 Ltr/Hr
Operating Pressure	0.3Bar - 6Bar (4.3 - 85 psi)
Pipe connection size	$^3/_4$" BSP male
Max Temperature	40^0C maximum
Unit Size	500 x 210 x 190 mm
Weight	2kg
Also available with PVDF body and/or external injection	

Hingerose Ltd
5 Ryder Court, Saxon Way East, Corby, Northants, NN18 9NX
Tel: **01536 461441** Fax: **01536 46100** E-Mail: **info@hingerose.co.uk**



Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

Cleaning Sanitising of Transportation Containers
The Use of ProSino 14TP and ProSino 14WPS2

The Problem

The cleaning and decontamination for the transportation of food stuffs is a very important element in the prevention of cross contamination from one load to another.

The cleaning of transport containers forms only one element of the problem. Cleaning is obviously a very important factor, as it gives the opportunity to not only eliminate gross debris but also to break down and dissolve / disperse organic film forming materials, including biofilm.

The second element in the cleaning process is the sanitising and elimination of pathogens.

The Solution

The use of the Citrox cleaning and sanitising products offers a truly holistic solution to the problem.

The use of ProSino 14TP as a primary cleaner / sanitiser and ProSino 14WPS2 as a terminal rinse sanitiser, offers a safe and reliable solution to the problem and is not specific to any given foodstuff (vegetables, fruit, meat, poultry and fish).

The Products

ProSino 14TP and 14WPS2 Plus (features and benefits)

ProSino 14TP

- Non corrosive
- Low foam
- Antibacterial
-

ProSino 14WPS2

- Safe to use on produce
- Effective against gram +ve, gram -ve bacteria, yeasts, moulds, fungi and viruses
- Conforms to organic farming EU regulations
- Manufactured from completely natural and renewable resources
- Effective in the presence of organic matter
- Breaks down biofilm
- Conforms to BSEN 1276 European suspension (test guarantees 5-log reduction)
- Contains a unique natural biocide
- Above 98% biodegradable

Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

Range of Pathogens ProGarda 14TP and 14WPS2 are Effective Against

Micro-bacteria and fungi

Alternaria spp	
Aspergillus flavus	
Aspergillus niger	
Aspergillus oizae	
Aspergillus terreus	
Campylobacter Jejuni	
Candida albicans	
Chaetomium golbosum	
Cholera (1)	
Dipiodia natalensis	
Escherichia coli	NCTC 10418 and 0157 variant
F. sp. Tuberosa	
Fuasarium oxysporum	
Fuasarium sambucinum	
Geotrichum candidum	
Klebsiella pneumonia	
Lactobacillus pentoaceticus	
Legionella pneumophila	
Listeria monocytogenes	
MRSA (clinical strain)	
Mycobacterium fortutium	NCTC 8573 for indication of tuberculocidal activity
Penicillium digitatum	
Penicillium funiculosum	
Penicillium italicum	
Penicillium roqueforti	
Penicillium sp.	
Phomopsis ortl	
Proteus vulgaris	
Pseudomonas aeruginosa	
Pullularia pullulans	
Salmonella chloreraesuis	
Salmonella typhimurium	
Scerotinia laxa	
Staphylococcus aureus	
Staphylococcus pyogenes	
Staphylococcus sp.	
Streptococcus faecalis	
Trichophyton interdigitale Typhus	

Viruses

General Orders	DEFRA Approval
SARS	
Rhino virus	
Human Influenza type B	

Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

Suggested Protocol

1) Inspect the containers and assess the state of the containers (walls, floors, roof and doors)

Assess - Levels of gross debris
- Basic cleanliness of surfaces

On the basis of the assessment follow the following protocol should be applied.

1.1 Pre-cleaning

Clean off all loose gross debris with brush or green pad - brush out debris to a collection bin or bags for disposal

2) Cleaning / primary sanitising

Clean all surfaces in the container using ProSino 14TP a pressure washer.
(Select concentration 3 - 7% depending upon the degree of soiling - usually 5% is an optimal concentration).

ProSino 14TP when applied at its in use concentration produces a stable foam which allows a contact time of between 10 - 15 minutes. The contact time is essential for the dissolution / dispersion of organic matter (including oils, fats and greases) and primary sanitising to occur.

The technique of application is to work from the back to the front of the container, applying evenly the foam to all surfaces. Finally foam should be applied to the doors including the internal surfaces which form a seal with the framework of the containers.

After allowing a contact time of between 10 - 15 minutes rinse off all surfaces following the above procedure (i.e.) work from back of the container to the front of the container - including doors and door frames.

The rinse of material should be directed to drain (there are no health and safety issues associated with disposal).

3) Terminal sanitising

After rinsing the last stage of decontamination can be completed by misting or fogging a 0.5% aqueous solution of ProSino 14WPS2 onto all the internal surfaces of the container. Again the misting or fogging should be carried out by working from the back to the front of the container.

Note: - The product should not be rinsed off as it is non toxic, non food tainting, hypoallergenic and will give residual protection for a period of up to 4 days.

Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

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TRIAL PROTOCOLS

Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

PROTOCOL – FISH TESTING

READY TO USE PRODUCT
(0.5% PROGARDA 14FP SOLUTION)

1. Rinse selected produce in cold water

2. Segregate into test produce and control produce – 3 sets of each

3. Spray apply ProGarda 14FP onto the test produce from the trigger pack ensuring complete coverage of the produce. Do not rinse off.

4. Take swabs from both the control produce and ProGarda 14FP test produce and measure TVC levels.

5. Take swabs every 24 hours thereafter for a further 5-day period and repeat TVC micro evaluation.

The results will show against the control the degree of protection the ProGarda 14FP gives against pathogens and pathogen re-growth.

NOTE.
The selection of a fatty fish such as Mackerel or Herring will show the most dramatic effects – as shelf life on these varieties is very short.

Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

PROTOCOL – FISH TESTING

PROGARDA FL003 IN ICE WATER

To be used with potable/process water only i.e. no other additive must be added.

1. Add 0.5% of ProGarda FL003 to the water prior to passing through the ice making machine.
2. To trial fish, take selected produce and split into two sections.
3. Immerse portion 1 in treated ice. Immerse portion 2 in untreated ice.
4. Take swabs from each and measure TVC levels.
5. Take swabs every 24 hours thereafter for a 5 day period and repeat TVC micro-evaluation.
6. The results will show against the control the degree of protection given by ProGarda FL003.

NOTE.
The selection of a fatty fish such as mackerel or herring will show the most dramatic effects as shelf life on these varieties is very short.

Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

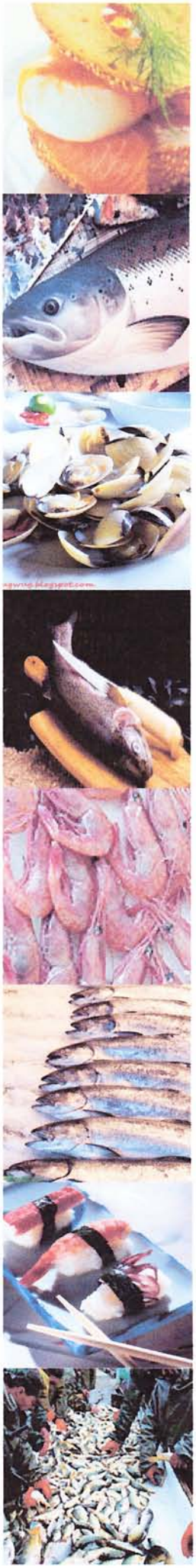
PROTOCOL – SHRIMP TANK DECONTAMINATION

PROSINO 14WPS2

To be used with potable/process water only i.e. no other additive must be added.

1. Drain pond and remove gross debris.
2. Spray walls and floor with clean water (high pressure water jetting).
3. Drain pond.
4. Make up 0.5% solution of ProSino 14WPS2 in water and high pressure jet apply to walls and floor of pond (quantity will depend on output rate of H. P. machine and size of pond).
5. Leave for 8 hours then refill pond with fresh clean water.
6. Allow pond to 'stabilise' for 48 hours then re-stock.
7. Follow feed programme on Aquacite
8. After harvest repeat cycle above.

Citrox Limited
Unit 9 River Court, Brighouse Road
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net



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F 3

TOXICITY DATA

Citrox Limited
Unit 9 River Court, Brighouse Road,
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

EVALUATION OF TOXIC EFFECTS OF PROGARDA 14FP ON FISH

RESULTS OF RANGE FINDING TESTS

Citrox Limited
Unit 9 River Court, Brighouse Road,
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

CONTENTS: -

ANNEX 1 & 2

Citrox Limited
Unit 9 River Court, Brighouse Road,
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

1. Introduction

The objective of this study is to assess the acute toxicity of ProGarda 14FP on fish (Brachydanio rerio). In the present toxicity tests the fish were exposed to various concentrations of the test substances under defined conditions.

The toxicity for these fish was determined over a period of days.

The tests were performed as range finding tests without the calculation of effect parameters. The end point of the tests was the mortality at the end of the tests.
The methods employed were based on OECD guidelines:

* Acute toxicity to fish

Tests with the Zebra fish Brachydanio rerio carried out according to OECD 203 (1992),

This report contains details of the methods, results and raw data of the performed bioassays.

2. Methods

2.1 Preparation of test solutions.

For the preparation on the concentration range for the acute toxicity tests, stock solutions of the test substances were used. These solutions were prepared freshly for the beginning of each test. The stock solutions were prepared by dissolving the test substances in Milli-Q water (Milli-pore, Etten-leur, Netherlands).
(For details see Annex-1)

2.2 Bioassays

In the following sections a description of the test methods is given. For each a more detailed description references should be made to the specific guidelines.

Brachydanio rerio.

The tests with the Zebra fish Brachydanio rerio were carried out according to OECD 203 (1992). The test duration was 96 hours, and after 24, 48, 72 and 96 hours the fish were observed for abnormal behavior and mortality. The fish were kept in quarantine for at least 12 hours before the start of the tests. The tests were performed using 7 fish per test vessel, in single fold. The fish used were 2.0 +/- 1.0 cms in length.

The test temperature 23 degrees Centigrade +/- degree. The light regime was 16 hours light and 8 hours dark. The test vessels were continuously aerated, and the test medium was not refreshed during the tests.

The test concentration range consisted of five concentration in a geometric series: 0.01, 0.10, 1.0, 10.0, and 100.00 mg/l of ProGarda 14FP.

The dilutions were made using DSW, which was also used as control. *

Citrox Limited
Unit 9 River Court, Brighouse Road,
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

The test volumes were 100 ml per vessel (glass). Before testing the following parameters were determined in the highest test concentration and in the control: oxygen saturation, acidity, nitrate ammonium and conductivity. Effect parameters were not determined.

* The dilution water used in the aquatic toxicity tests was Dutch Water Standard. This water is generally accepted as a standard dilution water for acute tests in the Netherlands. The compositions as follows: 200 mg/l calcium chloride, 180 mg/l magnesium sulphate, 100 mg/l sodium carbonate and 20 mg/l potassium carbonate.

2.3 Chemical Analyses.

Chemical analyses were not performed for this range finding test.

3. Results

3.1 Validity criteria and quality of test organisms.

Validity criteria are defined in the guidelines used. These are given in Table (1). All toxicity tests used in the investigations were found to be valid according to these criteria. The quality of the test organisms used in the acute toxicity is periodically tested using reference compounds. The results are checked with the criteria in the guidelines or ring test results. If the data are not valid the organisms will not be used for further testing.

	Criterion	Measured values
Brachydanio rerio Mortality in controls	< 10	0

3.2 Physical and chemical parameters.

During the toxicity tests several physical and chemical parameters (confounding factors) were determined. The confounding factors were determined in the controls and in the highest test concentration used. Throughout the tests the criteria of the OECD guidelines were met.

3.3 Results of acute toxicity tests.

The results of the acute toxicity tests performed with Brachydanio rerio are summarised in Annex 2 attached

Citrox Limited
Unit 9 River Court, Brighouse Road,
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net

4. Literature

OECD 203 (1992) Fish, acute toxicity test. OECD guideline for testing of chemicals. OECD, Paris, France. 203 adopted 17/07/92.

RIZA (2000) Handboek Toxicologie en lozingsvergunningen. RIZA-nota 2000.0007 RIZA. Lelystad.

Annex 1 : Physical and chemical parameters (confounding factors) in the tests with Brachydanio rerio.

	Parameters					
Ref. No.	Time Hrs	O^2 %	pH	NO^2 – mg/l	NH^4 + mg/l	Conductivity (uS/mm)
RIZA (2000) OECD (1992)	-	>60%	5-9	<20	<30	<1500
Control (DSW)	0	110	7.8	<2	0	53
-	96	100	8.0	<2	0	56
-	0	105	8.2	<2	0	53
	96	101	8.2	<2	0.5	54
Citrox 311487						

Annex 2 : Results of toxicity test with Brachydanio rerio

ProGarda 14FP Nominal concentration mg/l or ppm	Time (hours)						
		Survival					
	Number in test	24	48	72	96	Mortality % at end of test	Abnormalities
Control	7	7	7	7	7	0	None
0.01	7	7	7	7	7	0	None
0.10	7	7	7	7	7	0	None
1.00	7	7	7	7	7	0	None
10.0	7	7	7	7	7	0	None
100.0	7	0	0	0	0	100	None

Citrox Limited
Unit 9 River Court, Brighouse Road,
Riverside Park, Middlesbrough, TS2 1RT, UK
Tel. +44 (0) 1642 241777
Fax. +44 (0) 1642 219217
E-mail. info@citrox.net