UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF PREVENTION, PESTICIDES AND TOXIC SUBSTANCES

MEMORANDUM DATE: 07/19/2005

SUBJECT: **Apiguard EZ-3 (Thymol):** Review of Response to Deficiency Letter, Waiver Rationales, and Product Chemistry

DP Bar Code #:316472, EPA File Symbol Number: 79671-R, Decision #341453
MRIDs: 465245-01, 465245-02, 464856-01, administrative material with embedded waivers

FROM: Kent R. Carlson, Ph.D., Biologist /s/ 08/16/05 08-16-05
Biochemical Pesticides Branch
Biopesticide and Pollution Prevention Division (7511C)

THRU: Russell Jones, Senior Biologist /s/ 08/16/05 8/16/2005
Biochemical Pesticides Branch
Biopesticide and Pollution Prevention Division (7511C)

TO: Andrew Bryceland, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticide and Pollution Prevention Division (7511C)

ACTION REQUESTED: Vita (Europe) Limited in c/o Landis International, Inc. has submitted a package of administrative materials for the Section 3 registration of a food use product Apiguard EZ-3. Apiguard EZ-3 (active ingredient thymol) is proposed to be used in bee hives against Varroa mites.

SUMMARY RECOMMENDATIONS:

1) The supplier [redacted] submitted product chemistry information on [redacted] including confidential components and CAS numbers. Discussion regarding its similarity to [redacted] and/or product component information on [redacted] was not, however, submitted as requested (Andersen, 12/29/04; Landis 02/28/05). The current information does not allow bridging of toxicology information generated under the old formulation [redacted] **The registrant must submit discussion as to the physical/chemical/toxicological similarities of [redacted] in order to be considered for bridging.**

2) Minor label amendments must be made. These are stipulated in detail below (and

under **February 28th letter from Vita (Europe) International:**). In the February 28th letter, the registrant also replied to label changes suggested by EPA. The majority of suggested label changes were made by the registrant. Some new or unaddressed changes recommended by EPA are:

1) Agricultural Use Requirements Box:
   line 3; replace "insecticides" with "pesticides"
   line 4; replace "this" with "the"
   line 5; replace "(PPE) and restricted" with "(PPE), restricted"
   line 8; replace "allow worker entry" with "allow any worker to enter" ok
2) General Information subheading:
   line 4-5; delete "and provides a much more efficient control of varroa mite than was previously possible"
3) For 3 kg tubs:
   line 8; replace "nuclei may require" with "nuclei requires"
   line 15; delete "it is reccommended to"
4) For 50 g ready to use aluminum trays:
   line 11; delete "it is recommended to"

In addition, an REI of 4 hours is stipulated on the label without prior justification. Assignment of the REI is based on acute toxicity category unless alternate data is provided to demonstrate a reduced REI is possible. Apiguard EZ-1 has an acute eye irritation Toxicity Category of I, which makes the **REI 48 hours** [40 CFR §156.208 (c)]. **The restricted entry statement on the label must reflect this change in REI.**

3) The registrant has not formally submitted data for the TGAI or identified a registered source of thymol for manufacturing the EP product Apiguard EZ-3. **The registrant must submit physical, chemical, fate, and Tier I toxicology data or waiver rationales for each data requirement required for the TGAI (see Note to RAL below).**

4) **MRIDs: 465245-01, 465245-02; Preliminary Anaylsis of Apiguard EZ-3:** These studies were UNACCEPTABLE, but upgradable pending revision of the report. Specifically, the following clarifications should be made:

1) An expiration date must be provided for each test batch of Apiguard EZ-3.
2) The registrant must explain discrepancies between handwritten batch notes and the final certificates of analyses as reported in Table 1 of the review DER.
3) The registrant must explain why the methodological performance differed from batch protocols (see review DER), and if this new methodology will be used in preparing future batches of Apiguard EZ-3.

BACKGROUND: Apiguard EZ-3 (EPA Reg. No. 79671-R) is comprised of the active ingredient thymol (25.00%), [redacted] This product and its active ingredient have a ***terrestrial food use**, even though use classification suggests nonfood uses may be appropriate* (40 CFR §158, App. A, 10. Noncrop,

wide area, and general indoor/outdoor treatments; Directed pest control to pests' nests, etc, and for traps; Diseased beehives). Apiguard EZ-3, a gel in trays or sachets, is intended to be applied indirectly to beehives to control and suppress parasitic Varroa mites. Hives are to be treated with 50g of Apiguard EZ-3 gel during periods when there is no honey flow and the temperature is between 60 and 105°F. Fifty grams of Apiguard EZ-3 gel (12.5% thymol) in a tray holder can be set twice in the hive at a two week interval per season during the spring and late summer, depending on the hives' parasitic burden.

Thymol currently has an time-limited exemption from the requirement of a tolerance (§180.1240) in or on honeycomb in connection with use of the pesticide under section 18 emergency exemptions granted by EPA. Time limited exemptions from the requirement of a tolerance for residues of thymol expired on June 30, 2005.

REGULATORY HISTORY:

On **July 17th of 2003**, the registrant submitted an application for registration of Apiguard, administrative materials, physical and chemical properties, storage stability, product identity etc, acute oral, acute dermal, acute inhalation, eye irritation, skin irritation, contact hypersensitivity, residue studies and summaries (MRIDs 46043501 through -13), and a FQPA supplemental information document (MRID 46043514, diskette).

On **August 7th of 2003**, the registrant had a pre-registration meeting which reviewed the product and already submitted data.

On **September 9th of 2003**, EPA performed a science data screen on Apiguard.

On **September 18th of 2003**, the registrant submitted an application for registration and 4 publicly available scientific articles (mutagenicity, genotoxicity, subacute and chronic toxicity, antioxidant actions; MRIDs 46282801 through-04). These studies were briefly reviewed on **June 22nd of 2004**.

On **October 24th of 2003**, a deficiency letter regarding the application was sent to the registrant. This letter identified one component of concern, [redacted] a list 2 inert), which was listed as an inert on the CSF.

On **February 13th of 2004**, the registrant reapplied for Apiguard using a new formulation which did not contain [redacted] New ingredients, [redacted] were identified in this reapplication. Also submitted were draft labeling, and information on formulation and manufacturing processes (MRID 46198201).

On **April 21st of 2004**, the registrant submitted an application for registration and a magnitude of residue study (MRID 46254301).

On **May 13th of 2004**, EPA sent a letter detailing fee payment according to the PRIA action code of B63.

In **May-December of 2004**, EPA performed secondary (final) review of MRIDs containing information on product identity and composition, description of beginning materials and formulation process, discussion of the formation of impurities, preliminary analysis, certified limits, enforcement analytical methods, and physical and chemical characteristics (MRIDs 46043501, 46043502, 46043503, 46198201), acute oral toxicity, acute dermal toxicity, acute inhalation toxicity, primary eye irritation, primary dermal irritation, and skin sensitization (MRIDs 46043504 through -09), and magnitude of the residue in honey and beeswax (MRIDs 46254301, 46043510 through -13).

Inert ingredient information may be entitled to confidential treatment

On **October 9th of 2004**, EPA performed an abbreviated dietary exposure and risk assessment using residues of thymol in honey. EPA concluded that dietary exposure to thymol from non-pesticidal sources (cooking, chewing gum, candy, cakes, ice cream) was significantly higher than that from honey when using maximum thymol residue estimates from a magnitude of the residue study. Pesticidal uses of thymol, therefore, would not represent a significant increase in dietary exposure to total thymol.

On **December 29th of 2004**, EPA sent the registrant a letter detailing review of product identity, beginning materials, formulation process, formation of impurities, preliminary analysis, physical and chemical properties, mammalian toxicity, and magnitude of the residue data. Outstanding data requirements of avian acute oral (850.2100), avian dietary (850.2200), freshwater fish LC50 (850.1075), and freshwater invertebrate LC50 (850.1010) were also identified. Extensive label modifications were also detailed in this review. Identified study deficiencies included the lack of chemical information on [redacted] and the inability to bridge toxicology data generated with [redacted] information in this letter was summarized from a study review memo dated **December 21st of 2004**.

On **February 28th 2005**, the registrant sent a letter containing an application for registration, an updated CSF and data matrix, amended labeling, and three volumes on preliminary analysis and chemistry of thymol or Apiguard EZ-3. Specifically, a 5-batch analysis was submitted for the TGAI thymol, a 2-batch analysis for the EP Apiguard EZ-3, notice of a request for [redacted] to submit to the agency information and discussion on the chemical and physical similarities of [redacted] and waiver rationales for avian acute oral (850.2100), avian dietary (850.2200), freshwater fish LC50 (850.1075), and freshwater invertebrate LC50 (850.1010).

On **March 15th of 2005**, a confidential statement of ingredients for [redacted] was submitted by the supplier, [redacted] No information or discussion regarding [redacted] similarities was submitted.

On **April 21st 2005**, a renegotiated PRIA due date (December 15th , 2005) was finalized in letter form by EPA and the registrant.

On **April 27th 2005**, a Notice of Filing a Pesticide Petition to Establish a Tolerance was filed for the acaricide thymol (CAS No. 89-83-8) by Vita (Europe) Limited. The tolerance petition stated use in late summer and/or spring to reduce Varroa mite densities. Other information discussed included non-pesticidal uses, magnitude of the residue data, mammalian toxiciology data, information on aggregate and cumulative exposure and international tolerances (Swiss MRL based on taste at 0.8 mg/kg, WHO "safe" up to 50 mg/kg).

SUMMARIES AND CONCLUSIONS: The following commentary reviews the data waiver rationales that were embedded within administrative materials. It also briefly summarizes additional information collected by the EPA reviewer.

February 28th letter from Vita (Europe) International: Waiver requests for avian acute oral (850.2100), avian dietary (850.2200), freshwater fish LC50 (850.1075), and freshwater invertebrate LC50 (850.1010) toxicity testing discussed the obligate placement of the EP inside bee hive boxes and the reasonable expectation that no avian or aquatic organism would be exposed in such an environment.

Inert ingredient information may be entitled to confidential treatment
Product ingredient source information may be entitled to confidential treatment

EPA concurs with this expectation in situations where apiaries are maintained agriculturally. Placement of the product in cases of "wild nesting" (nesting not in hive boxes) may not, however, preclude exposure to avian or aquatic organisms since these nests (tree hollows) are not weather resistant and are accessible to a wide variety of animals. The incidence of wild nesting is presumed to be low in agricultural apiary settings designed to produce honey. Aquatic toxicity information retrieved by EPA from the EPA ECOTOX database (on *Asellus intermedius*, *Daphnia magna*, *Dugesia tigrina*, *Gammarus fasciatus*, *Helisoma trivolvis*, *Lumbriculus variegatus*, *Pimephales promelas*, Lymnaeidae, *Oncorhynchus kisutch*, *Onchorhynchus mykiss*, *Onchorhynchus tshawytscha*, and *Ptychocheilus oregonensis* supplements waiver rationales. The waiver rationales in combination with ECOTOX data are therefore acceptable and support their respective data requirements.

In this letter, the registrant also replied to label changes suggested by EPA. The majority of suggested label changes were made by the registrant. Some new or unaddressed changes recommended by EPA can be seen in the "SUMMARY RECOMMENDATIONS" above.

MRID: 464856-01; Preliminary Analysis of Thymol: Thymol is the active ingredient in Apiguard EZ-3, an end-use product (EP) applied indirectly as a gel in trays to beehives to control and suppress parasitic varroa mites. Content analyses of colorless to white crystalline thymol ranged from 99.9 to 100.0%. The average melting point of thymol was 50.1°C and no organic volatile impurities were detected.

MRIDs: 465245-01, 465245-02; Preliminary Anaylsis of Apiguard EZ-3: Apiguard EZ-3 (EPA Reg. No. 79671-R) is an end-use product (EP) applied indirectly as a gel in trays to beehives to control and suppress parasitic varroa mites. The CSF indicates that the active ingredient is 25.00% thymol. [redacted] are on the CSF as inert [redacted] A certificate of analysis for 2 batches of EP using inerts [redacted] No impurities of toxicological significance associated with the active ingredient are expected. Packaging specifications for trays (51g nominal), sachets (13 and 25g), and tubs (3.1 kg) were given. Discrepancies were noted between the handwritten batch notes and certificates of analysis and between the reported method and the performed analysis.

Data Table: Data requirements and relevant results for the EP Apiguard EZ-3 can be seen in Table 1.

TABLE 1. Physical and Chemical Properties for EP (Apiguard or Apiguard EZ-3)

Guideline Reference No./Property	Description of Result	Methods
Physical and Chemical Requirements		
Color	**Opalescent, colorless to pink.** *data matrix cited MRID 46043502*	Visual inspection, 830.6302

Inert ingredient information may be entitled to confidential treatment

Guideline Reference No./Property	Description of Result	Methods
Physical State	**Gel**, *data matrix cited MRID 46043502*, **Aqueous granular gel**, *cited in MSDS dated November 15th, 2001*	Visual inspection, 830.6303
Odor	**Strongly aromatic of thyme**, *data matrix cited MRID 46043502*	Olfactory inspection, 830.6304
Stability	*Not required for EP, data matrix cited MRID 46043502*, **stable-but avoid heating**, *cited in MSDS dated November 15th, 2001*	*Not required for EP*
Oxidation/Reduction:	*None of the components is an oxidizer or reducer*	*Not appropriate for this EP*
Flammability	*No combustible liquids in formulation;* **flashpoint >100°C**, *cited in MSDS dated November 15th, 2001*	*Not appropriate for this EP*
Explodability	*Not potentially explosive*	*Not appropriate for this EP*
Storage Stability	*data matrix cited MRID 46043503*, **maximum storage temperature of 30°C, shelf-life 24 months for 50g tray, 18 months for 3 kg tub**, *cited in MSDS dated November 15th, 2001*	830.6317
Miscibility	*Not an emulsifiable liquid to be diluted with petroleum solvents*	*Not appropriate for this EP*
Corrosion Characteristics	*data matrix cited MRID 46043503*	830.6320
Dielectric Breakdown Voltage	*Not to be used around electrical equipment*	*Not appropriate for this EP*
pH	**6.5 (EPA: 6.8 - 6.9)**, *data matrix cited MRID 46043502*	830.7000
Viscosity	**77,800 (EPA: 72,960 - 76,000) cps**, *cited MRID 46043502*; **95,000-125,000 cps @ 25°C**, *cited in MSDS dated November 15th, 2001*	830.7100
Melting Range	*Not required for EP*	*Not required for EP*
Boiling Range	*Not required for EP*	*Not required for EP*
Bulk Density	Specific gravity: **1.01 (EPA:1.009 - 1.010) g/cm³**, *data matrix cited MRID 46043502*; **1.005-1.025 g/cm³**, *cited in MSDS dated November 15th, 2001*	830.7300
Dissociation Constant	*Not required for EP*	*Not required for EP*
Partition Coefficient	*Not required for EP*	*Not required for EP*
Water Solubility	*Not required for EP, data matrix cited MRID 46043502*; **77% (w/w)**, *cited in MSDS dated November 15th, 2001*	*Not required for EP*
Vapor Pressure	*Not required for EP*; **$2.25*10^5$ Pa @ 20°C**, *cited in MSDS dated November 15th, 2001*	*Not required for EP*
Product Analysis Requirements		
Product Identity	*data matrix cited MRID 46043501, 46198201*	830.1550

Guideline Reference No./Property	Description of Result	Methods
Manufacturing Process	*data matrix cited MRID 46198201*	830.1620
Discussion of Formation of Unintentional Ingredients	*data matrix cited MRID 46198201*	830.1670
Analysis of Samples	*data matrix cited MRID 46043503*	830.1700
Certification of Limits	*data matrix cited MRID 46198201, 46043503*	830.1750
Analytical Methods	*data matrix cited MRID 46043501*	830.1800
[1] Residue Data Requirements		
Magnitude of the Residue	**Maximum thymol residues of 1.5 ppm in honey and 20.59 ppm in wax 30 days after treatment finish;** MRIDs 46043510 through -13	860.1500
[1] Toxicology Data Requirements		
Acute Oral Toxicity	**Oral LD50>2000mg/kg, Toxicity Category III;** *MRID 46043504*	870.1100
Acute Dermal Toxicity	**Dermal LD50>2000mg/kg, Toxicity Category III;** *MRID 460430505*	870.1200
Acute Inhalation Toxicity	**Unable to produce a suitable atmosphere for inhalation testing of gel product;** *MRID 46043506*	870.1300
Primary Eye Irritation	**Corneal opacity >28 days, resolving iritis and conjunctival irritation, Toxicity Category I;** *MRID 46043507*	870.2400
Primary Dermal Irritation	**Slight to well defined erythema and slight to moderate edema clearing by day 14, Toxicity Category IV;** *MRID 46043508*	870.2500
Hypersensitivity	**Not a sensitizer;** *MRID 46043509*	870.2600
Hypersensitivity Incidents	**Required for EP**	Incidents must be reported
Genotoxicity	*Not required for EP*	*Not required for EP*
Immune Response	*Not required for EP*	*Not required for EP*
90-day Feeding (1 spp)	*Not required for EP*	*Not required for EP*
90-day Dermal (1 spp)	*Not required for EP*	*Not required for EP*
90-day Inhalation (1 spp)	*Not required for EP*	*Not required for EP*
Teratogenicity (1 spp)	*Not required for EP*	*Not required for EP*
Nontarget Organism, Fate, and Expression Requirements		
Avian Acute Oral	*Not required for EP*	*Not required for EP*
Avian Dietary	*Not required for EP*	*Not required for EP*
Freshwater Fish LC50	*Not required for EP*	*Not required for EP*
Freshwater Invertebrate LC50	*Not required for EP*	*Not required for EP*
Nontarget Plant Studies	*Not required for EP*	*Not required for EP*
Nontarget Insect Studies	*Not required for EP*	*Not required for EP*

[1] Testing was done with previous formulations of Apiguard (using [redacted]

Note to RAL: The following information has been gleaned from submissions, online sources (MSDS), and peer-reviewed publications. The registrant has not presented a registered source for thymol on the CSF, provided required data for the TGAI, or has only incidentally provided waivers for this product (TGAI = thymol).

1) Waiver rationales presented on February 28th of 2005 for avian acute oral (850.2100), avian dietary (850.2200), freshwater fish LC50 (850.1075), and freshwater invertebrate LC50 (850.1010) toxicity testing in conjunction with data retrieved by EPA from ECOTOX (Carlson, 2005) and data submitted previously (MSDS dated November 15th, 2001), **support their respective data requirements for the TGAI.**

2) Genotoxicity and mutagenicity studies submitted on September 18th of 2003 (MRIDs 46282801 and -02), presumably as waiver rationales for genotoxicity (870.5000) and other peer-reviewed publications retrieved by EPA, **support the respective data requirement for the TGAI.**

3) Subacute and chronic studies submitted on September 18th of 2003 (MRIDs 46282803 and -04), presumably as waiver rationales for subchronic toxicity (870.3100), **do not support the respective data requirement for the TGAI.**

4) Data or waiver rationales for acute dermal toxicity (870.1200), acute inhalation toxicity (870.1300), dermal irritation(870.2500), eye irritation (870.2400), hypersensitivity (870.2600), immune response (870.7800), 90-day feeding (870.3100), 90-day dermal toxicity (870.3250), 90-day inhalation toxicity (870.3465), and teratogenicity (870.3700) have not been submitted by the registrant for the TGAI thymol. **The registrant must submit physical, chemical, fate, and Tier I toxicology data or waiver rationales for each data requirement required for the TGAI.**

TABLE 2. Data Requirements for TGAI (Thymol; CAS# 89-83-8; MW 150.24)

Guideline Reference No./Property	Description of Result	Methods
Physical and Chemical Requirements		
Color	**Colorless to white**, *cited MRID 46485601*	Visual inspection 830.6302
Physical State	**Crystalline**, *cited MRID 46485601*	Visual inspection 830.6303
Odor	**Thyme-like odor**, *cited in NOAA MSDS*	830.6304
Stability	**Stable under ordinary conditions of use and storage**, *cited in NOAA MSDS*	830.6313
Oxidation/Reduction:	*None of the components is an oxidizer or reducer*	*Not appropriate for this TGAI*

Guideline Reference No./Property	Description of Result	Methods
Flammability	*Not required for TGAI;* **Flammable**, *cited in NIH Toxnet*	*Not required for TGAI*
Explodability	*Not required for TGAI*	*Not required for TGAI*
Storage Stability	*Not required for TGAI*	*Not required for TGAI*
Miscibility	*Not required for TGAI*	*Not required for TGAI*
Corrosion Characteristics	*Not required for TGAI*	*Not required for TGAI*
Dielectric Breakdown Voltage	*Not required for TGAI*	*Not required for TGAI*
pH	**Not supplied by registrant**	830.7000
Viscosity	*Not required for TGAI*	*Not required for TGAI*
Melting Range	**50.1°C**, *cited MRID 46485601*, **49.5°C**, *cited in MSDS dated November 15th, 2001;* **51.5°C**, *cited in National Library of Medicine SIS ChemiID Plus;* **48-51°C**, *cited in NOAA MSDS*	GLC, 830.7200
Boiling Range	*Not a liquid*, **233°C**, *cited in MSDS dated November 15th, 2001 also NOAA MSDS;* **232.5°C**, *cited in National Library of Medicine SIS ChemiID Plus*	*Not appropriate for this TGAI*, 830.7220
Bulk Density	**Specific gravity - 0.97 @ 25°C/4°C**, *cited in NOAA MSDS*	830.7300
Dissociation Constant	**10.62 @ 20°C**, *cited in National Library of Medicine SIS ChemiID Plus*	830.7370
Partition Coefficient	**Log Kow = 3.30 @ 20°C**, *cited in MSDS dated November 15th, 2001;* **3.3**, *cited in National Library of Medicine SIS ChemiID Plus*	830.7550
Water Solubility	**900 mg/L water @ 20°C**, *cited in National Library of Medicine SIS ChemiID Plus;* **0.1g/100g water @ 25°C**, *cited in NOAA MSDS*	830.7840
Vapor Pressure	**0.0022 mmHg @ 25°C**, *cited in National Library of Medicine SIS ChemiID Plus;* **1 mm Hg @ 64°C**, *cited in NOAA MSDS;* **12.7 Pa @ 40°C**, *cited in NIH Toxnet*	830.7950
Product Analysis Requirements		
Product Identity	*Not required for TGAI*	*Not required for TGAI*
Manufacturing Process	**Not supplied by registrant**	830.1620
Discussion of Formation of Unintentional Ingredients	**Not supplied by registrant**	830.1670
Analysis of Samples	**Not supplied by registrant**	830.1700
Certification of Limits	*Not required for TGAI*	*Not required for TGAI*
Analytical Methods	*Not required for TGAI*	*Not required for TGAI*
Residue Data Requirements		

Guideline Reference No./Property	Description of Result	Methods
Magnitude of the Residue	*Not required for TGAI*	*Not required for TGAI*
Toxicology Data Requirements		
Acute Oral Toxicity	**Oral LD50=640, 750, 880, 980 mg/kg in mouse, rabbit, guinea pig, and rat, respectively, Toxicity Category III**, *cited in National Library of Medicine SIS ChemID Plus and NIOSH RTECS;* **Oral LD50 of chemical isomer of thymol, carvacrol=810 mg/kg in rat, Toxicity Category III**, *cited in Stammati et al., 1999*	870.1100
Acute Dermal Toxicity	**Not supplied by registrant**	870.1200
Acute Inhalation Toxicity	**Not supplied by registrant**	870.1300
Primary Eye Irritation	*Not required for TGAI* ; **Corrosive**, *cited in NIOSH RTECS*	*Not required for TGAI*
Primary Dermal Irritation	*Not required for TGAI* ; **Corrosive**, *cited in NIOSH RTECS*	*Not required for TGAI*
Hypersensitivity	*Not required for TGAI* ; **Not a sensitizer**, *cited in NIOSH RTECS;* **Sensitizer** - Thyme dust or Thymol, *cited in Lorenzi et al., 1995; Fisher, 1989; Spiewak et al., 2001; Basch et al., 2004.*	*Not required for TGAI*
Hypersensitivity Incidents	*Not required for TGAI*	*Not required for TGAI*
Genotoxicity	**Negative** - Ames test in TA97 w/wout S-9 activation, in TA98 w/wout S-9(X2), in TA100 w/wout S-9 (X2), in TA1535 w/wout S-9, in TA1537 w/wout S-9, *cited in Azizan and Blevins, 1995;* **Negative** - for chromosome aberrations in *Allium cepa;* **Negative** - for DNA damaging activity in *Bacillus subtilis rec-assay, cited in Zani et al., 1990;* **Marginally positive** - Ames test in TA98 w/wout S-9, in TA100 w/wout S-9, *cited in Stammati et al., 1999;* **Negative** - for SCE in chemical isomer of thymol, carvacrol, *cited in Evrim et al., 2003.*	870.5000, GC
Immune Response	**Not supplied by registrant**	880.3550
90-day Feeding (1 spp)	**Not supplied by registrant**	870.3100
90-day Dermal (1 spp)	**Not supplied by registrant**	870.3250
90-day Inhalation (1 spp)	**Not supplied by registrant**	870.3465
Teratogenicity (1 spp)	**Not supplied by registrant**	870.3700
Nontarget Organism, Fate, and Expression Requirements		
Avian Acute Oral	No anticipated exposure (Feb 28, 2005 admin materials)	Waiver rationale, 850.2100
Avian Dietary	No anticipated exposure (Feb 28, 2005 admin materials)	Waiver rationale, 850.2200

[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
[illegible]	[illegible]	[illegible]
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and Chemical Toxicology. 37:813-823.

Zani, F., Massimo, G., Benvenuti, S., Bianchi, A., Albasini, A., Melegari, M., Vampa, G., Bellotti, A., and P. Mazza. 1990. Studies on the genotoxic properties of essential oils with Bacillus subtilis rec-assay and Salmonella/microsome reversion assay. Planta Med. 57:237-241.

DATA EVALUATION RECORD

THYMOL
(APIGUARD)

STUDY TYPE: Magnitude of Residues in Honey and Beeswax (OPPTS 860.1500)

MRID 46254301

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 04-67

Primary Reviewer:
Sylvia Milanez, Ph.D., D.A.B.T. — Signature:_____ Date: _____

Secondary Reviewers:
Eric B. Lewis, M.S. — Signature:_____ Date: _____

Robert H. Ross, M.S., Group Leader — Signature:_____ Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. — Signature:_____ Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Magnitude of Residue in Honey and Beeswax (OPPTS 860.1500)
MRID NO:	46254301
DP BARCODE:	DP 306002 / 79671-R
CASE NO:	Not provided
SUBMISSION NO:	Not provided
TEST MATERIAL:	Thymol
STUDY NO:	44921A001
SPONSOR:	VITA (Europe) Ltd., 21/23 Wote Street, Basingstoke, Hants RG21 7 NE, United Kingdom
TESTING FACILITY:	Landis International, Inc., P.O. Box 5126, 3185 Madison Highway, Valdosta, GA 31603-5126
TITLE OF REPORT:	Magnitude of the residue of thymol in honey and beeswax following treatment of beehives with Apiguard.
AUTHOR:	Hattermann, D.R.
STUDY COMPLETED:	April 21, 2004
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed GLP statement was provided. The study was GLP compliant with some minor exceptions.

STUDY SUMMARY: Thymol levels were determined in honey and beeswax samples from field trials in MD, CA, and TX. Samples were collected 30 days and 60 days after treatment ended (0 to 30-day and 30 to 60-day samples, respectively). Samples were kept frozen for ≤35 days before extraction and storage stability studies were not conducted. The analytical method (GC-MSD) limit of quantitation (LOQ) was 0.01 ppm for honey and 0.10 ppm for wax. Honey residues were 0.019-2.59 ppm for the day 30 samples and <0.010-0.96 ppm for the day 60 samples. Beeswax residues were 0.11-97.6 ppm in the day 30 samples and <0.10-18.7 ppm in the day 60 samples. Residue levels were greatest at the MD site, possibly due to its combination of lower temperature and greater humidity. Residue levels declined with time, and no thymol residues were detected in untreated controls. The method validation residue recoveries were outside the acceptable range of 70-120% at the LOQ for honey (124-132% at 0.010 ppm) and beeswax (134-147% at 0.10 ppm), but all concurrent recovery samples were acceptable. The method was judged to have accurately determined thymol residues in honey and beeswax samples at the concentrations tested.

CLASSIFICATION: ACCEPTABLE, upon correction of footnote 2 of Table 2 (on page 22), and upon providing additional details of the preparation of the fortified samples used for method validation and concurrent recovery.

I. MATERIALS AND METHODS

1. Test Material

The test substance was Apiguard 25% gel, lot number 06Y0743, with an expiration date of April 2005. The label percent active ingredient (ai) thymol was 25%. The Apiguard was stored at ambient temperature (35-87°F) and was used within its stated shelf life. Thymol is used in bee hives to control the *Varroa* mite.

2. Test Methods

Field Trials: Field trials were conducted in Harmony, MD, Porterville, CA, and Weslaco, TX. Each site consisted of one control plot containing 4 untreated hives, and one treated plot containing 4 treated hives. The treated and control plots were separated by ≥80 feet at the CA and TX sites, and by ≥18 inches at the MD site. The small separation at the MD site did not affect the study results since control samples had no detectable thymol. Treatment consisted of placing one newly opened tray of 50 g Apiguard gel (25% thymol; for 12.5 g thymol/application) into each hive, and after 14 days replacing the first tray with a similar

second tray. In the MD field trial, ~25% of the material was left over in the two trays when they were removed, whereas in CA and TX, all the material was gone except for ~10% left in two trays at each site. [MD had lower temperatures and/or higher rainfall than CA and TX.] The maximum total application therefore ranged from ~19 - 25 g thymol per season. The second set of thymol trays was removed after 28 or 30 days, at which time new supers were placed in the hives, and honey and wax raw agricultural commodity (RAC) samples were collected ~ 30 and 60 days later and frozen immediately until analysis.

Sample Collection and Handling: Detailed sample collection procedures were provided individually for the TX, MD, and CA trials. At the TX site, honey + wax samples were collected using a clean stainless steel tablespoon from the center two trays of 3 untreated hives and one treated hive. Honey was strained out of the samples using clean nylon stockings and placed in a jar, and the remaining wax was placed in a separate jar. New frames were put in the supers for the 60-day (i.e., second) collection, which followed the same procedure as the first collection except that samples were collected from the center 4 trays from 4 untreated hives and 2 treated hives. Samples (100.9-135.8 g each) were placed on "blue ice" within a few minutes of collection, and into freezers within 2 hours of collection.

At the MD trial, three frames were taken each from the non-treated and treated hives (not stated from how many hives or supers), and driven 5 miles off site so bees would not interfere with sampling. Sections of the comb were cut from the frames and put into a wax press which squeezed out the honey into jars. The remaining wax was placed into separate jars. Samples were frozen within 50 minutes of collection. Control samples were pressed before treated samples, and the press was washed with soap and water and wiped with alcohol between samples. The frames were then returned to the hives, at which time the hives were stocked with fresh feed consisting of pink Kool-Aid®. This made it possible to distinguish the post-30 day honey at the 60-day sampling, when frames were collected from 3 control and 4 treated hives. Honey was sampled as at the first collection. Samples (100-240 g) were kept on ice and in freezers within 2.5 hours of collection.

The CA trial samples were collected from 4 untreated and 4 treated frames per hive. The honeycomb caps were removed from each frame, which was then centrifuged for several minutes in a stainless steel centrifuge to remove most of the honey. The honey was strained through clean nylon panty hose and put in a glass jar. The wax left on the frames was scraped off into a separate glass jar. The centrifuge was cleaned with hot water between each sample, and with hot water and soap between treated and untreated samples. New frames were put into the hives for collection of the 60-day samples, which were collected similarly to the 30- day samples except each sample consisted of 6 frames. The honey and wax samples (each 102-208 g) were placed on "blue ice" within 24 minutes of collection and in freezers within 78 minutes of collection.

All samples were shipped frozen either by air or by ACDS freezer truck to the University of California at Davis for analysis by the Department of Environmental Toxicology (Trace Analytical Laboratory). The samples arrived intact and still frozen. Samples were kept in frozen storage and extracted within 31 days (honey) and 26 days (wax) of shipping from the field site, where they had been kept frozen for 2-10 days.

Analytical Method: Thymol residues in honey and beeswax were measured using a method based on the report "Residues in Wax and Honey After Apilife VAR® Treatment" by S. Bogdanov, A. Imdof, and V. Kilchenmann, Apidologie 29: 513-524 (1998) with some minor modifications. This document was not provided. The validated method sensitivity for thymol is 0.01 ppm for honey and 0.10 ppm for beeswax. The analytical quantification method was gas chromatography with a mass selective detector (GC-MSD) and the instrument used was an HP 6890 gas chromatograph with a 5973 MSD and a PE-XLB, 15 m, 0.25 mm I.D. column with 0.25 µm film thickness. The thymol retention time was 7.21 minutes and the monitored ions were 139 and 150 m/z (100 ms dwell time). Thymol was quantitated in honey and wax samples by comparing the peak areas of the samples to a thymol standard curve generated using 2, 5, 10, 20, 50, and 100 pg/µL standards in ethyl acetate. Sample chromatograms and calibration curves were provided.

Honey samples (10 g) were mixed with 30 mL buffer (20% ethanol/water) in a flask and placed on a shaker platform at 175 RPM for 30 minutes. The extract, and a 5 mL rinse of the flask, were then loaded onto a C18 SPE column (1 g/6 mL Varian MegaBond) pre-conditioned with ethanol followed by water. The column eluate was discarded and the SPE was vacuum-dried for 10 minutes. Thymol-containing material was eluted from the SPE with ethyl acetate and subjected to gas chromatography with a mass selective detector (GC-MSD).

Wax samples (1.0 g) were sonicated for 1 hour in 10 mL ethanol, frozen for 10 minutes, centrifuged 15 minutes, and the supernatant decanted, and similarly frozen and centrifuged again. To 5 mL of the supernatant, 2 mL water was added, and the mixture was frozen and centrifuged as previously. The resulting supernatant was mixed with 20 mL water and loaded onto a C18 SPE column that was prepared and run as for the honey samples. Thymol-containing material was eluted with ethyl acetate and subjected to GC-MSD as for honey.

II. RESULTS:

1. **Analytical Method:** The analytical method limit of quantitation (LOQ) for honey was 0.01 ppm and for wax was 0.10 ppm. As shown in Table 1, the method validation residue recoveries for honey (89-132%) and beeswax (107-147%) were somewhat outside the acceptable range of 70-120% at the lowest fortification levels (0.010 ppm for honey and 0.10 ppm for beeswax). However, concurrent recoveries of all thymol-fortified samples were within the acceptable range for both honey (76-119%) and beeswax (72-105%). No thymol was detected above the LOQ in untreated controls. These results indicate the analytical method was capable of accurately determining thymol residues in honey and beeswax.

TABLE 1: Method validation and concurrent recoveries from honey and beeswax samples fortified with thymol						
Test location	Thymol residues (ppm)					
	Honey			Beeswax		
	Method validation					
	Fortification level (ppm)	Recovery of residues (%)	Mean recovery (%)	Fortification level (ppm)	Recovery of residues (%)	Mean recovery (%)
	5.00 0.10 0.010	99, 95, 89 100, 104, 105 124, 127, 132	94 ± 5 103 ± 3 128 ± 4	1.00 0.10	107, 112, 111 134, 137, 147	110 ± 3 139 ± 6
	Concurrent recoveries					
Trial 1: Harmony, MD	5.00 1.00 0.10	97 97 110, 119	106 ± 11 (n=4)[1]	150 100 50 10	80 84 87 90, 99	88 ± 7 (n=5)[1]
Trial 2: Porterville, CA	5.00 1.00 0.50 0.10 0.010	97 76, 87, 97 84, 83, 83 110, 119 110	95 ± 14 (n=10)[1]	150 100 50 10 1.0 0.10	80, 74, 72 84, 77, 77 87, 96, 92 99, 90, 80 86 92	85 ± 8 (n=14)[1]
Trial 3: Weslaco, TX.	5.00 1.00 0.50 0.10 0.010	97 97 84 110, 119 109	103 ± 12 (n=6)[1]	150 100 10 1.0 0.10	80 84 99, 80 86 93, 105, 92	90 ± 9 (n=8)[1]

Data from pages 51-58 of MRID 46254301.
LOQ = Limit of quantitation = 0.01 ppm for honey and 0.10 ppm for beeswax.
[1]The mean recoveries and standard deviations were calculated by the reviewer using data for all fortification levels.

2. **Storage Stability:** Honey and wax samples were kept in frozen storage and extracted within 31 days (honey) and 26 days (wax) of shipping from the field site, where they had been kept frozen for 2-10 days, for a total of ≤35 days from sample collection to extraction. Per OPPTS Guideline 860.1380 (Storage Stability Data), storage stability data is not needed for samples stored frozen <30 days unless the pesticide in known to be volatile or labile. The slightly greater freezer storage duration is not likely to have significantly affected the residue results, as the samples were collected in the field 30 days after the last application and thymol has a low vapor pressure (0.04 mm Hg per ChemFinder.com).

   The "days in storage" is defined in MRID 46254301, Table 2 (pages 20-22) as being the days "from sampling until extraction" (see footnote 2 on page 22). This disagrees with the stated dates of sample collection, shipping, and laboratory extraction in Table 2. The discrepancy is due to the fact that the report did not account for 2-10 days the samples were kept in frozen storage at the field after collection and before shipping. Therefore, footnote 2 on page 22 should define the "days in storage" as the time from the date shipped (and not the day collected) until extraction.

3. **Magnitude of Thymol Residues in Honey and Beeswax:** Thymol levels in honey and beeswax samples collected at the three field trial sites are shown in Table 2. Residue levels were greatest at the MD site and lowest at the TX site, possibly due to the combination of lower temperature and greater humidity at the MD site *vs.* the greatest temperature combined

with low humidity at the TX site. Residue levels decreased with time for both honey and wax. At the three sites, honey residues were 0.019-2.59 ppm after 30 days (0 to 30-day samples) and <0.010-0.96 ppm after 60 days (30 to 60-day samples). Beeswax residues were 0.11-97.6 ppm in day 30 samples and <0.10-18.7 ppm in day 60 samples. No thymol residues were detected above the LOQ in untreated controls and the concurrent fortified sample recoveries were all within the acceptable range of 70-120%.

TABLE 2: Thymol residues in honey and beeswax from hives treated twice with ~12.5 g thymol, and assayed approximately 30 and 60 days after the second treatment.

Test location	PHI (days)	Thymol residues (ppm)			
		Honey		Beeswax	
		Untreated	Treated	Untreated	Treated
Trial 1: Harmony, MD	30 61	<LOQ <LOQ	2.59; 2.11 0.90; 0.96	<LOQ <LOQ	97.6; 58.6 18.7; 14.1
Trial 2: Porterville, CA	30 59	<LOQ <LOQ	0.98; 0.93 0.046; 0.047	<LOQ <LOQ	20; 37 6.3; 5.1
Trial 3: Weslaco, TX	31 60	<LOQ <LOQ	0.020; 0.019 <LOQ; 0.013	<LOQ <LOQ	0.11; 0.11 <LOQ

Data from pages 23, 24, and 50 of MRID 46254301.
LOQ = 0.01 ppm for honey and 0.10 ppm for beeswax.

III. **STUDY AUTHOR'S CONCLUSIONS**: The study author concluded that this study appropriately and accurately determined thymol residues in honey and beeswax "when used according to the label."

IV. **REVIEWER'S CONCLUSIONS:** The reviewer concurs that the method accurately determined thymol residues in honey and beeswax at the concentrations tested. Several minor deficiencies need to be addressed that do not change the outcome or validity of this study (1) the registrant needs to correct footnote 2 of Table 2 (on page 22) to define the "days in storage" as the time from the date shipped (and not the day collected) until extraction, and (2) a description should be provided of how the method validation and concurrent recovery fortified samples were prepared. The ORNL reviewer could not locate OPPTS guidance as to the number of required field trials and their geographic distribution to establish tolerance levels for honey and beeswax.

CLASSIFICATION: ACCEPTABLE, upon correction of footnote 2 of Table 2 (on page 22), and upon providing additional details of the preparation of the fortified samples used for method validation and concurrent recovery.

DATA EVALUATION RECORD

THYMOL
(Apiguard EZ-3)

STUDY TYPES: Preliminary Analysis (OPPTS 830.1700)

DP# 316472, Decision #341453, Reg #79671-R
MRID 46485601

Prepared by
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

07/17/05

Primary Reviewer: /s/ 08/16/05
Kent R Carlson, PhD, Biologist

Signature: [signature]
Date: 08-16-05

Secondary Reviewers: /s/ 08/16/05
Russell Jones, PhD, Senior Biologist

Signature: [signature]
Date: 8/16/2005

Disclaimer:

This review may have been altered subsequent to the signatures above.

DATA EVALUATION RECORD

EPA Secondary Reviewer: Russell Jones, PhD; Senior Biologist /s/ 08/16/05

STUDY TYPE:	Preliminary Analysis (OPPTS 830.1700)
MRID NO:	46485601
DP BARCODE NO:	DP316472
CASE NO:	Material No. 600349
SUBMISSION NO:	Volume 21
TEST MATERIAL:	Thymol
PROJECT NO:	Material No. 600349
SPONSOR:	Vita (Europe) Limited c/o Landis International, Inc. P.O. Box 5126, Valdosta, GA 31603-5126
TESTING FACILITY:	SYMRISE GmbH & Co. KG Muhlenfeldstrasse 1, 37603 Holzminden, Germany
TITLE OF REPORT:	Preliminary Analysis - Thymol
AUTHOR:	Nicola Hosking and Peter Quinn
STUDY COMPLETED:	03 November, 2004
GOOD LABORATORY PRACTICE:	This study did not adhere to GLP compliance as published in 40 CFR §160
CONCLUSION:	Thymol is the active ingredient in Apiguard EZ-3, an end-use product (EP) applied indirectly as a gel in trays to beehives to control and suppress parasitic varroa mites. Content analyses of colorless to white crystalline thymol ranged from 99.9 to 100.0%. The average melting point of thymol was 50.1°C and no organic volatile impurities were detected.
CLASSIFICATION:	**ACCEPTABLE.**

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

Executive Summary: Thymol is the active ingredient in Apiguard EZ-3, an end-use product (EP) applied indirectly as a gel in trays to beehives to control and suppress parasitic varroa mites.

[redacted]

Test Material: The test substance was Thymol [redacted]

Quality control process information may be entitled to confidential treatment

Test Method: This was a preliminary analysis study. [redacted]

Results: [redacted]

Study Author's Conclusions: No study authors conclusions were present.

Primary Reviewer's Conclusion: The preliminary analysis of thymol reported thymol content, melting point, color, and impurities. No discrepancies were noted in the analyses.

Secondary Reviewer's Conclusion:

Deviations or Discrepancies: No study deviations were noted.

Classification: ACCEPTABLE.

DATA EVALUATION RECORD

THYMOL
(Apiguard EZ-3)

STUDY TYPES: Preliminary Analysis (OPPTS 830.1700), Certificate of Analysis (OPPTS Guideline Nos. 830.6302, 830.6303, 830.6304, 830.7000, 830.7100, 830.7300)

DP# 316472, Decision #341453, Reg #79671-R
MRID 46524501, 46524502

Prepared by
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

07/17/05

Primary Reviewer: /s/ 08/16/05
Kent R Carlson, PhD, Biologist

Signature: _____
Date: 08-16-05

Secondary Reviewers: /s/ 08/16/05
Russell Jones, PhD, Senior Biologist.

Signature: _____
Date: 8/16/2005

Disclaimer:

This review may have been altered subsequent to the signatures above.

DATA EVALUATION RECORD

EPA Secondary Reviewer: Russell Jones, PhD; Senior Biologist /s/ 08/16/05

STUDY TYPE:	Preliminary Analysis (OPPTS 830.1700); Certificate of Analysis (OPPTS 830.6302, 830.6303, 830.6304, 830.7000, 830.7100, 830.7300)
MRID NO:	46524501, 46524502
DP BARCODE NO:	DP316472
CASE NO:	Ref No. BK2364P
SUBMISSION NO:	Volume 22 (1), 23
TEST MATERIAL:	Thymol (Apiguard EZ3; File Symbol 79671-R)
PROJECT NO:	Ref. No. BK2364P
SPONSOR:	Vita (Europe) Limited c/o Landis International, Inc. P.O. Box 5126, Valdosta, GA 31603-5126
TESTING FACILITY:	Laleham Healthcare Limited Sycamore Park, Mill Lane Alton, Hampshire GU34 2PR, England
TITLE OF REPORT:	Preliminary Analysis - Apiguard EZ3 NaOH
AUTHOR:	A.M. Trelford
STUDY COMPLETED:	25 March, 2004
GOOD LABORATORY PRACTICE:	This study did not adhere to GLP compliance as published in 40 CFR §160, but was modified to be compliant with EC Directive 2001/83/EC
CONCLUSION:	Apiguard EZ-3 (EPA Reg. No. 79671-R) is an end-use product (EP) applied indirectly as a gel in trays to beehives to control and suppress parasitic varroa mites. The CSF indicates that the active ingredient is 25.00% thymol. [redacted] are on the CSF as [redacted] A certificate of analysis for 2 batches of EP using inerts [redacted] No impurities of toxicological significance associated with the active ingredient are expected. Packaging specifications for trays (51g nominal), sachets (13 and 25g), and tubs (3.1 kg) were given. Discrepancies were noted between the handwritten batch notes and certificates of analysis and between the reported method and the performed analysis.
CLASSIFICATION:	**UNACCEPTABLE**, but upgradable pending revision of the report.

Inert ingredient information may be entitled to confidential treatment
Quality control process information may be entitled to confidential treatment

Inert ingredient information may be entitled to confidential treatment
Quality control process information may be entitled to confidential treatment

Executive Summary: Apiguard EZ-3 (EPA Reg. No. 79671-R) is an end-use product (EP) applied indirectly as a gel in trays to beehives to control and suppress parasitic varroa mites. The CSF indicates that the active ingredient is 25.00% thymol. [redacted] are on the CSF as [redacted] A certificate of analysis for 2 batches of EP using inerts [redacted] No impurities of toxicological significance associated with the active ingredient are expected. Packaging specifications for trays (51g nominal), sachets (13 and 25g), and tubs (3.1 kg) were given. Discrepancies were noted between the handwritten batch notes and certificates of analysis and between the reported method and the performed analysis.

Test Material: The test substance was Apiguard EZ-3 (25.00% thymol; batches 03A0400 and 03A0401, manufactured March 18, 2004), described as a colorless to pink granular gel with a strong odor of thyme. No expiration date was provided for the batches

Test Method: This was a preliminary analysis study. [redacted]

Results: Two certificates of analysis for batches 03A0400 and 03A0401 of Apiguard EZ-3 (containing [redacted] were presented. [redacted] (w/w). Other physico-chemical parameters were also reported and can be seen below (Table 1). Reported values for both certificate of analyses were different than those reported in handwritten batch notes (shaded).

[redacted]

[redacted]

Study Author's Conclusions: No study authors conclusions were present.

Primary Reviewer's Conclusion: The preliminary analysis of Apiguard EZ-3 reported six physico-chemical parameters of the EP. Discrepancies were noted between handwritten notes and final certificates of analysis (Table 1) and in methodology reported and performed (page 10, mixer time duration). Both types of discrepancy did not result in exceedance of ranges reported on the certificates of analysis [redacted]

Secondary Reviewer's Conclusion: .

Deviations or Discrepancies: The following study deviations were noted. Deviations not addressed below in "Classification" were not thought to adversely affect study conclusions.

1) Information on the test material such as expiration date was not provided.
2) Handwritten batch notes and final certificates of analysis reported differing physico-chemical values (Table 1).
3) Methodology performed for batch 03A310400 was different from that reported in batch protocols. Mixing times for step 3 and 5 were substantially abbreviated from [redacted]

Classification: UNACCEPTABLE, but upgradable pending revision of the report. Specifically, the following clarifications should be made:

1) An expiration date must be provided for each test batch of Apiguard EZ-3.
2) The registrant must explain discrepancies between handwritten batch notes and the final certificates of analyses as reported in Table 1.
3) The registrant must explain why the methodological performance differed from batch protocols (see number 3 above), and if this new methodology will be used in preparing future batches of Apiguard EZ-3.

DATA EVALUATION RECORD

THYMOL
(APIGUARD)

STUDY TYPE: Product Identity and Composition (OPPTS 830.1550)
Description of Beginning Materials (OPPTS 830.1600)
Description of Formulation Process (OPPTS 830.1650)
Discussion of Formation of Impurities (OPPTS 830.1670)
Preliminary Analysis (OPPTS 830.1700)
Certified Limits (OPPTS 830.1750)
Enforcement Analytical Method (OPPTS 830.1800)
Physical and Chemical Characteristics (OPPTS 830.6302-830.7950)
MRIDs 46043501, 46043502, 46043503, and 46198201

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, Va. 22202 2

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 04-18

Primary Reviewer:
Susan Chang, M.S. — Signature: _____ Date: _____

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T. — Signature: _____ Date: _____

Robert H. Ross, M.S., Group Leader — Signature: _____ Date: _____

Quality Assurance:
Lee Ann Wilson, M.A. — Signature: _____ Date: _____

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Product Identity and Composition (OPPTS 830.1550) Description of Beginning Materials (OPPTS 830.1600) Description of Formulation Process (OPPTS 830.1650) Discussion of Formation of Impurities (OPPTS 830.1670) Preliminary Analysis (OPPTS 830.1700) Certified Limits (OPPTS 830.1750) Enforcement Analytical Method (OPPTS 830.1800) Physical and Chemical Characteristics (OPPTS 830.6302-830.7950)
MRID NOS:	46043501, 46043502, 46043503, and 46198201
DP BARCODE NO:	DP300905
CASE NO:	Not reported
SUBMISSION NO:	Not reported
TEST MATERIAL:	Apiguard (EPA Reg No. 79671-R; 25.00% thymol, a.i.)
PROJECT NO:	1C-001 (MRID 46043502), 2G-201 (MRID 46043503), Not assigned (MRIDs 46043501 and 46198201)
SPONSOR:	Vita (Europe) Limited, Basingstoke, Hampshire, UK
TESTING FACILITY:	Lalcham Healthcare Ltd, Alton, Hampshire, UK (MRIDs 46043502 and 46043503); Not applicable (MRIDs 46043501 and 46198201)
TITLES OF REPORTS:	Apiguard: Product Identity and Disclosure of Ingredients, including Manufacturing Process, Discussion of Apiguard: Formation of Impurities and Enforcement of Analytical Methods (MRIDs 46043501 and 46198201); Apiguard: Physical and Chemical Properties (MRID 46043502); Apiguard: Storage Stability with Corrosion Characteristics (MRID 46043503)
AUTHORS:	Aston Trelford (MRIDs 46043502 and 46043503), Wm. Ronald Landis (MRIDs 46043501 and 46198201)
STUDY COMPLETED:	March 11, 2002 (MRID 46043502), March 19 (MRID 46043503), July 17, 2003 (MRID 46043501), February 9, 2004 (MRID 46198201)

GOOD LABORATORY PRACTICE: Not GLP Compliant and not required (MRID 46043501 and 46198201);
Not GLP Compliant, studies conducted as basic research studies (MRIDs 46043502 and 46043503)

CONCLUSION: Apiguard is an EP used to treat varroatosis due to *Varroa destructor* in honeybees and is currently registered in Europe for the control of Varroa mites. Apiguard contains 25.00% w/w thymol as the active ingredient and [redacted] The chemical composition of [redacted] was not adequately defined. The label agrees with the CSF concerning the content of the active ingredient, but the number of the decimal places needs to be consistent. The beginning materials and the description of the formulation process are adequately presented, except that CAS No. and PC code for [redacted] are not present. No chemical reactions occur during the manufacturing process and formation of new impurities is not expected to occur. The active ingredient thymol is pending for registration, and unless it becomes registered, five certificates of analysis of thymol or preliminary analysis of five batches of Apiguard is needed. All certified limits are within the recommended range in the guideline proposed in OPPTS 830.1750, except that the upper certified limit (+4.8%) for the active ingredient is outside the recommended range (+3%), and no explanation was provided. The enforcement analytical method for thymol is reversed phase high performance liquid chromatography. The physical/chemical properties for Apiguard are not addressed, but pH, flammability, and bulk density are given on the CSF. MRIDs 46043501, 46043502, and 4043503 contain data for Apiguard containing [redacted] instead of [redacted] and [redacted]

CLASSIFICATION: **UNACCEPTABLE**, but upgradable if the registrant: 1) adjusts the number of the decimal places in the thymol content between the label and the CSF to be consistent, 2) provides the CAS No. of [redacted] on the CSF and provides the EPA with the ingredietns of [redacted] presents five certificates of analysis of the active ingredient thymol or performs a preliminary analysis of five batches of Apiguard, and 4) addresses the physical/chemical properties for Apiguard containing thymol. [redacted] In addition, the registrant needs to obtain a PC code for [redacted]

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

Test Material: Apiguard containing 25.00% thymol (5-methyl-2-isopropyl-1-phenol)

I. **PRODUCT IDENTITY AND COMPOSITION:** Apiguard (EPA Reg. No. 79671-R) is an end-use product (EP) used to treat varroatosis due to *Varroa destructor* in honeybees and is currently registered in Europe for the control of Varroa mites. The active ingredient is 25.00% w/w thymol (CAS No. 89-83-5, PC code 080402). The inerts are [redacted] The label agrees with the CSF concerning the nominal concentration of the active ingredient, but the number of decimal points need to be consistent. The registrant originally submitted "Apiguard" containing thymol, [redacted] Since [redacted] is a list 2 potentially toxic inert ingredient (see letter dated 10/24/03), the registrant replaced [redacted] This letter also stated that [redacted] is a list 4B inert ingredient, but the reviewer can not verify this claim because the [redacted] chemical composition was not reported. On the CSF dated 2/9/04 and in MRID 46198201 (p. 5 of 29), [redacted]

Deficiencies: The number of decimal places in the nominal concentration of the active ingredient is not consistent between the label and the CSF. The identity/chemical composition (and CAS No.) Of the inert [redacted] were not defined.

II. **DESCRIPTION OF BEGINNING MATERIALS:** The beginning materials are thymol (CAS No. 89-83-5, PC code 080402), [redacted] and [redacted] s not on the List 4 inert list. All MSDSs are provided in MRID 46198201.

Deficiencies: The CAS No. for [redacted] is not listed on the CSF and its PC code was not found.

Inert ingredient information may be entitled to confidential treatment

Manufacturing process information may be entitled to confidential treatment

III. **DESCRIPTION OF FORMULATION/PRODUCTION PROCESS:** [redacted]

Deficiencies: None

IV. **DISCUSSION OF FORMATION OF IMPURITIES:** No chemical reactions occur during manufacturing process and formation of new impurities is not expected to occur in the manufacturing process (MRID 46198201).

Deficiencies: None

V. **PRELIMINARY ANALYSIS:** Three certificates of analysis of three batches of Apiguard were presented. The thymol content was 25.6%, 26.2%, and 25.4%. Preliminary analysis is not required for a batch process with a registered active ingredient. The CSF indicates that the active ingredient thymol (100%) is pending for registration. The three batches (04T276, 04T305, and 04T306) were manufactured in April 1999 and apparently contained [redacted] (MRID 46043503).

Deficiencies: Unless the active ingredient becomes registered, the registrant needs to present five certificates of analysis of the active ingredient thymol or perform a preliminary analysis of five batches of Apiguard containing [redacted]

VI. **CERTIFIED LIMITS:** Apiguard contains 25.00% by weight (limits of 24.25-26.2%, by weight) thymol. The lower and upper certified limits for [redacted] respectively. The upper certified limit (+4.8%) for the active ingredient is outside the recommended range (+3%). All other certified limits are within the recommended range in the guideline proposed in OPPTS 830.1750.

In a letter dated 2/13/2004, the registrant indicates that the wide upper certified limit is "to reflect the variation in the active ingredient content based upon certified product analysis (MRID 46043503)." The three batches (04T276, 04T305, and 04T306) were manufactured in April 1999 and apparently contained [redacted] It is possible that replacing [redacted] in the manufacturing process would not alter the resulting thymol content in Apiguard.

Deficiencies: None

Table 1. Nominal concentrations and the upper and lower limits for the ingredients			
	Nominal	Lower Limit	Upper Limit
	% by weight		
Active ingredient			
Thymol, 100%	25.00%	24.25%	26.2%
Inert ingredient			
[redacted]			

VII. **ENFORCEMENT ANALYTICAL METHOD:** The active ingredient thymol in Apiguard® Gel is determined using reversed phase high performance liquid chromatography (CEMAS SOP CEM-1008/001). The HPLC column is 250 x 4.6 mm i.d. Hypersil ODS 5 μm or equivalent, the mobile phase is 70:30 v/v acetonitrile:water, flow rate is 1.0 mL/min, and the detector wavelength is 276 nm. Examples of chromatograms are presented in MRID 46043501 and the formulation tested was Apiguard® Gel, batch 05V581.

Validation of CEMAS SOP CEM-1008 for the analysis of thymol in Apiguard® Gel is also presented in MRID 46043501. The sample used was Apiguard® Gel, batch 05V581. System suitability, linearity, precision of the method, accuracy and range, limit of quantitation, limit of detection, specificity, and robustness were addressed. The results were satisfactory. The limit of quantitation was 0.5% w/w thymol and the limit of detection was 0.025% w/w thymol.

MRID 46043501 also presented "Validation of Laleham method LM122 for the determination of thymol content in Apiguard Gel." LM122 differs from CEMAS CEM-1008 with the column (200 x 4.6 mm i.d. Waters Spherisorb S5 ODS1 vs 250 x 4.6 mm i.d. Hypersil ODS 5 μm) and retention time (~2 minutes vs ~4 minutes). The thymol content in the storage stability study was determined using the Laleham LM 122 method. The results indicated that the use of a different column did not influence the measurement of thymol content in Apiguard gel.

Deficiencies: None

VIII. **PHYSICAL AND CHEMICAL CHARACTERISTICS:**

1. **Methods:** See the table below.

2. **Results:** The physical/chemical properties for Apiguard are not addressed, except bulk density (1.015), pH (6.5-8.0), and flammability (non-explosive, non-flammable) on the CSF dated 7/17/2003 and 2/9/2004.

3. **Deficiencies:** The registrant did not present the physical/chemical properties for Apiguard containing thymol, [redacted] It is not clear and questionable whether the physical/chemical properties listed on the CSF are just old data from the earlier formulation.

Parameters	Physical/Chemical Properties	Methods
Color	Not addressed.	
Physical State	Not addressed.	
Odor	Not addressed.	
Stability	Not required for EP	
Oxidation/Reduction	Not addressed.	
Flammability	Not addressed.	
Explodability	Not addressed.	
Storage Stability	Not addressed.	
Miscibility	Not addressed.	
Corrosion Characteristics	Not addressed.	
Dielectric Breakdown Voltage	Not required for EP	
pH	Not addressed.	
Viscosity	Not addressed.	
Melting Point	Not required for EP	
Boiling Point	Not required for EP	
Bulk Density	Not addressed.	
Dissociation Constant	Not required for EP	
Partition Coefficient (n-octanol/water)	Not required for EP	
Water Solubility	Not required for EP	
Vapor Pressure	Not required for EP	

IX. COMMENTS:

MRID 46043502 (Apiguard: Physical and Chemical Properties) was not used to write this DER. Apiguard described in MRID 46043502 contains thymol, [redacted] The registrant wishes to register Apiguard containing [redacted]

MRID 46043503 (Apiguard: Storage Stability with Corrosion Characteristics) presents the storage stability study of three batches of Apiguard that was carried out at 22°C and 35% relative humidity and 30°C and 35% relative humidity. The three batches (04T276, 04T305, and 04T306) were manufactured in April 1999 and apparently contained [redacted] The methods used were LM 08, LM 10, and LM 122 for pH, viscosity, and thymol content, respectively (see Table below). Appearance, odor, viscosity, and thymol content were measured at 0, 7, and 22 months. The pH and seal test/leaking test were checked at 0 and 22 months. The results showed the good stability of Apiguard when stored 22 months at 22°C and 30°C.

Summary of the storage stability study				
Storage at 22°C and 35% relative humidity				
Time point (months)	**0**	**7**	**22**	**Specification**
pH	7.3-7.8	-	6.6-6.9	6.5-8.0
Viscosity (cps)	109,100-119,700	110,200-119,500	85,900-95,600	70,000-130,000
Thymol content (% w/w)	25.4-26.2	23.8-26.2	24.6-25.2	23.75-26.25
Storage at 30°C and 35% relative humidity				
Time point (months)	**0**	**7**	**22**	
pH	7.3-7.8	-	6.5-6.6	6.5-8.0
Viscosity (cps)	109,100-119,700	110,200-122,200	85,700-88,000	70,000-130,000
Thymol content (% w/w)	25.4-26.2	24.0-25.3	23.8-24.9	23.75-26.25

DATA EVALUATION RECORD

CNEW

STUDY TYPE: ACUTE ORAL TOXICITY - RAT (870.1100)
MRID 46043504

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, Va. 22202 2

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 04-18

Primary Reviewer:
Susan Chang, M.S.

Signature: _____
Date: _____

Secondary Reviewers:
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.

Signature: _____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature: _____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature: _____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Oral Toxicity - Rat (OPPTS 870.1100)
MRID NO:	46043504
DP BARCODE NO:	DP300905
CASE NO:	Not reported
SUBMISSION NO:	Not reported
TEST MATERIAL:	CNEW
PROJECT NO:	197944
SPONSOR:	Vita (Europe) Limited, C/O Landis International, Inc., Valdosta, GA
TESTING FACILITY:	Notox B.V., Hambakenwetering 3, 5231 DD 's-Hertogenbosch, The Netherlands
TITLE OF REPORT:	CNEW: Assessment of Acute Oral Toxicity in the Rat
AUTHOR:	Dr. Ir. W.R. Pels Rijcken
STUDY COMPLETED:	March 25, 1997
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The oral LD_{50} for males, females, and combined was greater than 2000 mg/kg.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY III

I. STUDY DESIGN:

1. **Test Material:** CNEW (purity 25%), Batch No. 09701

2. **Test Animals:** Five male and ten female Wistar Crl:(WI) BR (outbred, SPF-quality) rats were received from Charles River, Sulzfeld, Germany, were assigned, and weighed 273-290 g (males) and 191-201 g (females) on the day of dosing. The young adult animals, approximately 8 weeks old, were housed in groups of five per sex per polycarbonate cage. The animals were fed standard pelleted laboratory animal diet (Carfil Quality BVBA, Oud-Turnhount, Belgium), *ad libitum*. Tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 21°C; relative humidity, 50%; air exchanges, approximately 15/hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** Rats were ear-marked: males (Nos. 1 to 5) and females (Nos. 6 to 10). The rats were quarantined for at least five days and fasted overnight prior to dosing. The test material

(2000 mg/kg body weight) was dosed at 10 mL/kg body weight by gavage. Body weights were recorded prior to dosing on day 1, and on days 8 and 15. The test animals were observed for clinical signs of toxicity at 2 and 4 hours post-dosing and once daily for 14 days. All animals were necropsied.

II. RESULTS:

1. **Mortality:** All animals survived the study.

2. **Body Weights:** All animals gained weight during the study.

3. **Clinical Observations:** Hunched posture was noted from one male and five females two hours after dosing with recovery by 4 hours after dosing. Alopecia on the neck of one female was noted on days 3 and 4 with recovery by day 5.

4. **Gross Necropsy:** No gross abnormalities were noted from any animal.

III. DISCUSSION:

The oral LD_{50} for males, females, and combined was greater than 2000 mg/kg. This places CNEW in TOXICITY CATEGORY III. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

CNEW

STUDY TYPE: ACUTE DERMAL TOXICITY - RAT (870.1200)
MRID 460430505

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, Va. 22202 2

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 04-18

Primary Reviewer: Susan Chang, M.S.	Signature: _____ Date: _____
Secondary Reviewers: H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.	Signature: _____ Date: _____
Robert H. Ross, M.S., Group Leader	Signature: _____ Date: _____
Quality Assurance: Lee Ann Wilson, M.A.	Signature: _____ Date: _____

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Dermal Toxicity - Rats (OPPTS 870.1200)
MRID NO:	46043505
DP BARCODE NO:	DP300905
CASE NO:	Not reported
SUBMISSION NO:	Not reported
TEST MATERIAL:	CNEW
PROJECT NO:	197955
SPONSOR:	Vita (Europe) Limited, C/O Landis International, Inc., Valdosta, GA
TESTING FACILITY:	Notox B.V., Hambakenwetering 3, 5231 DD 's-Hertogenbosch, The Netherlands
TITLE OF REPORT:	CNEW: Assessment of Acute Dermal Toxicity in the Rat
AUTHOR:	Dr. Ir. W.R. Pels Rijcken
STUDY COMPLETED:	March 25, 1997
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	The dermal LD_{50} for males, females, and combined was greater than 2000 mg/kg.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY III

I. STUDY DESIGN:

1. **Test Material:** CNEW (purity 25%), Batch No. 09701

2. **Test Animals:** Five male and ten female Wistar Crl:(WI) BR (outbred, SPF-quality) rats were received from Charles River, Sulzfeld, Germany, were assigned, and weighed 304-320 g (males) and 205-228 g (females) on the day of dosing. The young adult animals, approximately 8 weeks old, were individually housed in polycarbonate cages. The animals were fed standard pelleted laboratory animal diet (Carfil Quality BVBA, Oud-Turnhount, Belgium), *ad libitum*. Tap water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 21°C; relative humidity, 50%; air exchanges, approximately 15/hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** Rats were ear-marked: Male – Nos. 1 to 5; Female – Nos. 6 to 10. The rats were quarantined for at least five days and the back was clipped one day before treament. The test material (2000 mg/kg body weight) was "applied to an area of approximately 10% of the total body surface, i.e. approx. 25 cm^2 for males and 18 cm^2 for females, using a gauze patch (5 x 5 cm or 3.3 x 5 cm, respectively) fixed to aluminum foil (with a drop of petroleum) and held in place with Coban elastic bandage. A piece of Micropore tape was used for additional fixation of the bandage in females only." The coverings were removed after 24 hours and the excess test material was removed with tissue moistened with water. The test animals were observed twice daily for mortality and observed for clinical signs of toxicity 2 and 4 hours after treatment and once daily thereafter for 14 days. The rats were weighed prior to treatment on day 1 and on days 8 and 15. The decedent and surviving rats euthanized on day 14 were necropsied.

II. RESULTS:

1. **Mortality:** Two females (Nos. 6 and 8) were found dead on day 2. All other rats survived the study.

2. **Clinical Observations:** Two females were lethargic two hours post treatment and were found dead on day 2 and one female was lethargic four hours after treatment with recovery by day 2. Focal erythema, necrosis, wounds, scales, scabs, and/or scars on the back were noted on all surviving animals between days 3 and 15.

3. **Body Weight:** All surviving animals had normal body weight gains.

4. **Gross Necropsy:** One male (No. 1) had a yellow soft surface on the right kidney. The thoracic cavity of one decedent female (No. 8) contained watery fluid. No gross abnormalities were noted from other animals.

III. DISCUSSION:

The dermal LD_{50} for males, females, and combined was greater than 2000 mg/kg. This places CNEW in TOXICITY CATEGORY III. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

CNEW AND THYMOL

STUDY TYPE: ACUTE INHALATION TOXICITY - RAT (870.1300)
MRID 46043506

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, Va. 22202 2

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 04-18

Primary Reviewer: Susan Chang, M.S.	Signature: Date:	_____ _____
Secondary Reviewers: H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.	Signature: Date:	_____ _____
Robert H. Ross, M.S., Group Leader	Signature: Date:	_____ _____
Quality Assurance: Lee Ann Wilson, M.A.	Signature: Date:	_____ _____

Disclaimer

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Inhalation Toxicity - Rat (OPPTS 870.1300)
MRID NO:	46043506
DP BARCODE NO:	DP300905
CASE NO:	Not reported
SUBMISSION NO:	Not reported
TEST MATERIAL:	CNEW containing 25.00% w/w thymol; and thymol, 98% purity
PROJECT NO:	656021
SPONSOR:	Vita (Europe) Limited, C/O Landis International, Inc., Valdosta, GA
TESTING FACILITY:	RCC, Research and Consulting Company Ltd., c/o BRL Biological Research laboratories Ltd., CH-4414 Füllinsdorf, Switzerland and RCC Umweltchemie AG, CH-4452 Itingen, Switzerland
TITLE OF REPORT:	Technical Trials Preceding a Proposed 4-Hour Acute Inhalation Toxicity Study with CNEW and Thymol, the Active Ingredient of CNEW
AUTHOR:	C.N. Crouch, U. Decker, and K. Biedermann
STUDY COMPLETED:	November 13, 1997
GOOD LABORATORY PRACTICE:	Not GLP Compliant
CONCLUSION:	CNEW is a viscous gel and would not produce a suitable atmosphere for inhalation toxicity testing. Thymol produced a two-phase atmosphere, vapor and aerosol (containing crystals). The total concentration was 0.228 mg/L and was considered too low to justify the exposure of animals. The inhalation LC_{50} for males, females, and combined was not obtained due to the nature of the test material.
CLASSIFICATION:	ACCEPTABLE -- NOT CLASSIFIED

1. **Test Material:** CNEW containing 25% w/w thymol, [redacted] Batch No. 09702; thymol, 98% purity, Batch No. 11415-017

2. **Generation of Suitable Test Atmosphere:**
Four trials were made to obtain a suitable atmosphere for the four-hour acute inhalation toxicity in rats.

Trial 1 was attempted by diluting CNEW with water at room temperature and an inhomogeneous aqueous solution containing insoluble crystals that were crushed using a homogenizer. This could not be used for the generation of an aerosol or any test atmosphere.

Trail 2 was attempted by heating CNEW to 50°C to dissolve the crystals then transfer them to another glass flask at room temperature. Crystallization occurred immediately.

Trial 3 was attempted on the active ingredient thymol to achieve a target concentration of 1.25 mg/L air (equivalent to 5 mg CNEW/L) for 4 hours. Thymol was melted at approximately 75°C. The test atmosphere resulting from air (1.8 L/minute) being blown into the melted thymol was fed into the inhalation exposure system. PVC tubing of 9 mm inside diameter was used to connect the outlet of the wash-bottle to the inhalation exposure system. The resulting nominal concentration was 1.56 mg/L air and the aerosol concentration was 0.0013 mg/L air.

Trial 4 was also attempted on thymol. Modification was made on Trial 3 as following: air was preheated to 26.3°C before being blown into the melted thymol at 3.0 L/minute. PVC tubing of 25 mm inside diameter was used to connect the outlet of the wash-bottle to the inhalation exposure system. The resulting nominal concentration was 1.41 mg/L air and the aerosol concentration (solid phase) was 0.188 mg/L air and the vapor phase was 0.04 mg/L air.

3. **Conclusions:** CNEW is a viscous gel and would not produce a suitable atmosphere for inhalation toxicity testing. Thymol produced a two-phase atmosphere, vapor and aerosol (containing crystals). The total concentration was 0.228 mg/L and was considered too low to justify the exposure of animals. The inhalation LC_{50} for males, females, and combined was not obtained due to the nature of the test material.

The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

CNEW

STUDY TYPE: PRIMARY EYE IRRITATION - RABBIT (870.2400)
MRID 46043507

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, Va. 22202 2

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 04-18

Primary Reviewer:		
Susan Chang, M.S.	Signature:	_____
	Date:	_____
Secondary Reviewers:		
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.	Signature:	_____
	Date:	_____
Robert H. Ross, M.S., Group Leader	Signature:	_____
	Date:	_____
Quality Assurance:		
Lee Ann Wilson, M.A.	Signature:	_____
	Date:	_____

Disclaimer

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Acute Eye Irritation - Rabbit (OPPTS 870.2400)
MRID NO:	46043507
DP BARCODE NO:	DP300905
CASE NO:	Not reported
SUBMISSION NO:	Not reported
TEST MATERIAL:	CNEW
PROJECT NO:	197977
SPONSOR:	Vita (Europe) Limited, C/O Landis International, Inc., Valdosta, GA
TESTING FACILITY:	Notox B.V., Hambakenwetering 3, 5231 DD 's-Hertogenbosch, The Netherlands
TITLE OF REPORT:	CNEW: Acute Eye Irritation/Corrosion Study in the Rabbit
AUTHOR:	Dr. Ir. W.R. Pels Rijcken
STUDY COMPLETED:	April 18, 1997
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Corneal opacity was noted one hour after test material instillation with persistence through day 28. Iritis was noted one hour through 72 hours after test material instillation with resolution by day 7. Positive conjunctival irritation was noted one hour after test material instillation with resolution by day 14. The maximum irritation score was 57 at 72 hours after test material instillation. CNEW was severely irritating.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY I

I. STUDY DESIGN:

1. **Test Material:** CNEW (purity 25%), Batch No. 09701

2. **Test Animals:** One male young adult New Zealand White rabbit was received from Broekman Institute, Someren, The Netherlands. The animal was housed in a labelled cage with a perforated floor. The animal was fed standard laboratory rabbit diet (LKK-20, pellet diameter 4 mm, Hope Farms, Woerden, The Netherlands) approximately 100 g per day and,

in addition, hay was provided once a week. Tap water diluted with decalcified water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 21°C; relative humidity, 50%; air exchanges, approximately 15/hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** One male rabbit was ear-tagged (No. 1758). The rabbit was quarantined for at least five days. The test material (0.1 mL/eye/animal) was applied in the conjunctival sac of one eye, and the eye held closed for approximately one second. The other eye served as control. Based on the severity and persistence of the ocular lesion on the first animal, no additional animals were treated. The eyes were examined and scored 1, 24, 48 and 72 hours and 7, 14, 21, and 28 days after test material instillation.

II. RESULTS:

1. **Mortality:** The animal survived the study.

2. **Ocular Lesions:** Corneal opacity was noted one hour after test material instillation with persistence through day 28 (Table 1). Iritis was noted one hour through 72 hours after test material instillation with resolution by day 7. Positive conjunctival irritation (scores 2 and above) was noted one hour after test material instillation with resolution by day 14 (Table 2). The maximum irritation score was 57 at 72 hours after test material instillation (Table 3).

TABLE 1. Individual Male (M) and Female (F) Eye Scores w/ Time: Cornea (A=Density of Opacity, B=Area of Opacity)

Animal No.	1 hour		24 hours		48 hours		72 hours		7 days		14 days		21 days		28 days	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B
1758	1	1	3	1	3	1	2	4	2	2	1	1	1	1	1	1

TABLE 2. Summary of Eye Irritation Scores with Time: Conjunctiva and Iris

Score Conditions	1 hour	24 hours	48 hours	72 hours	7 days	14 days	21 days	28 days
Conjunctiva								
Erythema	1	3	3	3	2	1	0	0
Chemosis	4	3	2	1	0	0	0	0
Discharge	2	2	2	2	0	0	0	0
Iris	1	1	1	1	0	0	0	0

Irritation score is based on Draize Method

Scale for Scoring Ocular Lesions

CORNEAL IRRITATION

Opacity: degree of density (area most dense taken for reading)

- No ulceration or opacity (may include slight dulling of normal lustre) 0
- Scattered or diffuse areas of opacity, details of iris clearly visible 1
- Easily discernible translucent area, details of iris slightly obscured 2
- Nacreous area, no details of iris visible, size of pupil barely discernible 3
- Opaque cornea, iris not discernible through the opacity 4

Area of corneal involved:

- No ulceration or opacity 0
- One quarter or less but not zero 1
- Greater than one quarter, but less than half 2
- Greater than half, but less than tree quarters 3
- Greater than three quarters, up to whole area 4

IRIS

- Normal 0
- Marked deepened rugae, congestion, swelling, moderate circumcorneal hyperemia or injection, any of these or combination thereof, iris still reacting to light (sluggish reaction is positive) 1
- No reaction to light, hemorrhage, gross destruction (any or all of these). 2

CONJUNCTIVAL IRRITATION

Redness (refers to palpebral and SCLERA, excluding cornea and iris)

- Blood vessels normal 0
- Some blood vessels definitely hyperemic (injected) 1
- Diffuse, crimson color; individual vessels not easily discernible 2
- Diffuse beefy red 3

Chemosis (refers to lids and/or nictiating membranes):

- No swelling 0
- Any swelling above normal (includes nictitating membrane) 1
- Obvious swelling with partial eversion of lids 2
- Swelling with lids about half closed 3
- Swelling with lids about half closed to completely closed 4

Discharge:

- No discharge (may include small amounts observed in inner canthus of normal animals) 0
- Any amount different from normal and/or lacrimation 1
- Discharge with moistening of the lids and hairs just adjacent to lids 2
- Discharge with moistening of the lids and hairs (considerable area around the eye) 3

TABLE 3. Summary of Total[a] and Primary Eye Irritation Scores with Time

Animal #	1 h	24 h	48 h	72 h	7 d	14 d	21 d	28 d
1758	24	36	34	57	24	7	5	5
[b]Total	24	36	34	57	24	7	5	5

[a]Formula: Total Irritation Score = I + II + III, where,

I = Corneal Score = [Density (A) x Area (B)] x 5

II = Iris Score = Severity x 5

III = Conjunctival Score = [Erythema (A) + Chemosis (B) + Discharge (C)] x 2

[b]Primary Irritation = Sum of Total Irritation Scores ÷ 1

III. DISCUSSION:

Corneal opacity was noted one hour after test material instillation with persistence through day 28. Iritis was noted one hour through 72 hours after test material instillation with resolution by day 7. Positive conjunctival irritation was noted one hour after test material instillation with resolution by day 14. The maximum score was 57 at 72 hours after test material instillation. CNEW was severely irritating and is in TOXICITY CATEGORY I. The packet classification is **ACCEPTABLE.**

[redacted]

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Primary Dermal Irritation - Rabbit (OPPTS 870.2500)
MRID NO:	46043508
DP BARCODE NO:	DP300905
CASE NO:	Not reported
SUBMISSION NO:	Not reported
TEST MATERIAL:	CNEW
PROJECT NO:	197966
SPONSOR:	Vita (Europe) Limited, C/O Landis International, Inc., Valdosta, GA
TESTING FACILITY:	Notox B.V., Hambakenwetering 3, 5231 DD 's-Hertogenbosch, The Netherlands
TITLE OF REPORT:	CNEW: Primary Skin Irritation/Corrosion Study in the Rabbit
AUTHOR:	Dr. Ir. W.R. Pels Rijcken
STUDY COMPLETED:	March 25, 1997
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	Very slight to well defined erythema with very slight to moderate edema was noted on all rabbits one hour after patch removal. By 24 through 72 hours, well defined erythema with slight edema was noted on all rabbits that reduced to very slight erythema with no edema by day 7 and with clearance by day 14. Superficial, small, dry wounds, scaliness, and bald skin was noted during the observation period. The primary irritation index was 3.8. CNEW was moderately irritating.
CLASSIFICATION:	ACCEPTABLE -- TOXICITY CATEGORY IV

I. STUDY DESIGN:

1. **Test Material:** CNEW (purity 25%), Batch No. 09701

2. **Test Animals:** Three male young adult New Zealand White rabbits were received from Broekman Institute, Someren, The Netherlands. The animals, approximately 8-9 weeks old, were housed individually in labelled cages with perforated floors. The animals were fed

standard laboratory rabbit diet (LKK-20, pellet diameter 4 mm, Hope Farms, Woerden, The Netherlands) approximately 100 g per day and, in addition, hay was provided once a week. Tap water diluted with decalcified water was available *ad libitum*. The environmental conditions of the animal room were as follows: temperature, 21°C; relative humidity, 50%; air exchanges, approximately 15/hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** Rabbits were ear-tagged: Nos. 1729, 1739, and 1748 (males). The rabbits were acclimated for at least 5 days. The fur on the dorsal trunk (approximately 150 cm^2) of each rabbit was clipped on the day prior to treatment. The rabbits were given an approximate 0.5 mL of test material applied on a 2 x 3 cm gauze patch which was mounted on Micropore tape and placed on the clipped site. The patch and entire trunk were wrapped with a Coban elastic bandage. The covering was removed 4 hours later and the site wiped with tissue moistened with water to remove any residual test material. The animals were observed for mortality/viability twice daily and at least once daily for gross toxicity during the study. Dermal examination was recorded at 1, 24, 48, and 72 hours and 7 and 14 days after removal of the patch.

II. RESULTS:

1. **Mortality:** All rabbits survived the study.

2. **Dermal responses:** Very slight erythema or well defined erythema was noted on 2/3 and 1/3 rabbits, respectively, and very slight, slight, or moderate edema was noted on 1/3, 1/3, and 1/3 rabbits, respectively, one hour after patch removal. By 24 through 72 hours, well defined erythema with slight edema was noted on all rabbits that reduced to very slight erythema with no edema by day 7. Superficial, small, and dry wounds were noted on two rabbits (Nos. 1729 and 1748) 24, 48, and 72 hours after patch removal. Scaliness was noted on all rabbits on day 7. Bald skin was noted on one rabbit (No. 1748) on day 7 and on two rabbits (Nos. 1729 and 1748) on day 14. The primary irritation index was 3.8.

Irritation Scores:

TABLE 1. Summary of individual rabbit's dermal irritation scores with time

	Hours				Days	
Animal No.	1	24	48	72	7	14
1729	1/3 [a]	2/2	2/2	2/2	1/0	0/0
1739	1/1	2/2	2/2	2/2	1/0	0/0
1748	2/2	2/2	2/2	2/2	1/0	0/0

Data taken from pp. 12 and 13, MRID 46043508.
[a]Erythema/Edema

Description of rating method:

Evaluation of Skin Reaction:	**Score**
Erythema formation:	
No erythema	0
Very slight erythema (barely perceptible)	1
Well-defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness)	4

Edema Formation:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well-defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised by more than 1 mm extending beyond the area of exposure)	4

III. DISCUSSION:

Very slight to well defined erythema with very slight to moderate edema was noted on all rabbits one hour after patch removal. By 24 through 72 hours, well defined erythema with slight edema was noted on all rabbits that reduced to very slight erythema with no edema by day 7. Superficial, small, and dry wounds were noted on two rabbits 24, 48, and 72 hours after patch removal. Scaliness was noted on all rabbits on day 7. Bald skin was noted on one rabbit on day 7 and on two rabbits on day 14. The primary irritation index was 3.8. CNEW was moderately irritating and is in TOXICITY CATEGORY IV. The packet classification is **ACCEPTABLE.**

DATA EVALUATION RECORD

CNEW

STUDY TYPE: SKIN SENSITIZATION - GUINEA PIG (870.2600)
MRID 46043509

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, Va. 22202 2

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831
Task Order No. 04-18

Primary Reviewer:		
Susan Chang, M.S.	Signature:	_____
	Date:	_____
Secondary Reviewers:		
H. Tim Borges, M.T.(A.S.C.P.), Ph.D., D.A.B.T.	Signature:	_____
	Date:	_____
Robert H. Ross, M.S., Group Leader	Signature:	_____
	Date:	_____
Quality Assurance:		
Lee Ann Wilson, M.A.	Signature:	_____
	Date:	_____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Skin Sensitization - Guinea Pig (OPPTS 870.2600)
MRID NO:	46043509
DP BARCODE NO:	DP300905
CASE NO:	Not reported
SUBMISSION NO:	Not reported
TEST MATERIAL:	CNEW
PROJECT NO:	649732
SPONSOR:	Vita (Europe) Limited, C/O Landis International, Inc., Valdosta, GA
TESTING FACILITY:	Research & Consulting Company LTD., Itingen, Switzerland
TITLE OF REPORT:	Contact Hypersensitivity to CNEW in albino Guinea Pigs - Maximization Test
AUTHOR:	L. Ullmann
STUDY COMPLETED:	April 4, 1997
GOOD LABORATORY PRACTICE:	GLP Compliant
CONCLUSION:	After intradermal and epidermal inductions, the the test and control animals showed no signs of reactivity at 24 and 48 hours after challenge. CNEW was not a dermal sensitizer.
CLASSIFICATION:	ACCEPTABLE

I. STUDY DESIGN:

1. **Test Material:** CNEW containing 25% w/w thymol, [redacted]

2. **Test Animals:** Twenty female Dunkin Hartley guinea pigs received from Harlan Winkelmann, Borchen, Germany were assigned to groups and weighed 281-396 g (pretest group) and 453-605 g (main group) at experiment start. The young adult animals, approximately 5-7 weeks old, were housed individually in Makrolon type-3 cages. The animals were fed pelleted standard Nafag Ecosan 845 25W4, batch No. 130/96 guinea pig breeding/maintenance diet, *ad libitum*. Tap water was available *ad libitum*. Ascorbic acid (approximately 1 g/L) was supplied once weekly in the water. The environmental conditions of the animal room were as follows:

Inert ingredient information may be entitled to confidential treatment

temperature, 22±3°C, relative humidity, 40-70%; air changes, 10-15/hour; and photoperiod, 12 hour light/dark cycle.

3. **Methods:** Female guinea pigs were ear-tagged and grouped: Pretest – Nos. 676 to 680; Test – Nos. 666 to 675; Naive Control – Nos. 661 to 665. The guinea pigs were quarantined for 7 days. The animals were induced and challenged according to the Maximization Test. The dorsal skin (approximately 6 x 8 cm) from the scapular region of 10 test guinea pigs and 5 naive control animals was clipped prior to treatment. Three pairs of intradermal injections (0.1 mL/site) were made into a 4 x 6 cm clipped area on the guinea pigs on day one. The injectables were Freund's complete adjuvant (diluted with equal volume of physiological saline), 5% test material in PEG 400, and 5% test material in a 1:1 v/v mixture of Freund's complete adjuvant with physiological saline. One week after the injections, the same scapular region was clipped and shaved and approximately 0.3 g of test material (25% in vaselinum album), absorbed onto 2 x 4 cm filter paper, was applied to the intradermal injection area under occlusion for 48 hours. The vehicle control animals were treated similarly to the test animals with the exception that the test material was omitted from the intradermal injections and topical application. Two week after the epidermal induction, the animals were challenged. The flanks of the test animals and the control animals were clipped just prior to challenge. On day 22, the animals were challenged with one patch of filter paper saturated with 0.2 g of 1% test material in vaselinum album (left flank) and another patch of filter paper saturated with vaselinum album (right flank) using the same procedure as the topical induction for 24 hours. Approximately 21 hours after removal of the patches, the test sites treated with the test material were depilated with an approved depilatory cream (VEET Cream). The sites were evaluated 24 and 48 hours post exposure.

II. RESULTS:

1. **Mortality:** No deaths were observed in any group.

2. **Body Weight:** One animal (No. 669) of the test group lost weight during the treatment period. All other guinea pigs in the main study group gained weight during the study.

3. **Skin Effects:** Very slight erythema and well defined erythema were noted on 9/10 and 1/10 animals respectively at both 24 and 48 hours after the epidermal induction. No reaction was noted on any control or test animal after challenge. An alpha-hexylcinnamaldehyde positive control study carried out within six months of the current study was presented. The results were appropriate.

TABLE 1. Summary of Individual Erythema Challenge Scores with Time [a]

Time	24 hours				48 hours			
Erythema Score	0	0.5	1	2	0	0.5	1	2
Test	10	0	0	0	10	0	0	0
Control	5	0	0	0	5	0	0	0

[a]Number of animals affected

Evaluation score is based on the Draize Scale.

Scale for Scoring Skin Reaction

ERYTHEMA AND ESCHAR FORMATION:	Score
No erythema	0
Very faint slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4
EDEMA FORMATION:	
No edema	0
Very slight edema (barely perceptible)	1
Slight edema (edges of area well defined by definite raising)	2
Moderate edema (raised approximately 1 mm)	3
Severe edema (raised more than 1 mm and extending beyond the area of exposure)	4

III. DISCUSSION:

After intradermal and epidermal inductions, the the test and control animals showed no signs of reactivity at 24 and 48 hours after challenge. The study included an alpha-hexylcinnamaldehyde positive control study which was carried out within six month of the study and the results were appropriate. CNEW was not a dermal sensitizer. The packet is classified as **ACCEPTABLE.**

DATA EVALUATION RECORD

THYMOL
(APIGUARD)

STUDY TYPE: Magnitude of Residues in Honey and Beeswax

MRID 46043510

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 04-18

Primary Reviewer:
Eric B. Lewis, M.S.

Signature:_____
Date: _____

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature:_____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature:_____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:_____
Date: _____

Disclaimer

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Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Magnitude of Residues in Honey and Beeswax
MRID NO:	46043510
DP BARCODE:	DP300905
CASE NO:	Not provided
SUBMISSION NO:	Not provided
TEST MATERIAL:	Thymol
STUDY NO:	N/A
SPONSOR:	VITA (Europe) Ltd., c/o Landis International, Inc., Valdosta, GA 31603-5126
TESTING FACILITY:	RCC, Ltd., Environmental and Chemistry Division, CH-4452 Itingen, Switzerland
TITLE OF REPORT:	Summary of Three Studies on Determination of Residues of Thymol in/on Samples of Honey and Bees Wax, RCC Project Numbers, 668316, 708366 and 702663, Conducted in Europe in 1997 and 1998 After Application of Apiguard to Beehives
AUTHOR:	Hattermann, D.
STUDY COMPLETED:	N/A
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed GLP statement was provided. The study was summary of previous studies, and is not subject to GLP standards.

STUDY SUMMARY:	Results of previously-determined thymol residues in/on samples of honey and beeswax (MRIDs 46043511, 46043512, and 46043513) collected at 0 to 103 days after treatment of beehives with Apiguard are summarized. Treatments consisted of two or three applications of Apiguard (total thymol = 25 or 37.5 g) to the brood frames at 10 or 15 day intervals. The overall concentration of thymol in honey ranged from <0.03 to 4.61mg/kg, with a mean of 1.22 mg/kg. The overall concentration in beeswax ranged from 0.75 to 682.83 mg/kg, with a mean of 55.45 mg/kg. Concurrent recoveries were within the acceptable range of 70% to 120%. Overall thymol residues in honey and beeswax receiving either 2 or 3 applications appeared to be comparable.
CLASSIFICATION:	Acceptable

Introduction

Results from three studies that previously determined thymol residue in/on samples of honey and beeswax (MRIDs 46043511, 46043512, and 46043513) from hives treated with Apiguard are summarized in MRID 46043510. The hives were treated in Europe in 1997 and 1998. Two applications (total thymol = 25 g) in trays were made 15 days apart to brood frames in all three trials. In addition, one of the trials (3B-217) tested three applications at 10 day intervals (total thymol = 37.5 g). Apiguard was added in trays at the top or bottom of the brood frames. The supers were placed on the brood nest at the end of the treatment. In trial 3B-214, samples were collected from the brood nest on the last day of treatment and from the super 30 days after treatment. In trial 3B-215, samples were collected from the brood nest on the last day of treatment and from the super 103 days after treatment. In trial 3B-217, samples were collected from the brood nest 2 days and 14 days following treatment. No other details of the treatments were provided.

Results Summary

The thymol concentration in the samples was determined using gas chromatography with flame ionization detection (details of the method are provided in any of MRIDs 46043511-46043513). Overall results are provided in Table 1. Thymol in the honey samples ranged from <LOQ to 4.61 ppm, with a mean of 1.22 ppm. Thymol in beeswax samples ranged from 0.75 to 683 ppm. Concurrent recoveries in fortified samples ranged from 73.9 to 116.9% for honey and from 72.0 to 95.9% for beeswax.

TABLE 1. Thymol residues in honey and beeswax from hives treated with thymol						
Study no.	N	Days after treatment	Thymol conc. in honey (mg/kg)	Range (mg/kg) in honey	Thymol conc. in wax (mg/kg)	Range (mg/kg) in wax
3B-214[a] 2 applications	10	0	0.86 ± 0.69	0.20-2.14	97.62 ± 207.68	1.39-682.83
	10	30	0.10 ±0.08	<LOQ-0.26	5.64 ± 6.18	0.75-20.59
3B-215[b] 2 applications	10	0	1.64 ± 0.74	0.99-3.62	111.69 ± 76.21	22.68-263.72
	4	103	1.08 ± 0.27	0.86-1.41	-	-
3B-215[b] 2 applications	10	0	1.20 ±0.31	0.62-1.68	78.00 ± 97.77	8.81-291.85
	2	103	1.38 ± 0.14	1.28-1.48	-	-
3B-217[c] 2 applications	3	2	1.70 ± 2.53	<LOQ-4.61	26.74 ± 24.87	1.83-51.56
	3	14	0.23 ± 0.39	<LOQ-0.68	3.20 ± 3.26	1.18-6.96
3B-217[d] 3 applications	2	0	2.22 ± 0.37	1.95-2.48	100.70 ± 104.26	26.97-174.42
	2	14	1.8 ± 0.32	1.57-2.02	20.02 ± 26.04	1.60-38.43
Summary	56		mean =1.22	<LOQ-4.61	mean =55.45	0.75-682.83

Data from p. 5, MRID 46043510
N=number of samples
LOQ=limit of quantitation (0.03 mg/kg)
[a]Two applications on top of frames at 15 day interval, total thymol=25 g
[b]Two applications on bottom of frames at 15 day interval, total thymol=25 g
[c]Two applications of 1 tray at 15 day interval, total thymol=25 g
[d]Three applications of 1 tray at 10 day intervals, total thymol=37.5 g

Study Author's Conclusions

The study author concluded that the data were variable, but there did not appear to be a significant difference in thymol residues among the different treatments for honey or beeswax samples.

Reviewer's Conclusion

Based on the information presented, the reviewer agrees with the study author's conclusion. The reviewer notes that while the study author states that no significant differences in thymol residues among treatments were apparent, results from a statistical analysis were not provided.

DATA EVALUATION RECORD

THYMOL
(APIGUARD)

STUDY TYPE: Magnitude of Residues in Honey and Beeswax

MRID 46043511

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 04-18

Primary Reviewer:
Eric B. Lewis, M.S.

Signature:_____
Date: _____

Secondary Reviewers:
Sylvia Milanez, Ph.D., D.A.B.T.

Signature:_____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature:_____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:_____
Date: _____

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Magnitude of Residues in Honey and Beeswax
MRID NO:	46043511
DP BARCODE:	DP300905
CASE NO:	Not provided
SUBMISSION NO:	Not provided
TEST MATERIAL:	Thymol
STUDY NO:	668316
SPONSOR:	VITA (Europe) Ltd., c/o Landis International, Inc., Valdosta, GA 31603-5126
TESTING FACILITY:	RCC, Ltd., Environmental and Chemistry Division, CH-4452 Itingen, Switzerland
TITLE OF REPORT:	Determination of Residues of Thymol in/on Samples of Honey and Bees Wax
AUTHOR:	Wies, A.
STUDY COMPLETED:	November 16, 1998
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed GLP statement was provided. The study was not GLP compliant, but was compliant with GLP for Switzerland and complaint with OECD GLP.
STUDY SUMMARY:	Samples of honey and beeswax were analyzed for residues of thymol using gas chromatography with flame ionization detection. The limit of quantitation (LOQ) for both matrices was 0.030 mg/kg, and the mean recovery in samples fortified at the LOQ (n=9) was 82.0 ± 9.2%, with a coefficient of variation of 11.2%. Thymol residues in/on the honey test samples (n=30) ranged from <0.03 to 8.99 mg/kg, and in/on the beeswax test samples (n=30) from 0.07 to 682.83 mg/kg.
CLASSIFICATION:	Acceptable

Test Material

The test substance, Thymol, Batch No. Aldrich 61028029, consisted of colorless crystals supplied by the sponsor with a reported 98% purity. It was stored in a well-closed container away from light prior to the test. The expiration date of April 17, 1998 was assigned by the study director. Based on the expiration date, the analyses were performed within the expected shelf life.

Thirty samples each of beeswax and honey from Apiguard beehive trials conducted on behalf of the sponsor were received at ambient temperature and maintained at room temperature for approximately 2 months, then frozen until analysis. Control honey was a commercial Swiss product; control beeswax was supplied by the sponsor.

Test Methods

Standard solutions were prepared by dissolving the thymol in acetone to obtain a stock solution of 500 μg/mL, which was in turn diluted with hexane/acetone (800/200 mL) to obtain standard solutions of 100 to 0.05 μg thymol/mL. The solutions were frozen prior to use. To validate the method, untreated samples were fortified with different volumes of the standard solutions.

Beeswax and honey samples (1.5 g each) were weighed into a flask (fortified samples were spiked at this stage), to which 150 mL distilled water and 2 mL hydrochloric acid were added. The flask was placed in a heating mantle and connected to a distillation bridge and an Erlenmeyer receiving flask. The mixture was heated until 100 mL of distillate was collected in the receiver (1-2 hrs). The distillate was adjusted to pH >10 with ~1M sodium hydroxide solution, transferred to a separatory funnel, and partitioned with 50 mL hexane. The aqueous phase was drained back into the Erlenmeyer flask and the upper hexane phase was discarded.

The solution was transferred back to the separatory funnel and 50 mL dichloromethane was added. After partitioning and phase separation, the lower dichloromethane phase was drained through a funnel containing 20-30 g sodium sulphate into a 250 mL flask and the liquid/liquid separation was repeated. The dichloromethane was evaporated to 2 to 3 mL on a rotary evaporator, and the remaining solvent was evaporated into a stream of nitrogen.

Bakerbond cartridges were connected to the solid-phase extraction manifold and 10 to 20 mL of hexane were drawn through until the adsorbent became transparent. Hexane (5 mL) was added to the sample residue in the flask, which was placed in an ultrasonic bath prior to being transferred to the cartridge and allowed to move into the adsorbent. The cartridge was treated with 10 mL hexane/dichloromethane (950/50 mL) mixture. The thymol was eluted with 20 mL hexane/dichloromethane (700/300 mL) and collected in a volumetric cylinder. The eluate was transferred to a 50 mL conical flask using 5-10 mL dichloromethane as a rinse and concentrated to 2-3 mL on a rotary evaporator. Remaining solvent was removed in a nitrogen stream, and the residue was reconstituted with 0.5 mL hexane/acetone (800/200 mL). This solution was analyzed using gas chromatography with flame ionization detection.

The gas chromatograph was a Hewlett-Packard 5890 Ser. II with a 30 m x 0.25 mm DB 1701 column and a HP MS5971A ChemStation detector. The flame ionization detection

temperature was 280°C. Typical chromatograms of a standard solution, fortified sample, and test sample were provided in MRID 46043511.

Injected samples were quantified by peak height, with reference to the calibration curve (p. 25 of MRID 46043511) that was obtained by correlating the peak height (in counts) of the analytical standards with their corresponding concentrations in µg/mL. Samples and standards were injected in duplicate. The correlation was performed using a least squares fit of a non-linear function. Thymol residues and percent recovery were then calculated.

Results Summary

The limit of quantitation for both matrices was 0.030 mg/kg. Recovery in honey samples (n=5) fortified with 0.030 mg thymol/kg ranged from 73.9 to 89.6%, with a mean recovery of 80.2 ± 6.8% and a coefficient of variation (cv) of 8.5%. In the fortified beeswax samples (n=4), recovery ranged from 72.0% to 95.9%, with a mean of 84.2 ± 12.3% and a cv of 14.7%. Overall results for recovery in fortified samples of both matrices was 82.0 ± 9.2%, with a cv of 11.2%. No thymol was detected in the controls.

Thymol residues in/on the honey test samples ranged from <0.03 to 8.99 mg/kg, and in/on the beeswax test samples ranged from 0.07 to 682.83 mg/kg.

Study Author's Conclusions

The study author did not draw any conclusions.

Reviewer's Conclusion

The reviewer believes the method accurately determines the amount of thymol residue in samples of honey and beeswax at the concentrations tested.

DATA EVALUATION RECORD

THYMOL
(APIGUARD)

STUDY TYPE: Magnitude of Residues in Honey and Beeswax

MRID 46043512

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 04-18

Primary Reviewer:
Eric B. Lewis, M.S.

Signature:_____
Date: _____

Secondary Reviewers:
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Signature:_____
Date: _____

Robert H. Ross, M.S., Group Leader

Signature:_____
Date: _____

Quality Assurance:
Lee Ann Wilson, M.A.

Signature:_____
Date: _____

Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

Oak Ridge National Laboratory managed and operated by UT-Battelle, LLC., for the U.S. Department of Energy under Contract No. DE-AC05-00OR22725.

DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Magnitude of Residues in Honey and Beeswax
MRID NO:	46043512
DP BARCODE:	DP300905
CASE NO:	Not provided
SUBMISSION NO:	Not provided
TEST MATERIAL:	Thymol
STUDY NO:	708366
SPONSOR:	VITA (Europe) Ltd., c/o Landis International, Inc., Valdosta, GA 31603-5126
TESTING FACILITY:	RCC, Ltd., Environmental and Chemistry Division, CH-4452 Itingen, Switzerland
TITLE OF REPORT:	Determination of Residues of Thymol in/on Samples of Honey and Bees Wax
AUTHOR:	Wies, A.
STUDY COMPLETED:	June 28, 1999
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed GLP statement was provided. The study was not GLP compliant, but was compliant with GLP for Switzerland and complaint with OECD GLP.

STUDY SUMMARY: Twenty-six samples of honey and twenty samples of beeswax were analyzed for residues of thymol using gas chromatography with flame ionization detection. The limit of quantitation for both matrices was previously found to be 0.030 mg/kg, but due to matrix interference and high concentrations in the samples, the lowest fortification level was 3.0 mg/kg for honey and 30 mg/kg for beeswax. Thymol recovery in fortified honey samples (n=5) ranged from 88.7 to 116.9%, with a mean of 100.5 ± 10.8% and a coefficient of variation (cv) of 10.7%. Recoveries in fortified beeswax samples (n=2), were 75.8 and 84.6%, for a mean of 80.2 ± 6.2% and a cv of 7.8%. Overall results for recovery in fortified samples of both matrices was 94.7 ± 13.5%, with a cv of 14.2%. No thymol was detected in the controls. Thymol residues in/on honey test samples (n=26) ranged from 0.86 to 3.62 mg/kg, and in/on the beeswax test samples (n=20) from 8.81 to 292 mg/kg.

CLASSIFICATION: Acceptable

Test Material

The test substance, Thymol, Lot No. Haarmann & Reimer 8020016, consisted of colorless to white crystals supplied by the sponsor with a reported >98% purity. It was reportedly protected from atmospheric moisture and water prior to the test. The expiration date of September 23, 1999 was assigned by the study director. Based on the expiration date, the analyses were performed within the expected shelf life.

Samples of beeswax and honey from trials conducted on behalf of the sponsor were received at ambient temperature and stored frozen until analysis. Control honey was a commercial Swiss product; control beeswax was supplied by the sponsor.

Test Methods

Standard solutions were prepared by dissolving thymol in hexane/acetone (800/200 mL) to obtain a stock solution of 99.17 µg/mL. Defined volumes of the stock solution were diluted with hexane/acetone to obtain standard solutions ranging from 50 to 0.05 µg thymol/mL. The solutions were frozen prior to use. To validate the method, untreated samples were fortified with different volumes of the standard solutions.

Beeswax and honey samples (1.5 g each) were weighed into a flask (fortified samples were spiked at this stage), to which 150 mL distilled water and 2 mL hydrochloric acid were added. The flask was placed in a heating mantle and connected to a distillation bridge and an Erlenmeyer receiving flask. The mixture was heated until 100 mL of distillate was collected in the receiver (1-2 hrs). The distillate was adjusted to pH >10 with 1M sodium hydroxide solution, transferred to a separatory funnel, and partitioned with 50 mL hexane. The aqueous phase was drained back into the Erlenmeyer flask and the upper hexane phase was discarded.

The solution was transferred back to the separatory funnel and 50 mL dichloromethane was added. After partitioning and phase separation, the lower dichloromethane phase was drained through a funnel containing 20-30 g sodium sulphate into a 250 mL flask and the liquid/liquid separation was repeated. The dichloromethane was evaporated to 2 to 3 mL on a rotary evaporator, and the remaining solvent was evaporated into a stream of nitrogen.

Bakerbond cartridges were connected to the solid-phase extraction manifold and 10 to 20 mL of hexane were drawn through until the adsorbent became transparent. Hexane (5 mL) was added to the sample residue in the flask, which was placed in an ultrasonic bath prior to being transferred to the cartridge and allowed to move into the adsorbent. The cartridge was treated with 10 mL hexane/dichloromethane (950/50 mL) mixture. The thymol was eluted with 20 mL hexane/dichloromethane (700/300 mL) and collected in a volumetric cylinder. The eluate was transferred to a 50 mL conical flask using 5-10 mL dichloromethane as a rinse and concentrated to 2-3 mL on a rotary evaporator. Remaining solvent was removed in a nitrogen stream, and the residue was reconstituted with 0.5 mL hexane/acetone (800/200 mL). This solution was analyzed using gas chromatography with flame ionization detection.

The gas chromatograph was a Hewlett-Packard 5890 Ser. II with a 23 m x 0.25 mm DB 1701 column. The flame ionization detection temperature was 280°C. Typical chromatograms of a standard solution, fortified sample, and test sample were provided in MRID 46043512.

Injected samples were quantified by peak height, with reference to the calibration curve (p. 24 of MRID 46043512) that was obtained by correlating the peak height (in counts) of the analytical standards with their corresponding concentrations in μg/mL. Samples and standards were injected in duplicate. The correlation was performed using a least squares fit of a non-linear function. Thymol residues and percent recovery were then calculated.

Results Summary

The limit of quantitation for both matrices was previously validated as 0.030 mg/kg. Due to matrix interference and high concentrations in the samples, the lowest fortification level was 3.0 mg/kg for honey and 30 mg/kg for beeswax. Recovery in honey samples (n=5) fortified with 3.0 mg thymol/kg ranged from 88.7 to 116.9%, with a mean recovery of 100.5 ± 10.8% and a cv of 10.7%. In the fortified beeswax (n=2), recoveries were 75.8 and 84.6%, for a mean of 80.2 ± 6.2% and a cv of 7.8%. Overall results for recovery in fortified samples of both matrices was 94.7 ± 13.5%, with a cv of 14.2%. No thymol was detected in the controls.

Thymol residues in/on honey test samples (n=26) ranged from 0.86 to 3.62 mg/kg, and in/on the beeswax test samples (n=20) from 8.81 to 292 mg/kg.

Study Author's Conclusions

The study author did not draw any conclusions.

Reviewer's Conclusion

The reviewer believes the method accurately determines the amount of thymol residue in samples of honey and beeswax at the concentrations tested.

DATA EVALUATION RECORD

THYMOL
(APIGUARD)

STUDY TYPE: Magnitude of Residues in Honey and Beeswax

MRID 46043513

Prepared for
Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
1801 Bell Street
Arlington, VA 22202

Prepared by
Toxicology and Hazard Assessment Group
Life Sciences Division
Oak Ridge National Laboratory
Oak Ridge, TN 37830
Task Order No. 04-18

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Disclaimer

This review may have been altered subsequent to the contractor's signatures above.

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DATA EVALUATION RECORD

EPA Secondary Reviewer:

STUDY TYPE:	Magnitude of Residues in Honey and Beeswax
MRID NO:	46043513
DP BARCODE:	DP300905
CASE NO:	Not provided
SUBMISSION NO:	Not provided
TEST MATERIAL:	Thymol
STUDY NO:	702663
SPONSOR:	VITA (Europe) Ltd., c/o Landis International, Inc., Valdosta, GA 31603-5126
TESTING FACILITY:	RCC, Ltd., Environmental and Chemistry Division, CH-4452 Itingen, Switzerland
TITLE OF REPORT:	Determination of Residues of Thymol in/on Samples of Honey and Bees Wax
AUTHOR:	Wies, A.
STUDY COMPLETED:	June 29, 1999
CONFIDENTIALITY CLAIMS:	None
GOOD LABORATORY PRACTICE:	A signed GLP statement was provided. The study was not GLP compliant, but was compliant with GLP for Switzerland and complaint with OECD GLP.
STUDY SUMMARY:	Twelve samples each of honey and beeswax were analyzed for residues of thymol using gas chromatography with flame ionization detection. The limit of quantitation was 0.030 mg/kg for honey and 30.0 mg/kg for beeswax. Thymol recovery in fortified honey samples (n=3) was 108.1 ± 9.2%, with a coefficient of variation (cv) of 8.5%. Recovery in fortified beeswax (n=2) was 75.0 ± 0.8%, with a cv of 1.0%. Overall recovery in fortified samples of both matrices was 94.9 ± 19.3%, with a cv of 20.0%. No thymol was detected in the controls. Thymol residues in/on honey test samples (n=12) ranged from <0.03 to 4.61 mg/kg, and in/on the beeswax samples (n=12) from 0.96 to 174 mg/kg.
CLASSIFICATION:	Acceptable

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[redacted]

The gas chromatograph was a Hewlett-Packard 5890 Ser. II with a 23 m x 0.25 mm DB 1701 column. The flame ionization detection temperature was 280°C. Typical chromatograms of a standard solution, fortified sample, and test sample were provided in MRID 46043513.

Injected samples were quantified by peak height, with reference to the calibration curve (p. 24 of MRID 46043513) that was obtained by correlating the peak height (in counts) of the analytical standards with their corresponding concentrations in µg/mL. Samples and standards were injected in duplicate. The correlation was performed using a least squares fit of a non-linear function. Thymol residues and percent recovery were then calculated.

Results Summary

The limit of quantitation for honey was 0.030 mg/kg and for beeswax was 30.0 mg/kg, due to matrix interference and high concentrations in the samples. Overall mean recovery in honey samples (n=3) fortified with 0.030 or 5.000 mg thymol/kg was 108.1 ± 9.2%, with a cv of 8.5%. Overall mean recovery in beeswax (n=2) fortified with 30.00 or 175.0 mg thymol/kg was 75.0 ± 0.8%, with a cv of 1.0%. Overall recovery in fortified samples of both matrices was 94.9 ± 19.3%, with a cv of 20.0%. No thymol was detected in the controls.

Thymol residues in/on honey test samples (n=12) ranged from <0.03 to 4.61 mg/kg, and in/on the beeswax samples (n=12) from 0.96 to 174 mg/kg.

Study Author's Conclusions

The study author did not draw any conclusions.

Reviewer's Conclusion

The reviewer believes the method accurately determines the amount of thymol residue in samples of honey and beeswax at the concentrations tested.