

US EPA ARCHIVE DOCUMENT

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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

March 28, 1997

Memorandum

Subject: Ecological Toxicity Review for Potassium Salts of Phosphorus Acid

From: Frank W. Ellis, Jr., Entomologist *Frank W. Ellis Jr.*
Biopesticides and Pollution Prevention Division (7501W)

Thru: Roy D. Sjoblad, Ph.D., Microbial Team Leader *Roy D. Sjoblad*
Biopesticides and Pollution Prevention Division (7501W)

To: Rita Kumar, Regulatory Action Leader
Biopesticides and Pollution Prevention Division (7501W)

Action: 145 New Biol-Non-Food/Feed
DP Barcode: D226397
ID#: 069579-R
MRID #: 439058-12, 13, 14, 16, 17

Case: Registration
Submission: S505790
Chemical #: 076416 mono- and di-potassium
salts of phosphorous acid

Action Requested

BPPD has been requested to review the ecological toxicity package studies submitted by U.I.M. Agrochemicals Pty. Ltd. These studies support the registration of their end-use product, Foli-R-Fos®, which contains potassium salts of phosphorous acid. The primary reviews were conducted by Oak Ridge National Laboratory.

Conclusions

Guidelines 154-6, 7, 8, and 9 are adequately fulfilled by the submitted studies. The studies indicate that use of the product should result in no significant adverse effects on avian or aquatic organisms. The freshwater invertebrate study raised questions regarding potential sublethal effect of the product on *Daphnia*. However, the finding that the product is, at most, slightly toxic to *Daphnia* is unchallenged and label language is proposed to prevent direct application to water.

While the honey bee study addressing Guideline 154-11 is classified as supplemental, it does not have to be repeated. The data presented in that study are adequate to assess the acute toxicity of the product to honey bees.

Discussion

The proposed use of Foli-R-Fos® is as a systemic fungicide for the suppression of *Phytophthora* and *Pythium* in ornamentals and bedding plants, conifers, and turf. The proposed label includes the following statement under the Environmental Hazards section:

Do not apply directly to water, to areas where surface water is present or to intertidal areas below the mean high water mark. Do not contaminate water when disposing of rinsate or equipment washwaters.

The following table summarizes the results from studies submitted in accordance with the Subdivision M Guidelines Series 154-6, 7, 8, 9, 11 (nontarget organism fate and expression Tier 1 data requirements) and their respective risk evaluation from the data evaluation reports. A discussion of studies with slight adverse risks is also provided.

| Guideline # | Type of Test | MRID # | Findings | Classification |
|-------------|---|-----------|--|-------------------|
| 154-6 | avian acute oral (bobwhite quail) | 439058-12 | LD ₅₀ > 2250 mg/kg NOEL = 1350 mg/kg Practically non-toxic | acceptable (core) |
| 154-7 | avian dietary (bobwhite quail) | 439058-13 | LC ₅₀ > 5620 ppm NOEC ≥ 5620 ppm Practically non-toxic | acceptable (core) |
| 154-8 | freshwater fish LC ₅₀ (rainbow trout) | 439058-14 | LC ₅₀ > 96.4 mg wm/L NOEL ≥ 96.4 mg wm/L Practically non-toxic | acceptable (core) |
| 154-9 | freshwater invertebrate LC ₅₀ (<i>Daphnia magna</i>) | 439058-16 | EC ₅₀ > 100 mg wm/L NOEC < 100 mg wm/L - (requires explanation) Slightly or non-toxic practically | acceptable (core) |
| 154-11 | nontarget organism testing (honeybee) | 439058-17 | LD ₅₀ > 100 µg/bee NOEC inconclusive Slightly toxic practically non-toxic (see next page) | supplemental |

Guideline 154-9: Freshwater Invertebrate LC₅₀

While the submitted study was acceptable and adequately assessed the acute toxicity of the test substance to *Daphnia*, the following point was raised in the data evaluation:

“Further explanation is needed regarding the 2 floating daphnids in test chambers with the test material. Was any effort made to determine if the daphnids were capable of submerging? If so, this should be noted in the report. If not, it may be necessary to run further tests to determine if there are indeed sublethal effects.”

Resolving this question would clarify the findings of the study, however, it would not significantly alter the findings that the test substance is, at most, slightly toxic to daphnids. Additionally, the language under the Environmental Hazards section of the label is adequate, in conjunction with the submitted study, to address all freshwater invertebrate concerns.

Guideline 154-11: Nontarget Organism Testing

The submitted study was classified as supplemental for the following reason:

“Due to the high incidental mortality of honeybees in these types of tests (e.g., 8% in both negative and solvent control groups), more bees should be tested per group in order to provide a clearer statistical picture of the results. Although 10% mortality is acceptable in some studies, it has been demonstrated that honeybees can be tested outside the hive environment with only 8% control mortality for up to 30 days. Control mortalities of 8% seem extreme in a 48-hour test.”

It was stated that the study could be repaired in the following manner:

“Repeat the tests using a greater number of bees (at least 50 bees/group) to provide conclusive results for sublethal effects and no-mortality levels. Comparisons between groups should be made statistically rather than by general observation.”

While the results of the study did not permit the calculation of an LC_{50} for the test substance, it did demonstrate that this value was greater than the maximum tested dose of 100 $\mu\text{g}/\text{bee}$. This indicates that the potassium salts of phosphorus acid are ~~slightly toxic~~ to honey bees.

practically no-toxic gth 4/17/97

DATA EVALUATION REPORT

POTASSIUM SALTS OF PHOSPHOROUS ACID

STUDY TYPE: AVIAN SINGLE-DOSE ORAL LD₅₀ TEST - BOBWHITE QUAIL (71-1)

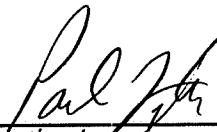
Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Crystal Station I
2800 Jefferson Davis Highway
Arlington, VA 22202

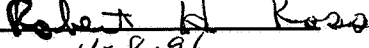
Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

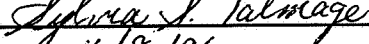
Primary Reviewer:
Paul G. Forsyth, Ph.D.

Signature: 
Date: 11-11-96

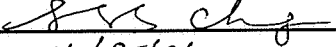
Secondary Reviewers:
Robert H. Ross, M.S., Group Leader

Signature: 
Date: 11-8-96

Sylvia S. Talmage, Ph.D., D.A.B.T.

Signature: 
Date: 11/8/96

Quality Assurance:
Susan Chang, M.S.

Signature: 
Date: 11/8/96

Disclaimer

This Data Evaluation Report may have been altered by the Biopesticides and Pollution Prevention Division subsequent to signing by Oak Ridge National Laboratory personnel.

POTASSIUM SALTS OF PHOSPHOROUS ACID

Avian Single-Dose Oral LD₅₀ Test (71-1)

EPA Reviewer: Frank W. Ellis, Jr., M.S.
Biopesticides and Pollution Prevention Division
EPA Team Leader: Roy D. Sjoblad, Ph.D.
Biopesticides and Pollution Prevention Division

Frank W. Ellis Jr.

Date: 3/14/97

Date: _____

DATA EVALUATION REPORT

MRID# & TITLE OF STUDY: MRID 43905812, Potassium Salts of Phosphorus Acid: An Acute Oral Toxicity Study with the Northern Bobwhite. FIFRA Guideline 71-1.

DP BARCODE: D226397
REG./FILE#: 069579-R

CASE: 046750
CHEMICAL/BIOLOGICAL#: 076416 Mono- and di-potassium salts of phosphorous acid

COMPANY/SPONSOR: U.I.M. Agrochemicals (Aust.) Pty. Ltd.
P.O. Box 72, Brisbane Markets, Queensland, Australia 4106

TEST MATERIAL: Identified as potassium salts of phosphorous acid

TEST FACILITY: Wildlife International, Ltd., 8598 Commerce Drive, Easton, MD.

REVIEW CONCLUSION: This study was conducted according to prescribed procedures, is acceptable, and determined that the acute oral LD₅₀ for this formulation of potassium salts of phosphorus acid (41.0%) to northern bobwhite quail is >2250 mg/kg and the no-observed-effect dosage is 1350 mg/kg.

RECOMMENDATIONS: On page 10 under heading "Dosing Solution Analyses," change units of dosing solution analyses from "mg a.i./kg" to "mg a.i./mL."

ADEQUACY OF STUDY: Core

REPORTED MATERIALS & METHODS: The study procedures followed those of Section 71-1, EPA Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms and were conducted following GLP regulations 40 CFR 160; OECD Guidelines for Testing of Chemicals (ISBN 92-84-12367-9); and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau. The test substance was a clear liquid, identified on the label as "m-dkP BRAND OF foli-r-fOS 400 FUNGICIDE; ACTIVE CONSTITUENT 400 g/L PHOSPHOROUS ACID; pH 5.7-6.0." The reported purity of the substance was 41.0% (wt/vol). The test substance was stored under refrigerated conditions.

Groups of 10 birds (5 per sex) received a single dose of the test substance diluted with deionized water. Liquid volumes given to each bird corresponded to 4 mL/kg body weight. Nominal dosages were 292, 486, 810, 1350, and 2250 mg/kg of the test substance with no correction for purity (i.e., dosages were not based on active ingredient). Control birds were treated with deionized water. The test material was intubated directly into the crop or proventriculus of each bird using a stainless steel cannula. The birds were observed for 14 days and weighed on days 3, 7, and 14. Average feed consumption was determined for each dosage group and the controls for day 0-3, 4-7, and 8-14. Samples of the dosing solutions were collected and shipped to Compliance Services International (Tacoma, WA) for dosage concentration verification. The study was conducted according to prescribed procedures and was acceptable.

REPORTED RESULTS: In the control group, one male was found dead on Day 3; this mortality was considered incidental. Two other birds exhibited injuries related to pen wear, and not attributable to treatment. There were no mortalities in any of the treatments. Sublethal effects in 4 birds (1 male, 3 females) in the 2250 mg/kg dosage group were shallow and rapid respiration immediately following dosing on Day 0; these effects were not observed after Day 0. No sublethal effects were noted in any other group. Treatments had no apparent effect on either body weights or food consumption. Dosing solutions were 103.3% to 112.6% of nominal concentrations and were considered satisfactory.

DISCUSSION: The study was conducted according to prescribed procedures, is acceptable, and determined that the formulation of potassium salts of phosphorus acid used in this study is practically nontoxic to northern bobwhite quail under these test conditions. The no-observed-effect dosage of potassium salts of phosphorus acid in this study was 1350 mg/kg and the LD₅₀ was >2250 mg/kg. Sublethal effects (shallow and rapid respiration) were observed only in 4 of 10 birds in the highest dosage group, indicating that any toxic effects in bobwhite quail from the test substance occurred only at very high levels.

DATA EVALUATION REPORT

POTASSIUM SALTS OF PHOSPHOROUS ACID

STUDY TYPE: AVIAN DIETARY LC₅₀ TEST - BOBWHITE QUAIL (71-2)

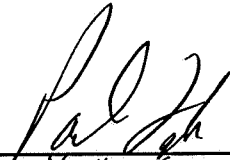
Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Crystal Station I
2800 Jefferson Davis Highway
Arlington, VA 22202

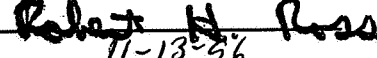
Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

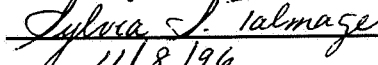
Primary Reviewer:
Paul G. Forsyth, Ph.D.

Signature: 
Date: 11-11-96

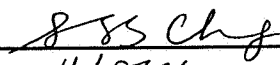
Secondary Reviewers:
Robert H. Ross, M.S., Group Leader

Signature: 
Date: 11-13-96

Sylvia S. Talmage, Ph.D., D.A.B.T.

Signature: 
Date: 11/8/96

Quality Assurance:
Susan Chang, M.S.

Signature: 
Date: 11/8/96

Disclaimer


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7

POTASSIUM SALTS OF PHOSPHOROUS ACID

Avian Dietary LC₅₀ Test (71-2)

EPA Reviewer: Frank W. Ellis, Jr., M.S.
Biopesticides and Pollution Prevention Division
EPA Team Leader: Roy D. Sjoblad, Ph.D.
Biopesticides and Pollution Prevention Division



Date: 3/14/97

Date: _____

DATA EVALUATION REPORT

MRID# & TITLE OF STUDY: MRID 43905813, Potassium salts of phosphorus acid: A dietary LC₅₀ study with the northern bobwhite. FIFRA Guideline 71-2, OECD Guideline 205.

DP BARCODE: D226397

CASE: 046750

REG./FILE#: 069579-R

CHEMICAL/BIOL#: 076416 Mono- and di-potassium salts of phosphorus acid

COMPANY/SPONSOR: U.I.M. Agrochemicals (Aust.) Pty. Ltd.,
P.O. Box 72, Brisbane Markets, Queensland, Australia 4106

TEST MATERIAL: Identified as potassium salts of phosphorous acid

TEST FACILITY: Wildlife International, Ltd., 8598 Commerce Drive, Easton, MD

REVIEW CONCLUSION: This study was conducted according to prescribed procedures, is acceptable, and determined that the oral LC₅₀ of this formulation of potassium salts of phosphorous acid to the northern bobwhite is >5620 ppm. The no observed effect dietary concentration is greater than or equal to 5620 ppm.

RECOMMENDATIONS: None

ADEQUACY OF STUDY: Core

REPORTED MATERIALS & METHODS: The study procedures followed those of Section 71-2, EPA Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms; OECD Guideline 205, Guideline for Testing of Chemicals, Avian Dietary Toxicity Test; and ASTM Standard E857-87 "Standard Practice for Conducting Subacute Dietary Toxicity Tests with Avian Species" and were conducted following GLP regulations 40 CFR 160; OECD, ISBN 92-84-12367-9; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau. The test substance was a clear liquid, identified on the label as "m-dkP BRAND OF foli-r-fOS 400 FUNGICIDE; ACTIVE CONSTITUENT 400 g/L PHOSPHOROUS ACID; ph 5.7-6.0." The reported purity of the test substance was 41 %.

Ten northern bobwhite (*Colinus virginianus*) chicks, all 10 days old, were randomly assigned to each treatment and control group. The test substance was administered to the chicks by mixing with a game bird ration and feeding *ad libitum* for 5 days. Nominal dietary test concentrations were 562, 1000, 1780, 3160, and 5620 ppm of the test substance with no correction for purity. Control chicks were fed only basal ration. Following the 5 day treatment, chicks were fed basal ration for an additional 3 days. Chicks were observed at least twice daily for 8 days for abnormal behavior, signs of toxicity, and mortality. Body weights were measured by group on Days 0, 5, and 8. Average feed consumption for each treatment and

control group was measured for Days 0-5 and Days 6-8. No mortalities throughout the tests eliminated the need for LC₅₀ calculations. Analyses were run by an indirect analytical method (Minnesota Valley Testing Laboratories), which measures the amount of phosphorous acid, on test rations to verify test substance concentrations. Samples were taken from the top, middle, and bottom of each batch of treated ration to test for homogeneity of the test substance throughout the batch. Samples of control ration were also analyzed.

REPORTED RESULTS: There were no mortalities, abnormal signs of behavior, or clinical signs of toxicity noted for any chicks in the test or control groups throughout the test. There appeared to be no treatment related effect on either feed consumption or body weights. Chemical analyses of rations indicated that the test substance was fairly homogeneously mixed, with average percent nominal levels for different strata within the batches ranging from 81.5 to 119.9 and the average percent nominal levels for entire batches ranging from 100.9 to 110.1. The LC₅₀ was greater than 5620 ppm. The no mortality and no observed effect concentrations were both greater than or equal to 5620 ppm.

DISCUSSION: This study was conducted according to prescribed procedures, is acceptable, and determined that the dietary LC₅₀ value for this formulation of potassium salts of phosphorus acid in the northern bobwhite is >5620 ppm. This value, coupled with no clinical symptoms at that level, indicates that this formulation of potassium salts of phosphorous acid is virtually non-toxic to the northern bobwhite under these test conditions.

DATA EVALUATION REPORT

POTASSIUM SALTS OF PHOSPHOROUS ACID

STUDY TYPE: FRESHWATER FISH ACUTE BIOASSAY - RAINBOW TROUT (154-8)

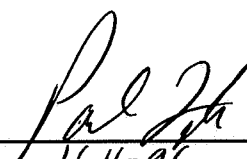
Prepared for

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Office of Pesticide Programs
U.S. Environmental Protection Agency
Crystal Station I
2800 Jefferson Davis Highway
Arlington, VA 22202

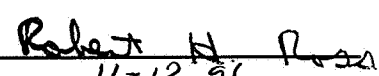
Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

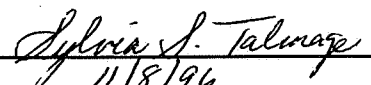
Primary Reviewer:
Paul G. Forsyth, Ph.D.

Signature: 
Date: 11-11-96

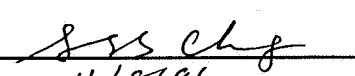
Secondary Reviewers:
Robert H. Ross, M.S., Group Leader

Signature: 
Date: 11-13-96

Sylvia S. Talmage, Ph.D., D.A.B.T.

Signature: 
Date: 11/8/96

Quality Assurance:
Susan Chang, M.S.

Signature: 
Date: 11/8/96

Disclaimer

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Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464

POTASSIUM SALTS OF PHOSPHOROUS ACID

Freshwater Fish Acute Bioassay (154-8)

EPA Reviewer: Frank W. Ellis, Jr., M.S.
Biopesticides and Pollution Prevention Division
EPA Team Leader: Roy D. Sjoblad, Ph.D.
Biopesticides and Pollution Prevention Division

Frank W. Ellis, Jr.

Date: 3/14/97

Date: _____

DATA EVALUATION REPORT

MRID# & TITLE OF STUDY: MRID 43905814, Potassium Salts of Phosphorous Acid: Acute Toxicity to Rainbow Trout, *Oncorhynchus mykiss*, Under Static Test Conditions. FIFRA Guideline 154-8.

DP BARCODE: D226397

CASE: 046750

REG./FILE#: 069579-R

CHEMICAL/BIOL#: 076416 Mono- and di-potassium salts of phosphorous acid

COMPANY/SPONSOR: U.I.M. Agrochemicals (Aust.) Pty. Ltd., P.O. Box 72, Brisbane Markets Queensland, Australia 4106

TEST MATERIAL: Identified as potassium salts of phosphorous acid

TEST FACILITY: Toxikon Environmental Services, 106 Costal Way, Jupiter, FL

REVIEW CONCLUSION: This study was conducted according to prescribed procedures, is acceptable, and determined that the 96-hour static freshwater LC₅₀ for this formulation of potassium salts of phosphorus acid (65.26%) to Rainbow trout (*Oncorhynchus mykiss*) is > 96.4 mg whole material (wm)/L and the no-observed-effect dosage is greater than or equal to 96.4 mg wm/L.

RECOMMENDATIONS: Clarify units for the reported purity of the test substance. It is reported as "65.26% m/v....". Is this mass/volume?

ADEQUACY OF STUDY: Core

MATERIALS & METHODS: The study procedures followed those of Section 154-8, EPA Assessment Guidelines, Subdivision E, Hazard Evaluation: Wildlife and Aquatic Organisms and were conducted following GLP regulations 40 CFR 160.105. The test substance (Batch #2244-3, Lot No. CSI #64-F5B) received from Compliance Services International (Tacoma, WA) was a clear, colorless liquid with a pH of 5.7 and specific gravity of approximately 1.38 (as reported by the Sponsor). The test substance was 410 g/L of phosphorus acid present as mono- and di-potassium phosphite (65.26% m/v potassium phosphite salts). The test substance was stored in the dark at ambient room temperature.

Three grams of the test substance (no correction for purity) were directly added to test chambers containing 30 L each of dilution water, giving a nominal test concentration of 100 mg wm/L. Groups of 10 randomly selected trout were placed into each test chamber. Each treatment group was replicated 3 times for a total of 30 trout per treatment. Test chambers were covered throughout the exposure period to reduce evaporation. Control trout were placed in dilution water only. The trout were monitored daily for any abnormalities in behavior or physical appearance and for mortality. All required physical parameters were

monitored daily. Water samples were removed from each test chamber and analyzed for the test substance by Toxikon Environmental Sciences (Jupiter, FL). Due to lack of any clinical symptoms, no statistical analysis was conducted. The study was conducted according to prescribed procedures and was acceptable.

REPORTED RESULTS: The mean measured concentration of potassium salts of phosphorous acid was 96.4 mg wm/L, which was 96% of nominal. One trout died in 1 control chamber after 72 hours, and 1 trout died in 1 test chamber after 96 hours, resulting in a 3% mortality rate for both control and test trout. No sublethal effects were observed. The 96-hour LC_{50} was >96.4 mg wm/L. Based on the lack of mortality above control levels and the absence of sublethal effects, the no-observed-effect concentration was 96.4 mg wm/L.

DISCUSSION: The study was conducted according to prescribed procedures, is acceptable, and determined that the formulation of potassium salts of phosphorous acid used in this study is practically nontoxic to Rainbow trout under these test conditions. The no-observed-effect dosage of potassium salts of phosphorous acid in this study was greater than or equal to 96.4 mg wm/L and the LD_{50} was >96.4 mg wm/L. Although there was measurable mortality in one test chamber, this mortality did not exceed that in control chambers and was considered incidental.

DATA EVALUATION REPORT

POTASSIUM SALTS OF PHOSPHOROUS ACID

STUDY TYPE: FRESHWATER AQUATIC INVERTEBRATE ACUTE
BIOASSAY - DAPHNIA MAGNA (154-9)


Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Crystal Station I
2800 Jefferson Davis Highway
Arlington, VA 22202

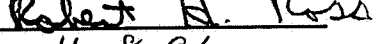
Prepared by

Chemical Hazard Evaluation Group
Biomedical and Environmental Information Analysis Section
Health Sciences Research Division
Oak Ridge National Laboratory
Oak Ridge, TN 37831

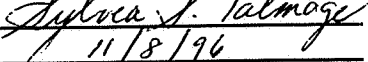
Primary Reviewer:
Paul G. Forsyth, Ph.D.

Signature: 
Date: 11-11-96

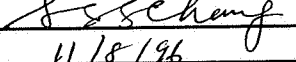
Secondary Reviewers:
Robert H. Ross, M.S., Group Leader

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Date: 11-8-96

Sylvia S. Talmage, Ph.D., D.A.B.T.

Signature: 
Date: 11/8/96

Quality Assurance:
Susan Chang, M.S.

Signature: 
Date: 11/8/96

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POTASSIUM SALTS OF PHOSPHOROUS ACID

Freshwater Aquatic Invertebrate Acute Bioassay (154-9)

EPA Reviewer: Frank W. Ellis, Jr., M.S.
Biopesticides and Pollution Prevention Division
EPA Team Leader: Roy D. Sjoblad, Ph.D.
Biopesticides and Pollution Prevention Division

Frank W. Ellis Jr.

Date: 3/14/97

Date: _____

DATA EVALUATION REPORT

MRID# & TITLE OF STUDY: MRID 43905816, Potassium Salts of Phosphorus Acid: Acute Toxicity to the Water Flea, *Daphnia magna*, Under Static Conditions. FIFRA Guideline 154-9, Subdivision M.

DP BARCODE: D226397
REG./FILE#: 069579-R

CASE: 046750
CHEMICAL/BIOL#: 076416, Mono- and di-potassium salts of phosphorous acid

COMPANY/SPONSOR: U.I.M. Agrochemicals (Aust.) Pty. Ltd., P.O. Box 72, Brisbane Markets Queensland, Australia 4106

TEST MATERIAL: Identified as potassium salts of phosphorous acid

TEST FACILITY: Toxikon Environmental Services, 106 Costal Way, Jupiter, FL

REVIEW CONCLUSION: This study was conducted according to prescribed procedures, is acceptable, and determined that the 48 hour EC₅₀ for this formulation of potassium salts of phosphorous acid to *Daphnia magna* under static conditions is >100 mg wm (whole material)/L. The NOEC may be <100 mg wm/L, pending further explanation. The test material is only very slightly toxic or non-toxic to daphnids under these test conditions.

RECOMMENDATIONS: Clarify units used for purity of test substance, reported as "65.25 % m/v....". Is this mass/volume? Further explanation is needed regarding the 2 floating daphnids in test chambers with the test material. Was any effort made to determine if the daphnids were capable of submerging? If so, this should be noted in the report. If not, it may be necessary to run further tests to determine if there are indeed sublethal effects.

ADEQUACY OF STUDY: Core

MATERIALS & METHODS: The study procedures followed those of U.S. EPA FIFRA Guideline 154-9, and were conducted in accordance with GLP regulations 40 CFR 160.105. The test substance (Batch #2244-3; Lot No. CSI #64-F5B), identified as potassium salts of phosphorous acid (Foli-R-Fos 400), was received from Compliance Services International (Tacoma, WA), and was a clear, colorless liquid with a pH of 5.7 and specific gravity of approximately 1.38 as reported by the Sponsor. The test substance was 410 g/L of phosphorus acid present as mono- and di-potassium phosphite (65.26 % m/v potassium phosphite salts) and was stored in the dark at ambient room temperature. The test organism, *Daphnia magna*, was a subculture of animals received from the U.S. EPA (Duluth, MN) in 1989. The cultures were daily fed the green algae, *Selenastrum capricornutum*, and a solution prepared from cereal leaves. Less than 24 hours prior to test initiation, the adults were reisolated in food-free dilution water and neonates (<24 hours old) were collected for the tests. The test animals were cultured and isolated in moderately hard

POTASSIUM SALTS OF PHOSPHOROUS ACID Freshwater Aquatic Invertebrate Acute Bioassay (154-9)

freshwater. Dilution water was moderately hard freshwater from the municipal water supply of the Town of Jupiter, Florida and was treated by vigorous aeration, filtration to 5 μm , passing through activated carbon and re-aerating prior to use. At test initiation the dilution water had a hardness of 66 mg/L as CaCO_3 , alkalinity of 12 mg/L as CaCO_3 , and specific conductivity of 382 micromhos per centimeter.

The test solution was prepared by adding 0.0999 g of the test chemical to a 1 L volumetric flask and bringing to volume with dilution water. Dilution water was used for controls. Ten water fleas were added to each 300 ml test chamber containing 200 ml of the test solutions (3 replicates each). A total of 30 water fleas was used for each solution. Test chambers were then covered to limit evaporation and placed into temperature-controlled environmental chambers which received 16 hours of fluorescent light and 8 hours of darkness each day. Survival and abnormal appearance or behavior were monitored daily for 48 hours. No feed was supplied during the test.

Samples (100 ml) were collected from the control and test solutions at test initiation and termination and analyzed for concentrations of potassium salts of phosphorous acid.

No mortality eliminated the need for statistical analyses.

REPORTED RESULTS: Test substance concentrations remained stable throughout the test and averaged 100 percent of the nominal concentration. There was no daphnid mortality in either test or control chambers after 48 hours exposure, resulting in an EC_{50} of >100 mg wm/L. The text reports no sublethal effects during the test, resulting in a no-observed-effect concentration (NOEC) of 100 mg wm/L. However, Table 2 (page 19) indicates that 2 daphnids were observed floating after 48 hours.

DISCUSSION: This study was conducted according to prescribed procedures with one minor deviation, is acceptable, and determined that the 48 hour EC_{50} for *Daphnia magna* exposed to this formulation of potassium salts of phosphorous acid under static conditions is >100 mg wm/L.

The authors report no sublethal effects; however, 2 daphnids were reported floating after 48 hours in chambers containing the test material while no floaters were observed in the controls. No explanation was given as to the cause of this behavior, or even if this behavior was abnormal. An effort to lower them into the water column with a drop of test solution should have been made. When 2 of 60 total test units exhibit a particular behavior, this behavior seems abnormal. Since the behavior occurred only in chambers containing the test material and not in control chambers, it could be considered a sublethal effect of the test material unless otherwise explained. Therefore, the NOEC of 100 mg wm/L, as reported by the authors, is questioned by the reviewer until further explanation is given. The NOEC may actually be somewhere between 0 and 100 mg wm/L. Even so, this formulation of potassium salts of phosphorous acid is only very slightly toxic to *Daphnia magna*.

DATA EVALUATION REPORT

POTASSIUM SALTS OF PHOSPHOROUS ACID

STUDY TYPE: ACUTE CONTACT LD₅₀ - HONEY BEE (141-1)

Prepared for

Biopesticides and Pollution Prevention Division
Office of Pesticide Programs
U.S. Environmental Protection Agency
Crystal Station I
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Arlington, VA 22202

Prepared by

Chemical Hazard Evaluation Group
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Primary Reviewer:

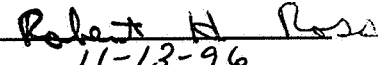
Paul G. Forsyth, Ph.D.

Signature: 

Date: 11-11-96

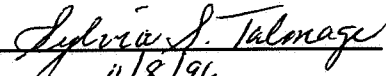
Secondary Reviewers:

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

Signature: 

Date: 11-13-96

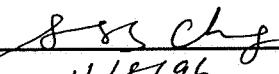
Sylvia S. Talmage, Ph.D., D.A.B.T.

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Date: 11/8/96

Quality Assurance:

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Date: 11/8/96

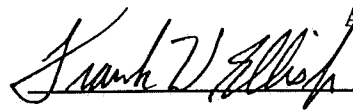
Disclaimer

This Data Evaluation Report may have been altered by the Biopesticides and Pollution Prevention Division subsequent to signing by Oak Ridge National Laboratory personnel.

Oak Ridge National Laboratory, managed by Lockheed Martin Energy Research Corp. for the U.S. Department of Energy under contract number DE-AC05-96OR22464

POTASSIUM SALTS OF PHOSPHOROUS ACID

EPA Reviewer: Frank W. Ellis, Jr., M.S.
Biopesticides and Pollution Prevention Division
EPA Team Leader: Roy D. Sjoblad, Ph.D.
Biopesticides and Pollution Prevention Division



Acute Contact LD₅₀ (141-1)

Date: 3/14/97

Date: _____

DATA EVALUATION REPORT

MRID# & TITLE OF STUDY: MRID 43905817, Potassium Salts of Phosphorous Acid: An Acute Contact Toxicity Study with the Honey Bee. Guideline References: FIFRA Subdivision L, Section 141-1 and EPPO Guideline 170.

DP BARCODE: D226397

CASE: 046750

REG./FILE#: 069579-R

CHEMICAL/BIOL#: 076416 Mono- and di-potassium salts of phosphorous acid

COMPANY/SPONSOR: U.I.M. Agrochemicals (Aust.) Pty.Ltd., P.O. Box 72,
Brisbane Markets, Queensland, Australia 4106

TEST MATERIAL: Identified as potassium salts of phosphorous acid

TEST FACILITY: Wildlife International, Ltd., 8598 Commerce Drive, Easton, MD.

REVIEW CONCLUSION: This study was conducted according to acceptable procedures; however, results were mixed. The 48-hour acute contact LD₅₀ of this formulation of potassium salts of phosphorous acid to honeybees was >100 µg/bee. The no-observed-effect concentration was inconclusive, as was the no-mortality level.

RECOMMENDATIONS: Repeat the tests using a greater number of bees (the reviewer recommends at least 50 bees/group) in order to provide conclusive results for sublethal effects and no-mortality levels. Comparisons between groups should be made statistically rather than by general observation.

ADEQUACY OF STUDY: Supplemental

MATERIALS & METHODS: The study protocol was based upon procedures outlined in FIFRA Subdivision L, Section 141-1, Hazard Evaluation: Nontarget Insects, and EPPO Guideline 170 and was conducted following GLP regulations 40 CFR 160; OECD, ISBN 92-84-12367-9; and Japan MAFF, 59 NohSan, Notification No. 3850, Agricultural Production Bureau. The test substance was received from Compliance Services International (Tacoma, WA) and was a clear, colorless, waterlike liquid identified on the label as "m-dKP BRAND OF foli-r-fos 400 FUNGICIDE; ACTIVE CONSTITUENT 400 g/L PHOSPHORUS ACID; ph 5.7-6.0". The reported purity of the test substance was 41%. The test substance was stored under ambient conditions for approximately 2 months, then under refrigeration. A positive control test substance, dimethoate, was received from Chem Service, Inc. (address not reported) and was a crystalline solid identified on the label as "Dimethoate; Purity:98%; LOT:146-14B; POISON-STENCH; F989; EXP:12/99; 100mg". The positive control substance was stored under refrigeration.

Groups of 20 bees (3 replicates) were randomly selected for each treatment group. Each bee (*Apis mellifera*) received a single 2 µl dose of either the test substance diluted in methanol, the positive control

substance diluted in methanol, methanol alone, or no treatment (negative control). Doses were administered by placing the 2 μ l droplet on the abdomen and/or thorax of each bee. Negative control bees were handled precisely as the other treatment groups with the exception of the dosing process. Nominal dosages for the test substance were 6.25, 12.5, 25.0, 50.0, and 100 μ g/bee. There was no correction for purity of the test substance. Nominal dosages for the positive control substance were 0.05, 0.10, and 0.20 μ g a.i./bee. The bees were housed in environmental chambers set to maintain 28-32°C, approximately 50% relative humidity, and continuous darkness except during dosing and observations. The bees were observed at approximately 1, 2.25, 24, and 48 hours following treatment for signs of toxicity, abnormal behavior, or mortality. Test data was analyzed by a computer program designed to calculate the LD₅₀ and the 95% confidence interval by probit analysis, the moving average method or binomial probability with nonlinear interpolation. The mortality pattern in test substance treatment groups was not calculable and was, therefore, estimated via visual inspection of the mortality data. The no-observed-effect dose was determined by visual inspection of the mortality and clinical observation data.

REPORTED RESULTS: All immobile bees at test termination were included in mortality calculations. At test termination, there was 8% mortality among bees in both negative control and solvent control groups. With the exception of one immobile bee in the negative control group on Day 1, all surviving bees in these control groups appeared normal in appearance and behavior throughout the test. Mortalities in the positive control dosage groups of 0.05, 0.10, and 0.20 μ g a.i./bee were 20%, 27%, and 92%, respectively. The LD₅₀ for the positive control substance was 0.13 μ g/bee with a 95% confidence interval of 0.10 to 0.20 μ g/bee. One bee in the 0.20 μ g/bee group exhibited loss of equilibrium on Day 1. Mortalities in the test substance dosage groups of 6.25, 12.5, 25.0, 50.0, and 100 μ g/bee were 7%, 13%, 15%, 12%, and 12%, respectively. Two bees in the 25 μ g/bee group exhibited loss of equilibrium on Day 1. The LD₅₀ was determined to be greater than 100 μ g/bee, the highest dose tested. The no observed effect dose was determined to be 100 μ g/bee.

DISCUSSION: Because there is no single protocol for acute contact toxicity in honeybees recommended by the EPA, this study was conducted according to a protocol considered acceptable to the reviewer, with one exception. Due to the high incidental mortality of honeybees in these types of tests (e.g., 8% in both negative and solvent control groups), more bees should be tested per group in order to provide a clearer statistical picture of the results. Although 10% mortality is acceptable in some studies, it has been demonstrated that honeybees can be tested outside the hive environment with only 8% control mortality for up to 30 days. Control mortalities of 8% seem extreme in a 48-hour test.

The 48-hour acute contact LD₅₀ for this formulation of potassium salts of phosphorous acid (41.0% wt/vol) was greater than 100 μ g/bee, the highest dose tested. The use of bees found to be immobile after 48 hours in mortality calculations is viewed with some skepticism by the reviewer since the bees were not actually determined to be dead. If bees are immobile after 48 hours, the test should be extended until affected bees either recover or die. The reviewer considers immobility to be a sublethal effect and does not agree with the no-observed-effect dose of 100 μ g/bee as stated by the authors. There was 1 immobile bee after 48 hours in the lowest dose tested, indicating that the no-observed-effect dose could be as low as 6.25 μ g/bee or less. One bee represents 5% (statistically significant) of a replicate using 20 bees. The effects on one test unit when testing social insects, which behave more as a colony than as individuals, should not significantly affect results, further indicating a need for increasing the number of bees tested.

Also in question is the conclusion by the authors that there was no increase in mortality in those treatment groups receiving greater than 6.25 μ g/bee. Statistical comparisons of the mean mortalities of these groups indicate that 1 group, possibly 2 groups (depending on the model used), have significantly higher

POTASSIUM SALTS OF PHOSPHOROUS ACID

Acute Contact LD₅₀ (141-1)

mortalities than the lowest dose used. These higher mortalities do not occur in a dose-response fashion, but may occur through slow, yet steady, uptake of the test chemical, or by slow, steady production of a toxic metabolite. Again, using a higher number of bees per group should provide a clearer result.

Productivity in honeybee colonies (i.e., honey production and pollination) relies heavily on maintenance of a large population of worker bees with good vitality. The sublethal effects and possible low-level mortality exhibited in this study indicate a need for further testing that will provide more definitive results. However, results of these tests do indicate the test substance is, at best, only slightly lethal to honeybees.