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

OFFICE OF
PREVENTION, PESTICIDES AND
TOXIC SUBSTANCES

MAR 2 0 2006

MEMORANDUM

SUBJECT: Science Review to support registration of new end-use product, **Resist (EPA No. 82940-R)**, containing 57.0 % w/w mono-and di-potassium phosphate as its active ingredient.

PC code: 076416
DP Barcode: DP 326475.
Decision No. 362983.

FROM: Clara Fuentes, Ph.D., Biologist
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

TO: Raderrio Wilkins, Regulatory Action Leader
Biochemical Pesticides Branch
Biopesticides & Pollution Prevention Division (7511C)

ACTION REQUESTED

Actagro LLC. is requesting registration of a new end-use product, ***Resist*** **(EPA Reg. No. 82940-R)**, containing 57.0 % w/w mono- and di-potassium phosphate as its active ingredient. The new end-use product is a systemic fungicide to control phytophthora, downy mildew, pythium, and other diseases on agricultural and greenhouse crops, indoor and outdoor ornamentals, bedding plants, turf, commercial forests, and domestic trees.

RECOMMENDATIONS AND CONCLUSIONS

*Actagro LLC.*has submitted the following data in fulfillment of registration requirements: Product-specific chemistry data (MRIDs 467082-01 to –03), acute mammalian toxicity studies for oral, dermal, inhalation, and eye routes of exposure (MRDs 467082-12 to 467082-17), and ecological effects and non-target effect studies, aquatic invertebrate acute toxicity (MRID 467082-04), Fish acute toxicity (MRID 467082-05), Analytical Method for *Resist* in freshwater (467082-06), Avian dietary toxicity (467082-08), Analytical Method avian diet (467082-09), and data waiver requests for subchronic toxicity studies, 90-Day Feeding, 90- day Dermal, 90-Day

Inhalation, Immune response, Teratogenecity (**MRID 467082-19),** Residues data (**MRID 467082-20**), and Terrestrial plant toxicity (467082-11). The registrant also submitted copies of CSF, and proposed product label supporting registration of new end-use product, ***Resist*** **(EPA Reg. No. 82940-R**), containing 57% w/w mono- and di-potassium phosphate as its active ingredient (**PC code: 076416**).

I. The product specific chemistry data (**MRIDs 467082-01, -02 and -03**) are **unacceptable.** To upgrade the studies, the registrant needs to correct the following deficiencies:

1. Resolve the discrepancy in the beginning materials described on p. 12, and on pp. 7 of MRID 467082-01. [redacted] is listed in page 7 of MRID 467082-01, as one of the starting materials used to manufacture [redacted] a precursor for the end-use product. This chemical is not mentioned in *Description of the Formulation Process*, page 12 of MRID 46708-01.

2. Describe the quality control criteria and the product packaging.

3. Submit results from a five-batch analysis of the end use product. The preliminary analysis submitted by the registrant is for five lots of [redacted] evidently obtained from the supplier, not for five lots of the end-use product.

4. Submit results from a five-batch analysis of the end use product, and include any impurities identified at >0.1% w/w on the CSF.

5. Provide data on storage stability and corrosion characteristics.

6. No CSF is needed for [redacted] and it should be deleted from the submission.

7. Explain how the volume of titrant, as described in the Enforcement Analytical Method, correlates to the concentration of active ingredient (presumably measured as phosphorous acid equivalent).

II. The submitted data for acute mammalian toxicity, oral, dermal, inhalation, and eye routes of exposure (**MRDs 467082-12 to 467082-17**) are **acceptable**.

Waiver requests for subchronic toxicity studies, 90-Day Feeding, 90- day Dermal, 90-Day Inhalation, Immune response, and teratogenecity (**MRID 467082-19),** and Residues data (**MRID 467082-20**) are **acceptable.**

Data submitted for Genotoxicity, *Salmonella/Escherichia*/Mammalian Activation Gene Mutation Assay; OPPTS 870.5100 [§84-2] ((**MRID 467082-18)** are **acceptable** although the investigators did not report that the doses were adjusted for the percentage of active ingredients; therefore, the test material was likely tested as the 57% commercial product.

III. The waiver request for Terrestrial Plant Toxicity (MRID 467082-11) are **acceptable.** Based on public literature and infromatioin on phosphorus acid, phosphates/phosphonate products, the active ingredients in Resist are not phytotoxic. Therefore, terrestrial plant toxicity studies with Resist will yield little useful information.

STUDY SUMMARY

I. PRODUCT CHEMISTRY

(MRIDs 467082-01, 467082– 02, and 467082-03)

A. Description of Beginning Materials and Formulation Process.
Deficiencies: The description of the manufacturing process for the end use product (p. 12) does not mentio[redacted]is evidently used in the production of[redacted]by the supplier.[redacted]should not be included in the list of beginning materials. It is mentioned in page 7 of MRID 467082-01, *Description of Materials Used to Produce the Product.* The quality control criteria and the product packaging are not described.

B. Discussion of Formation of Impurities.
Deficiencies: None.

CONTAINS CONFIDENTIAL BUSINESS INFORMATION

C. Preliminary Analysis of Active Ingredient. The preliminary analysis submitted by the registrant is for five lots of phosphorous acid, evidently obtained from the supplier, not for five lots of the end use product.
Deficiencies: Results from a five-batch analysis of the end use product must be submitted, and any impurities identified at >0.1% w/w must be included on the CSF.

D. Certified Limits.
Deficiencies: None.

TABLE 1. Nominal CSF concentrations and certified limits for Resist[a]					
Ingredients (CAS number)	PC Code	Purpose	Concentration (% by weight)		
			Nominal	Lower	Upper
Active Ingredient					
Mono- and di-potassium phosphite (CAS Nos. 13977-65-6 and 13492-26-7, respectively)	076416	Active ingredient	57.0	55.29	58.71
Inert Ingredients					
[redacted]					

[a]Data from CSF.

G. Enforcement Analytical Method.
Deficiencies: The description of the method does not explain how the volume of titrant correlates to the concentration of active ingredient (presumably measured as [redacted] equivalent).

H. Physical and Chemical Characteristics.
Deficiencies: Data will need to be provided for storage stability and corrosion characteristics.

TABLE 2. Physical and Chemical Properties for Resist[a]			
Guideline Reference No./Property		Description of Result	Methods
830.6302	Color	Clear to light tan	Visual inspection
830.6303	Physical State	Liquid	Visual inspection
830.6304	Odor	Not required for EP	
830.6313	Stability	Not required for EP	
830.6314	Oxidation/Reduction: Chemical Incompatibility	Compatible with water, 10% monoammonium phosphate, iron powder, and kerosene. Incompatible with 10% potassium permanganate.	44 FR 16267
830.6315	Flammability	Not expected to be flammable	Product knowledge
830.6316	Explodability	Not expected to be explosive	Product knowledge
830.6317	Storage Stability	**Not provided**	
830.6319	Miscibility	N/A, product is not to be used with oil based solvents/diluents	
830.6320	Corrosion Characteristics	**Not provided**	
830.6321	Dielectric Breakdown Voltage	N/A, not for use around electrical equipment	

TABLE 2. Physical and Chemical Properties for Resist[a]			
Guideline Reference No./Property		Description of Result	Methods
830.7000	pH	5.91 ± 0.01 (1% w/w solution in distilled water)	pH meter (VWR Model 8005) CIPAC MT-75
830.7050	UV/Visible Absorption	Not required for EP	
830.7100	Viscosity	4.941 ± 0.016 cS @ 20°C 2.915 ± 0.002 cS @ 40°C	ASTM D445/D446
830.7200	Melting Range	Not required for EP	
830.7220	Boiling Range	Not required for EP	
830.7300	Density/Relative Density/Bulk Density	1.430 ± 0.001 g/mL @ 20°C	CIPAC MT-3 OECD No. 109 ASTM D891-95
830.7370	Dissociation Constant in Water	Not required for EP	
830.7520	Particle Size/Distribution	N/A, product is a liquid	
830.7550	Partition Coefficient	Not required for EP	
830.7840	Water Solubility	Not required for EP	
830.7950	Vapor Pressure	Not required for EP	

[a] Data from MRID 46708203, CSF.

II. MAMMALIAN TOXICITY

Table 3. Mammalian Toxicity Categories

Mammalian Toxicity Studies	Guideline	Study Results	Toxicity Categories
Acute Oral Toxicity	870.1100	LD_{50} > 5000 mg/kg; In female rats	IV CAUTION
Acute Dermal Toxicity	870.1200	LD_{50} > 5000 mg/kg in male, female rats and combined.	IV CAUTION
Primary Dermal Irritation In rabbit	870.2500	Very slight erythema. irritation index = 0.5	IV CAUTION

Mammalian Toxicity Studies	Guideline	Study Results	Toxicity Categories
Primary Eye Irritation In Rabbit	870.2400	Maximum average score was 6.0 at 1 hour. Minimally irritating.	IV CAUTION
Skin sensitization-Guinea pig	870.2600	Not a sensitizer	Not a sensitizer
Acute Inhalation Toxicity	870.1300	LC_{50} for male, female rats, and combined was > 2.02 mg/L.	IV CAUTION
Hypersensitivity Incidents		No incidents reported	No incidents reported

The registrant is requesting a waiver for residue data (**MRID 467082-20**), and for the following mammalian toxicity studies: Inmune response, 90-Day Feeding, 90-Day Dermal, 90-Day Inhalation, and Teratogenicity (**MRID 467082-19**) under the following rationale:

a) The proposed product is practically non-toxic (oral LD_{50} >5000 mg/kg).
b) The proposed use pattern is lower than the highest dose tested.
c) Residues from fertilizer use in commercial commodities are indistinguishable from fungicide use, and no ill effects have been reported.

(MRID 467082-18) Genotoxicity, *Salmonella/Escherichia*/Mammalian Activation Gene Mutation Assay; OPPTS 870.5100 [§84-2]

In a reverse gene mutation assay in bacteria (MRID 46708218), strains TA97a, TA98, TA100 and TA1535 of *S. typhinurium* and strain WP2(uvrA) of *E.* coli were exposed to Resist (57.0% a.i., Lot No. 5-143) in deionized, ultra filtered water at concentrations of 0, 5, 10, 50, 100, 500, 1000, 2500 or 5000 μg/plate in the presence and absence of mammalian metabolic activation (S9-mix) in a standard plate incorporation assay. The same strains were exposed to Resist at concentrations of 0, 100, 500, 1000, 2500 or 5000 μg/plate with and without S9-mix in a confirmatory 30-minute preincubation assay. The investigators did not report that the doses were adjusted for the percentage of active ingredients; therefore, the test material was likely tested as the 57% commercial product. The S9-fraction was obtained from Aroclor 1254 induced male Sprague-Dawley rat liver.

Resist was tested up to the limit dose of 5000 μg/plate for the assay. No cytotoxicity or test material precipitation was seen in either assay. The number of revertants per plate was not increased over the concurrent solvent control value at any Resist concentration with or without S9-mix in any strain using either the plate incorporation or the preincubation protocol. The

solvent and positive controls induced the appropriate responses in the corresponding strains. **There was no evidence of induced mutant colonies over background.**

III. ECOLOGICAL EFFECTS AND NON-TARGET EFFECTS.

MRID 46708204 *Aquatic Invertebrate Acute Toxicity Test.*

The test organisms were *Daphnia magna* which are continuously bred in the facilities of the testing laboratory. Seven adult daphnids were held 14 days prior to collection of juveniles to monitor health and reproduction. The adults showed no signs of disease or stress during the holding period. At test initiation, young daphnids were separated and transferred via 10 mL glass beakers to test chambers until each chamber contained 10 daphnids. Test chambers were 25-L stainless steel aquaria filled with approximately 22 L of test water. Each test chamber contained one test compartment constructed from a glass beaker approximately 6.5 cm in diameter and 12 cm in height with nylon screen attached to two holes on the sides of the beaker. The test compartment contained the daphnids. Test water (dilution water) was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International property. A continuous flow diluter was used to deliver each concentration of the test substance and a negative control. The stock solutions were mixed with dilution water in mixing chambers and the diluter adjusted so that each test chamber received approximately five volume additions of test water every 24 hours.

One stock solution was prepared for each of the five concentrations tested from a primary stock with a nominal concentration of 186 mg a.i./mL. Secondary stock solutions were prepared at nominal concentrations of 11.63, 23.25, 46.5, and 93 mg a.i./mL by proportional dilution. The five stock solutions were injected into the diluter mixing chambers (at a rate of 0.1 mL/minute) where they were mixed with well water (at a rate of 155 mL/minute) to achieve desired concentrations. Water samples were collected from one test chamber of each treatment and control group before test initiation, at test initiation, and at termination to measure the concentration of the test substance. The method for analyses of Resist in freshwater was developed by Wildlife International using GC/FPD. The method LOQ for Resist in freshwater was set at 5.00 mg a.i./L.

Fluorescent lighting similar to natural sunlight was used for illumination of test chambers with a photoperiod of 16 hours light/8 hours darkness. Water temperatures were 20 $\pm$ 1°C and dissolved oxygen remained >8.1 mg/L throughout the test. Measurements of pH ranged from 7.3 to 8.3.

Nominal test concentrations selected for use in the study were 7.5, 15, 30, 60 and 120 mg a.i./L. Measured concentrations of Resist in the test solution samples prior to test initiation, at test initiation and termination ranged from approximately 92 to 100% of nominal concentrations. When measured concentrations collected at 0 and 48 hours were averaged the mean measured concentrations for the study were 7.4, 14, 29, 56 and 115 mg a.i./L representing 99, 93, 97, 93 and 96% of nominal concentrations, respectively.

Daily observations for mortality, immobility and signs of toxicity were conducted during the test. Daphnia in the negative control group appeared normal throughout the test with the exception of

one lethargic daphnid noted at test termination. Percent mortality/immobility at test termination in the 7.4, 14, 29, 56 and 115 mg a.i./L treatment groups was 5,5, 0, 0, and 5%, respectively. All other daphnia in the Resist treatment groups appeared normal except for the one lethargic daphnid in the 56 mg a.i./L treatment group. Since there was <50% mortality/immobility in all of the Resist treatment groups, the 48-hour EC50 value was >115 mg a.i./L.

Daphnia magna were exposed for 48 hours under flow-through conditions to five mean measured concentrations of Resist ranging from 7.4 mg/L to 115 mg/L. There was <10% mortality/immobilization at any concentration tested. The 48-hour EC_{50} was estimated to be above 115 mg/L, the highest mean measured concentration tested.

MRID 467082-05. *Fish Acute Toxicity Test*

The test species was the rainbow trout, *Oncorhynchus mykiss,* obtained from Thomas Fish Company, Anderson, California. All fish were from the same source and year class and length of the longest fish was no more than twice the length of the shortest. The average total length of 10 negative control fish measured at the end of the test was 4.4 cm, with a range of 4.0 to 5.0 cm. The average wet weight (blotted dry) of 10 negative control fish measured at the end of the test was 0.62 grams, with a range of 0.40 to 1.1 grams. Loading was defined as the total wet weight of fish per liter of test water that passes through the test chamber in 24 hours and was 0.06 g fish/L/day. Instantaneous loading was 0.42 g fish/L of test water present in the test chambers at any given time. The rainbow trout were held for 14 days prior to the test, in the water from the same source and temperature as the definitive test. The trout showed no signs of disease or stress during the holding period. At test initiation, 10 trout were segregated to replicate test chambers for a total of 20 trout per test concentration. Test chambers were 25-L stainless steel aquaria filled with approximately 15 L of test water. Test water (dilution water) was freshwater obtained from a well approximately 40 meters deep located on the Wildlife International property. A continuous flow diluter was used to deliver each concentration of the test substance and a negative control. The stock solutions were mixed with dilution water in mixing chambers and the diluter adjusted so that each test chamber received approximately seven volume additions of test water every 24 hours.

One stock solution was prepared for each of the five concentrations, from a primary stock with a nominal concentration of 186 mg a.i./mL. Secondary stock solutions were prepared at nominal concentrations of 11.63, 23.25, 46.5, and 93 mg a.i./mL by proportional dilution. The five stock solutions were injected into the diluter mixing chambers (at a rate of 0.1 mL/minute) where they were mixed with well water (at a rate of 155 mL/minute) to achieve desired concentrations. Water samples were collected from one test chamber of each treatment and control group before test initiation, at test initiation and at termination to measure the concentration of the test substance. The method for analyses of Resist in freshwater was developed by Wildlife International using GC/FPD. The method LOQ for Resist in freshwater was set at 5.00 mg a.i./L

Fluorescent lighting similar to natural sunlight was used for illumination of test chambers with a photoperiod of 16 hours light/8 hours darkness with a 30-minute transition period. Water temperature was 12 ± 1°C and dissolved oxygen remained >7.9 mg/L (73% of saturation)

throughout the test. Measurements of pH ranged from 7.3 to 8.3.

Nominal test concentrations selected for use in the study were 7.5, 15, 30, 60 and 120 mg a.i./L. Results of analyses of Resist in the test solution samples prior to test initiation, at test initiation and termination ranged from approximately 93 to 110% of nominal concentrations. When measured concentrations collected at 0 and 48 hours were averaged the mean measured concentrations for the study were 7.9, 15, 29, 62 and 127 mg a.i./L representing 105, 100, 97, 103 and 106% of nominal concentrations, respectively.

Daily observations for mortality, immobility and signs of toxicity were conducted during the test. No mortalities occurred during the test and all rainbow trout in the negative control group and Resist treatment groups appeared normal throughout the test. LC_{50} values at 24, 48, 72 and 96 hours were estimated to be >127 mg a.i./L. The no-mortality concentration and the NOEC were both 127 mg a.i./L.

Rainbow trout, *Oncorhynchus mykiss* were exposed for 96 hours under flow-through conditions to five mean measured concentrations of Resist ranging from 7.9 mg/L to 127 mg a.i./L. There were no mortalities or signs of toxicity observed in trout at any concentration tested. The 96-hour LC_{50} value was estimated to be >127 mg a.i./L. The no-mortality concentration and the NOEC were both 127 mg a.i./L.

MRID 467082-06 *Analytical Method for RESIST in Freshwater*

Freshwater was fortified with Resist at three different concentrations and analyzed based on a method developed by Wildlife International, Ltd. A stock solution of Resist was prepared and serially diluted in HPLC-grade bottled water. Freshwater used for the calibration standards and method verification samples was obtained from a well approximately 40 meters deep located on the Wildlife International property. Fortified samples were prepared by volumetrically adding aliquots of potassium phosphite stock solutions to freshwater in flasks. To a second set of flasks, 1 mL of isopropanol: 10% H_2SO_4 solution was added. The requisite quantity of each aqueous sample was transferred to the flasks containing IPA/H_2SO_4. Trimethylsilyldiazomethane was added to each sample and allowed to react for approximately 10 minutes. All samples were adjusted to final volume with isopropanol and transferred for analysis by GC/FPD (Hewlett-Packard Model 5890 Gas Chromatograph, Flame Photometric Detector operated in phosphorus mode).

Freshwater was fortified at 7.50, 50.0 and 200 mg a.i./L using the stock solution of Resist in HPLC-grade bottle water. Samples fortified at 7.50, 50.0 and 200 mg a.i./L yielded mean recoveries of 93, 98 and 99% of nominal concentrations, respectively (Table 1). Two reagent blanks and two matrix blanks were analyzed to determine possible interferences. No interferences were observed at or above the LOQ during the sample analyses.

The method developed by Wildlife International for analysis of Resist in freshwater was verified at concentrations ranging from 7.50 to 200 mg a.i./L. Samples of Resist in freshwater fortified at 7.50, 50.0 and 200 mg a.i./L yielded mean percent recoveries of 93, 98 and 99%, respectively.

The overall mean recovery was 96.6±5.66% (RSD=5.86%).

TABLE 1. Method verification recovery of Resist in freshwater.

Sample		Concentration (mg a.i./L)			Mean Measured (x) Std. Dev (SD) Relative SD(RSD)	Mean Percent (%)
Number	Type	Fortified	Measured[1]	Percent Recovery[2]		
VREB-1	Reagent Blank	0.0	< LOQ	–	–	
VREB-2	Reagent Blank	0.0	< LOQ	–		
VMAB-1	Matrix Blank	0.0	< LOQ	–	–	
VMAB-2	Matrix Blank	0.0	< LOQ	–		
VMAS-1	Fortification	7.50	7.66	102.0		
VMAS-2	Fortification	7.50	6.94	92.5	(x) = 6.97	93
VMAS-3	Fortification	7.50	6.56	87.4	SD = 0.43	
VMAS-4	Fortification	7.50	6.66	88.8	RSD = 6.19%	
VMAS-5	Fortification	7.50	7.03	93.7		
VMAS-6	Fortification	50.0	47.8	95.6		
VMAS-7	Fortification	50.0	49.1	98.3	(x) = 48.8	98
VMAS-8	Fortification	50.0	48.9	97.8	SD = 0.676	
VMAS-9	Fortification	50.0	48.5	96.9	RSD = 1.39%	
VMAS-10	Fortification	50.0	49.6	99.2		
VMAS-11	Fortification	200	179	89.6		
VMAS-12	Fortification	200	207	103.0	(x) = 199	99
VMAS-13	Fortification	200	188	94.2	SD = 14.2	
VMAS-14	Fortification	200	209	105.0	RSD = 7.13%	
VMAS-15	Fortification	200	210	105.0		
Mean = 96.6 Standard Deviation = 5.66 Relative Standard Deviation = 5.86% N = 15						

[1] The limit of quantitation (LOQ) was calculated to be 5.00 mg a.i./L based upon the product concentration of the lowest calibration standard (0.05 mg a.i./L) and the dilution factor of the matrix blank samples (100).
[2] Results were generated using Excel 2000 in full precision mode. Manual calculations may differ slightly.

MRID 467082-07 *Avian Acute Oral Toxicity*

The test species was the northern bobwhite, *Colinus virginianus*, obtained from K&L Quail, Oroville, CA, 95965. Test birds were maintained separately by sex at Wildlife International under conditions that would not facilitate reproduction. Northern bobwhite ranged in weight from 172 – 217 grams and were approximately 21 weeks of age and appeared to be in good health at test initiation. All birds were from the same hatch, pen-reared and phenotypically indistinguishable from wild birds. Birds were assigned to five test groups and one control group. Each treatment or control group contained five males and five females. Throughout acclimation and testing, birds were allowed access to game bird ration and water *ad libitum*.

The test substance was dispersed in reverse osmosis water and the concentration of the test substance in the diluent was adjusted to provide a constant volume to body weight dosage for all treatment birds. The dosages were adjusted to 100% active ingredient and all dosages and LD_{50} values are reported as milligrams of active ingredient per kilogram of body weight. Nominal dosages used in this study were 0, 292, 486, 810, 1350 and 2250 milligrams active ingredient of Resist per kilogram of body weight (mg a.i./kg). The birds were fasted for 17 hours prior to dosing. At test initiation, a single dose of the test substance in diluent was orally intubated directly into the crop or proventiculus of each bird. Each bird was weighed and dosed on the

basis of milligrams of test substance per kilogram of body weight.

Test birds were housed indoors in pens with floor space of approximately 78 x 51 cm. The walls, ceilings and floor of the pens were wire mesh with solid metal walls between pens. Each dosage group was assigned two pens, one pen contained five males and the other five females. Birds were maintained at ambient room temperature (23.0 ± 0.4°C) with an average relative humidity of 68 ± 7%. The photoperiod was eight hours light/16 hours dark during acclimation and throughout the test.

Following dosing, multiple observations were performed on Day 0 with particular attention being paid for signs of regurgitation. From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity and abnormal behavior. Body weight was measured individually at test initiation, Days 3, 7 and 14 of the test. Average feed consumption was determined by pen for each dosage group and the control group for Days 0-3, 4-7 and 8-14.

There were no mortalities in the control group. On day 3 of the test, one bird was noted with an ankle lesion attributed to toe-picking and was bandaged for the remainder of the test. All other control birds were normal in appearance and behavior throughout the test. There were no mortalities in the 292, 486, 810 or 1,350 mg a.i./kg treatment groups. There was 10% mortality (1/10) in the 2,250 mg a.i./kg treatment group.

There were no signs of toxicity in the 292, 486, 810 or 1,350 mg a.i./kg treatment groups and all birds at those dosage levels were normal in appearance and behavior throughout the test. At the 2,250 mg a.i./kg dosage level, one female was found dead approximately three hours after dosing. In addition, one male in the 2,250 mg/a.i./kg treatment group was noted with a ruffled appearance 4.5 hours after dosing but appeared normal the next day. When compared to the control group, there were no treatment related effects on body weight of birds dosed at the 292, 486, 810, 1350 and 2250 mg a.i./kg levels. All birds gained weight during the 14 day test period. Mean weights increased by 3 to 6 grams in control birds and 4 to 14 grams in test birds. Likewise, there were also no apparent treatment-related effects on feed consumption at any of the dosage levels tested.

Since there was only a single mortality, it was not possible to calculate the LD_{50}. The LD_{50} value was determined to be greater than the highest dosage tested and the no-observed-effect level was determined by detailed observations of the birds during testing.

The acute oral LD_{50} value for northern bobwhite exposed to Resist at a single oral dose was determined to be greater than 2,250 mg a.i./kg body weight, the highest dose tested. The no-mortality level and the no-observed effect level were 1,350 mg a.i./kg.

MRID 467082-08 *Avian Dietary Toxicity Test*

The test organism was northern bobwhite (*Colinus virginianus)* chicks which were 10 days of age and appeared in good health at test initiation. Northern bobwhite chicks ranged in weight from 21 – 25 grams and were hatched from the Wildlife International flock. All birds were from the same hatch, pen-reared and phenotypically indistinguishable from wild birds. Birds were

assigned to five test groups and one control group. Each treatment or control group contained 10 chicks and the control group contained 30 chicks. Birds were housed in brooding pens and throughout acclimation and testing, birds were allowed access to game bird ration and water *ad libitum*.

Test diets were prepared by mixing the test substance directly into the game bird ration. Dietary test concentrations were corrected to 100% a.i. based on the purity of Resist. Nominal dietary test concentrations were 0, 562, 1000, 1780, 3160 and 5620 ppm a.i. Resist. Samples of the test diet were analyzed for concentration and homogeneity of test substance in the diet at Day 0 and Day 5.

Test birds were housed indoors in brooding pens with floor space of approximately 72 x 90 cm with ceiling height of 23 cm. The walls, ceilings and floor of the pens were wire mesh with solid metal walls. During the test, each treatment group was assigned two pens that contained five chicks each. The control group was assigned six pens that contained five chicks each. Chicks were acclimated for 10 days, exposed for 5 days and post-exposure observed for 3 days. Birds were maintained in brooding compartments at 38.4 $\pm$ 1.8°C. The photoperiod was 16 hours light/8 hours dark during acclimation and throughout the test.

Following dosing, multiple observations were performed on Day 0. From test initiation until termination, all birds were observed at least twice daily. A record was maintained of all mortality, signs of toxicity and abnormal behavior. Body weight was measured individually at test initiation, the end of exposure on day 5, and at test termination on day 8. Average feed consumption was determined by pen for the exposure period (Days 0-5) and during the post-exposure observation period (Days 6-8).

Diet analyses indicated that none of the control samples showed the presence of Resist. Diet samples collected from the 562 and 5620 ppm a.i. test concentrations were analyzed for homogeneity. Means and standard deviations for the two test concentrations were 531$\pm$31.3 ppm a.i. and 5217$\pm$267 ppm a.i., respectively. Samples collected during the test to verify test substance concentration for the 1000, 1780 and 3160 ppm a.i. diets had means of 867 ppm a.i., 1459 ppm a.i. and 2836 ppm a.i., respectively. These values represented 86.7, 81.9, and 89.8% of nominal concentrations. Analysis of diet samples collected from feeders after being held at ambient temperature for 5 days averaged 86.6%, 99%, 106%, 102% and 108% of the Day 0 values for the 562, 100, 1780, 3160 and 5620 ppm a.i. test concentrations, respectively.

There were no mortalities in the control group. One control group bird was noted lame; otherwise, all control birds were normal in appearance and behavior throughout the test. In addition, there was one bird with an ankle lesion attributed to toe-picking that was bandaged for the remainder of the test. All other control birds were normal in appearance and behavior throughout the test. There were no mortalities or overt signs of toxicity noted in the 562, 1000, 1780, 3160 and 5620 ppm a.i. Resist treatment groups. At the 562 ppm a.i. test concentration, three birds developed lesions from toe-picking and one bird in the 3160 ppm a.i. test concentration had a leg fracture on Day 5. None of these injuries were treatment related. All other birds were normal in appearance and behavior.

When compared to the control group, there were no treatment related effects on body weight of birds dosed at the 562, 1000, 1780, 3160 and 5620 ppm a.i. Resist test concentrations. All birds

gained weight during the 8 day test/observation period. Mean weight increased by 22 grams in control birds and 21 to 22 grams in test birds. Likewise, there were also no treatment-related effects on feed consumption at any of the Resist concentrations tested in the diet.

Since there were no mortalities, it was not possible to calculate the LD_{50}. The LD_{50} value was determined to be greater than the highest dosage tested and the no-observed-effect level (NOEL) was determined by detailed observations of the birds during testing.

The dietary LC_{50} value for northern bobwhite chicks exposed to Resist was greater than 5,620 ppm, the highest concentration tested. The no-mortality concentration and the no-observed effect (NOEL) concentration were both 5,620 ppm a.i.

MRID 467082-09 *Analytical Method Avian Diet*

A stock solution of Resist was prepared and serially diluted in HPLC-grade bottled water. Avian diet (game bird ration) was fortified at 60.0 and 6,000 µg a.i./g. Ten grams of each test diet and control diet (no Resist) were placed in French square bottles. HPLC-grade bottled water was added to each sample. Samples were sonicated and allowed to sit undisturbed for 10-15 minutes to allow fine particles to precipitate. To a second set of bottles, 1mL of isopropanol/H_2SO_4. Trimethylsilyldiazomethane was added to each sample and the bottles were stoppered and allowed to react for approximately 10 mminutes. All samples were adjusted to final volume with isopropanol and transferred for analyses by GC/ FPD (Hewlett-Packard Model 5890 gas Chromatograph, Flame Photometric Detector operated in phosphorus mode). The LOQ for the method verification analyses in avian diet was set at 33.4 µg a.i./g based upon the product of the concentration of the lowest calibration standard (0.05 mg a.i./L) and the dilution factor of the matrix blank samples (667). One reagent blank and 3 matrix lanks were analyzed concurrently with the verification set. Calibration curves were prepared from external standards of Resisit.

Analysis of avian diet (game bird ration) fortified at 60.0 and 6,000µg a.i./g with a stock solution of Resist resulted in mean percent recoveries of 84 and 94%, respectively. One reagent blank and 3 matrix blanks were analyzed to determine possible interferences. No interferences were observed at or above the LOQ (33.4 µg a.i./g) during the sample analyses.

The method developed by Wildlife International for analysis of Resist in avian diet was verified at concentrations of 60.0 and 6,000 µg a.i./g. The overall mean recovery was 87.4 ± 6.16 % (RSD= 7.05 %).

MRID 467082-10 *Honey Bee Acute Contact Toxocity test*

The test organisms were the honey bees, (*Apis mellifera*) obtained as young adult worker bees from the University of Maryland hives. Bees were collected from hives the day of the test and held in a screened box supplied with a 50% sucrose solution. Bees in the holding box were immobilized with nitrogen, impartially distributed, dosed with the appropriate dosing solution and 20 dosed bees were placed in the appropriate test chamber. Three replicates of each dosing solution were tested. Dosing solutions were prepared by mixing Resist in reverse osmosis water or acetone to generate doses of 13, 22, 36, 60 and 100 µg a.i./bee. Positive controls were prepared by dissolving dimethoate in acetone to produce dosing solutions of 0.05, 0.10 and 0.30 µg dimethoate/bee. Bees in the test substance, positive control and solvent control groups were

dosed by administering a 2 μL droplet of the appropriate dosing solution to the abdomen or thorax of each bee. Negative control bees were handled identically to bees in the treatment and control groups but were not administered any test or control substance.

During the test, bees were maintained in perforated stainless steel cylinders approximately 9 cm in diameter by 9 cm high. Each end of the cylinder was covered with a plastic Petri dish. An inverted 20 mL glass vial containing 50% sucrose provided an *ad libitum* food source to the test bees. Test chambers were maintained at 26-28°C with a relative humidity of 56-77% in continuous darkness during the test, except during periods of dosing and observation. Bees were observed at ¼, 1¼, 24 and 48 hours after dosing.

Results of the contact test with Resist are presented in Table 1. Mortality at the end of the test ranged form 3 to 15%. The mortality was 8% in the negative control and 7% in the solvent control at test termination. There were no treatment related effects in the test. Based on the results, the 48-hour LD_{50} for honey bees exposed to Resist was determined to be greater than 100 μg a.i./bee

The 24-hour LD_{50} value for honey bees exposed to dimethoate was determined to be 0.139 μg a.i./bee with a 95% confidence interval of 0.122 and 0.161 μg a.i./bee indicating the test methods and procedures were valid for determining contact toxicity to honey bees.

The 48-hour acute contact LD_{50} value for honey bees exposed to Resist was determined to be greater than 100 μg a.i./bee. Therefore, Resist was classified as relatively non-toxic to honey bees according to the toxicity categories of Atkins.

TABLE 1. Cumulative mortality and observations of honey bees after topical exposure to Resist.

Group	Rep	¼ Hour[1]		1¼ Hours		24 Hours		48 Hours		Replicate Mortality (%)	Group Mortality (%)
		Mortality[2]	Effects[3]	Mortality	Effects	Mortality	Effects	Mortality	Effects		
Negative Control	A	0	20 AN	0	20 AN	0	20 AN	1	19 AN	5	8
	B	0	20 AN	0	20 AN	0	20 AN	3	17 AN	15	
	C	0	20 AN	0	20 AN	1	19 AN	1	19 AN	5	
Solvent Control	A	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	7
	B	0	20 AN	0	20 AN	0	20 AN	3	17 AN	15	
	C	0	20 AN	0	20 AN	0	20 AN	1	19 AN	5	
13 µg a.i./bee	A	0	20 AN	0	20 AN	1	19 AN	3	17 AN	15	15
	B	0	20 AN	0	20 AN	1	19 AN	2	18 AN	10	
	C	0	20 AN	0	20 AN	0	20 AN	4	16 AN	20	
22 µg a.i./bee	A	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	5
	B	0	20 AN	0	20 AN	0	20 AN	1	19 AN	5	
	C	0	20 AN	0	20 AN	0	20 AN	2	18 AN	10	
36 µg a.i./bee	A	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	3
	B	0	20 AN	0	20 AN	0	20 AN	1	1 L, 19 AN	5	
	C	0	20 AN	0	20 AN	0	20 AN	1	19 AN	5	
60 µg a.i./bee	A	0	20 AN	0	20 AN	1	19 AN	2	18 AN	10	7
	B	0	20 AN	0	20 AN	1	19 AN	1	19 AN	5	
	C	0	20 AN	0	20 AN	1	19 AN	1	19 AN	5	
100 µg a.i./bee	A	0	20 AN	0	20 AN	0	20 AN	0	20 AN	0	3
	B	0	20 AN	0	20 AN	0	20 AN	1	19 AN	5	
	C	0	20 AN	0	20 AN	0	20 AN	1	19 AN	5	

[1] Observation times represent the approximate number of hours after dosing was completed.
[2] Mortality data are presented as the cumulative number dead of 20 bees originally exposed.
[3] Number of bees exhibiting clinic signs: AN=appear normal, L=lethargic
Data reproduced from Table 1, pg. 15, MRID 46708210.

MRID 467082-11 *Waiver request for terrestrial Plant toxicity*

Actagro LLC is requesting a data waiver for terrestrial toxicity testing of Resist under the rationale that: a)Resist contains 57 % mono-and di-potassium phosphate that have been used extensively as fungicides and fertilizers since 1930's without adverse effect incidents reported, and b) studies cited from public literature (Brunings et al. 2005; Landschoot and Cook, 2005; McDonald et al. 2001; and Thizy et al. 1978) showed no phytotoxicity among phosphorus acid, phosphates/phosphonate products.

BACKGROUND AND REVIEWER COMMENTS

Resist is an end use product for use as a systemic fungicide to control phytophthora, downy mildew, pythium, and other diseases on agricultural and greenhouse crops, indoor and outdoor ornamentals, bedding plants, turf, commercial forests, and domestic trees. The active ingredient is 57.0% by weight mono- and di-potassium phosphite. The sole inert ingredient is [redacted]. A separate CSF for [redacted] was submitted but is not required. The certified

limits for the active and inert ingredients are within the OPPTS-recommended ranges. There is a discrepancy between the identity of the beginning materials and the description of the materials used in the production process. Quality control procedures for the production process were not discussed, and the product packaging was not described. No impurities present at a concentration >0.1% w/w are expected in the product. A five-batch preliminary analysis was provided for one of the beginning materials, but must instead be provided for the end use product. Additional explanation of the enforcement analytical method is needed. The physical and chemical characteristics were adequately addressed, except that results for storage stability and corrosion characteristics tests were not provided.

Bibliography

Brunings, A.,. Datnoff ., Lawrence E. and Simonne Eric H.2005. Phosphorus Acid and Phosphoric Acid: When all P Sources are not Equal. University of Florida IFAS Extension HS1010, pp. 1-8.

Landschoot, P. and Cook J. 2005. Sorting Out the Phosphonate Products. Golf Course Management. Pp. 73-77.

McDonald A. E., Bruce R.G., Plaxton C. W. 2001. Phosphite (Phosphorus Acid): Its Revelance in the Environment and Agriculture and Influence on Plant Phosphate Starvation Response. Journal of Plant Nutrition. Vol. 24 (10), pp. 1505-1519.

Thizy A., Pillon D., Debourge J.C., and Bernard G. 1978. Fungicidal Compositions Containing Phosphorus Acid and Derivatives Tereof. U.S. Patent 4,119,724.

13544

R133191

Chemical: *Mono- and di- potassium salts of phosphorous acid*

PC Code:
076416
HED File Code: 41500 BPPD Tox/Chem
Memo Date: 3/20/2006
File ID: DPD326475
Accession #: 412-07-0026

HED Records Reference Center
1/3/2007