Appendix D: Ecological Effects

Table D-1: Acute Toxicity of Avitrol to Freshwater Fish and Amphibians						
Test Organism	**Test Substance**	**Test Type**	**Endpoint**	**96-Hr. Value mg/L**	**Comments on Study**	**MRID Classification**
Channel catfish *Ictulurus Punctutus*	4-aminopyridene (purity not reported)	Acute	LC_{50} NOAEC LOAEC	2.43 – 5.80 3 (mortality) 4 in soft water at 12°C	Nominal concentrations utilized. Groups of 10 fish/concentration at 11 levels. Tested in 4 different water hardnesses and 3 temperatures. LC_{50}'s range from 2.43 in very hard water at 12°C; 5.80 in soft water at 22°C.	00004083, 00003985, 00004101 Supplemental (final review pending)
Bluegill sunfish *Lepomis macrochirus*	4-aminopyridene (purity not reported)	Acute	LC_{50} NOAEC LOAEC	2.82 – 7.56 4 (mortality) 5 in soft water at 12°C.	Nominal concentrations utilized. Groups of 10 fish/concentration. Tested in 4 different water hardnesses and 3 temperatures. LC_{50}'s range from 2.82 in hard water at 12°C; 7.56 in soft water at 22°C.	00004083, 00003985, 00004101 Supplemental (final review pending)
Southern leopard frog larvae (*Rana sphenocephala*)	4-aminopyridene (purity not reported)	Acute static	LC_{50}	2.4 (2.0-2.9)	16°C. Spring water reconstituted to yield hardness of 24 mg/L as $CaCO_3$.	ECOTOX Ref. # 7412 Supplemental (final review pending)
Southern leopard frog eggs and larvae (*Rana sphenocephala*)	4-aminopyridene (purity not reported)	Static at 16°C	NOAEC	1.0 for larval survival	Hatching success ≤ 5% at 10 mg/L; larval survival ≥ 95% at 1.0 mg/L; ≤ 5% at 2.0 mg/L. Range of concentrations tested not provided.	ECOTOX Ref. # 7412 Supplemental (final review pending)

Table D-2 Acute Toxicity of Avitrol to Marine/Estuarine Fish				
Test Organism	**Test Substance**	**Endpoint**	**Comments and Value (mg/L)**	**MRID Classification**
Cowfish (*Lactophyrs tricornis*) Globe fish (*Chilomycterus sp.*)	Compound 1861 hydrochloride (4-aminopyridine – purity not reported)	LC_{50} NOAEC LOAEC	Four 72-hour static renewal limit tests conducted on 2 fish of each species at nominal concentrations ranging from 4.6 to 15.4 mg/L. No negative controls; however, no effects at lowest concentration. Continuously aerated. Fish transferred to fresh solutions every 24 hours. Mortality: 0, 50, 75 and 100% in 4.6, 6.8, 10.2 and 15.4 mg/L solutions. LC_{50} from combined tests and species: 7.6 ± 1.4 NOAEC 4.6; LOAEC 6.8 (mortality and sublethal effects)	00004111 Supplemental (final review pending)

Table D-3 Acute Toxicity of Avitrol to Freshwater Invertebrates				
Test Organism	**Test Substance**	**Endpoint**	**Value (mg/L)**	**MRID Classification**
Water flea *Daphnia magna*	4-aminopyridene (purity not provided)	EC_{50}	3.2 (2.3-4.5) at 48 hours in 21°C. Neither sublethal effects nor details on mortality provided in study.	ECOTOX Ref. # 7412 Supplemental (final review pending)
Juvenile glass shrimp *Palaemonetes kadiakensis*	4-aminopyridene (purity not provided)	LC/EC_{50}	0.37 (0.25-0.56) at 96 hours in 16° C temp.	ECOTOX Ref. # 7412 Supplemental (final review pending)
Juvenile crayfish *Procamarus acutus acutus*	4-aminopyridene (purity not provided)	LC/EC_{50}	2.2 (1.7-2.8) at 96 hours in 16° C temp.	ECOTOX Ref. # 7412 Supplemental (final review pending)
Mayfly nymph *Isonychia sp.*	4-aminopyridene (purity not provided)	LC/EC_{50}	0.58 (0.45-0.74) at 96 hours in 12° C temp.	ECOTOX Ref. # 7412 Supplemental (final review pending)
Caddisfly larvae *Hydropsyche sp.*	4-aminopyridene (purity not provided)	LC/EC_{50}	15 (9.8-22) at 96 hours in 16° C temp.	ECOTOX Ref. # 7412 Supplemental (final review pending)

Table D-3 Acute Toxicity of Avitrol to Freshwater Invertebrates				
Test Organism	**Test Substance**	**Endpoint**	**Value (mg/L)**	**MRID Classification**
Adult river horn snail *Oxytrema catenaria*	4-aminopyridene (purity not provided)	LC/EC_{50}	62 (53-73) at 96 hours in 16° C temp. Sublethal effects (inability to right themselves when dislodged and inability to cling to sidewalls) observed at concentrations < 62 mg/L (not stated in study at what concentrations)	ECOTOX Ref. # 7412 Supplemental (final review pending)
Adult Asiatic clam *Corbicula manilensis*	4-aminopyridene (purity not provided)	LC/EC_{50}	45 (40-50) at 96 hours in 16° C temp.	ECOTOX Ref. # 7412 Supplemental (final review pending)

Table D-4 Acute, Repeated Dose and Short-Term Dietary Toxicity of Avitrol to Birds						
Test Organism	**Test Substance**	**Test Type**	**Endpoint**	**Value mg/kg or ppm**	**Comments on Study**	**MRID Classification**
Mallard duck *Anas platyrhynchos*	4-aminopyridene (purity not reported - assumed to be 100%)	8-Day Dietary	LC_{50} NOAEC	681 (517-896) ppm < 464 ppm	Tested up to 10000 ppm (nominal). No vehicle control (corn oil in diet). 10 birds/group. Decrease in body weight gain and food consumption observed at lowest concentration level.	00147985 Supplemental (final review pending)
Mallard duck *Anas platyrhynchos*	4-aminopyridene (99.9%)	Acute oral	LD_{50}	5.19 (4.00-6.73) mg/kg	Twelve 3-4 month old male ducks per dose. Value found in summary document by USFWS. Clinical signs listed but no NOAEL/LOAEL provided.	00160000 Supplemental
Mallard duck *Anas platyrhynchos*	4-aminopyridene (95%)	Acute oral	LD_{50}	4.36 (3.36-5.66) mg/kg	Twelve 3-4 month old male ducks per dose. Value found in summary document by USFWS. Clinical signs listed but no NOAEL/LOAEL provided.	00160000 Supplemental
Bobwhite quail *Colinus virginianus*	4-aminopyridene (purity not reported)	Acute Oral	LD_{50}	15.0 mg/kg	Value found in a literature study by the same author; however, original value was from a review. Not possible to classify.	Schafer et al. 1973: a review summarized in 00004083, 00003985, 00004101

Table D-4 Acute, Repeated Dose and Short-Term Dietary Toxicity of Avitrol to Birds						
Test Organism	**Test Substance**	**Test Type**	**Endpoint**	**Value mg/kg or ppm**	**Comments on Study**	**MRID Classification**
Bobwhite quail *Colinus virginianus*	4-aminopyridene (purity not reported) in cracked corn bait with different dilution ratios of treated and untreated bait	Dietary (32 days)	LC_{50}	Not calculable	4 birds tested per concentration level. 100% mortality at ratio of 1:29, 0% mortality at ratio of 1:99 (3% bait – 300 ppm). Single baits not lethal.	00004083, 00003985, 00004101 Supplemental (final review pending)
Mourning dove *Zenaida macroura*	4-aminopyridene (purity not reported)	Acute oral	LD_{50}	8.1 mg/kg	Value found in a literature study by the same author; however, original value was from a review. Not possible to classify.	Schafer et al. 1973: a review summarized 00004083, 00003985, 00004101
Mourning dove *Zenaida macroura*	4-aminopyridene (purity not reported) in cracked corn bait with different dilution ratios of treated and untreated bait	Dietary (7 days)	LC_{50}	Not calculable	7 birds tested per concentration level; 43 controls. 72% mortality at ratio of 1:99 (3% bait); 43% mortality at same ratio but with 2% bait (200 ppm). Single baits not lethal.	00004083, 00003985, 00004101 Supplemental (final review pending)
Mourning dove *Zenaida macroura*	DRC-1327 (4-aminopyridene - purity not reported). Dietary study	Dietary (30 days)	LC_{50} NOAEC	316 (100 – 1000) ppm 100 ppm	6 birds/concentration level. 0, 31.6, 100, 316 ppm tested. 50% mortality at 316 ppm (2.96 mg avitrol/bird/day). No mortalities or sublethal effects at 100 ppm and below.	00004083, 00003985, 00004101 00003998 Supplemental (final review pending)
Coturnix quail *Coturnix coturnix*	4-aminopyridene (purity not reported).	Dietary (28 days)	LC_{50} NOAEC	479 (354-645) ppm 100 ppm	Treatment levels: 0, 31.6, 100, 316, 1000 ppm. 12 birds/ concentration level; 24 controls. 100% mortality at 1000, 3 died at 316 ppm. No mortalities or sublethal effects at 100 ppm and below.	00004083, 00003985, 00004101 Supplemental (final review pending)
Coturnix quail *Coturnix coturnix*	4-aminopyridene (purity not reported)	Acute oral	LD_{50}	7.65 (M), 8.05 (F) mg/kg	Value found in a literature study by the same author; however, original value was from a review. Not possible to classify.	Schafer et al. 1973: a review summarized 00004083, 00003985,

Table D-4 Acute, Repeated Dose and Short-Term Dietary Toxicity of Avitrol to Birds						
Test Organism	**Test Substance**	**Test Type**	**Endpoint**	**Value mg/kg or ppm**	**Comments on Study**	**MRID Classification**
						00004101
Coturnix quail *Coturnix coturnix*	4-aminopyridene (purity not reported).	Dietary (40 days)	LC_{50}	>316 ppm	Treatment levels: 0, 316 ppm. 24 birds/ concentration level. 8% mortality at 316 ppm, 4% mortality at 0 ppm	00004083, 00003985, 00004101 Supplemental (final review pending)
Starling *Sturnus vulgaris*	4-aminopyridene (purity not reported).	Dietary (25 days)	LC_{50}	>1.78 mg/kg/day	Treatment levels: 0, 1.78 mg/kg/day; 10 birds/concentration level. No mortalities.	00004083, 00003985, 00004101 Supplemental (final review pending)
Starling *Sturnus vulgaris*	4-aminopyridene (purity not reported)	Acute oral	LD_{50}	4.9 mg/kg	Value found in a literature study by the same author; however, original value was from a review. Not possible to classify.	Schafer et al. 1973: a review summarized 00004083, 00003985, 00004101
Coturnix quail *Coturnix coturnix*	4-aminopyridene (purity not reported)	4-week oral gavage	NOAEC/ LOAEC	LOAEC 5.62 mg/kg	Only 0 and 5.62 mg/kg tested. Slight to moderate intoxication symptoms, some mortality, reduction in egg production at 5.62; however, hatchability not affected.	05003186 Supplemental (final review pending)
Quelea *Quelea quelea*	4-aminopyridene (purity not reported)	Acute oral	LD_{50}	5.6 mg/kg	2 birds/dose level, 4 day observation period. Data not available, so value could not be verified.	05003191 Supplemental (final review pending)
House sparrow *Passer domesticus*	4-aminopyridene (purity not reported)	Acute oral	LD_{50}	7.5 mg/kg	2 birds/dose level, 4 day observation period. Data not available, so value could not be verified.	05003191 Supplemental
Red Winged blackbird *Agelaius phoeniceus*	4-aminopyridene (purity not reported)	Acute oral	LD_{50}	2.4 mg/kg	2 birds/dose level, 4 day observation period. Data not available, so value could not be verified.	05003191 Supplemental
Blackbilled and Yellowbilled	4-aminopyridine	Acute oral	LD_{50}	2.4 mg/kg (95% CI could	2 blackbilled and 1 yellowbilled /dose level at 1.8 and 3.2 mg/kg. Affected at	00004006 Supplemental

Table D-4 Acute, Repeated Dose and Short-Term Dietary Toxicity of Avitrol to Birds						
Test Organism	**Test Substance**	**Test Type**	**Endpoint**	**Value mg/kg or ppm**	**Comments on Study**	**MRID Classification**
magpies *Pica pica* and *Pica nuttalli*			NOAEL	not be calculated) 1.8 mg/kg	NOAEL but no significant clinical signs.	(final review pending)
Sparrow hawk *Falco sparverius*	4-aminopyridine	Acute oral	LD_{50} NOAEL	5.6 mg/kg (4.2-7.5 mg/kg) 3.2 mg/kg	2 birds/dose level at 4.2, 5.6, 7.5 mg/kg and 1 bird at 1.0, 1.8 & 3.2 mg/kg.	00004006 Supplemental (final review pending)

Table D-5 Acute Toxicity of Avitrol to Mammals					
Test Organism	**Test Substance**	**Test Type**	**Endpoint**	**Value (mg/kg)**	**MRID Classification**
Rat *Rattus norvegicus*	Compound 1861 Hydrochloride (purity not reported)	Acute oral Gavage	LD_{50}	28.7 very steep dose-response curve	00004024
Dog Mongrel	Compound 1861 Hydrochloride (purity not reported)	Acute oral Capsule	LD_{50}	3.7 ± 0.2. Range of doses: 2.0 – 6.8 mg/kg. Clinical signs (ataxia, hyperactivity) observed at two lowest dose levels (2.0 and 3.0), beginning ½ hour following dosing and persisting to 8 hours post-dosing. Normal by 24 hours. More severe clinical signs at higher dose levels. No gross pathologic lesions.	00004024

Table D-6 Chronic Toxicity of Avitrol to Birds

Test Organism	Test Substance	Endpoint	Value	Affected Endpoints	MRID Classification
Coturnix quail *Coturnix coturnix*	4-aminopyridene (purity not reported)	NOAEC LOAEC	31.6 ppm 100 ppm for sublethal effects	Up to 8 week feeding: 6 pairs/concentration level; 6 pairs each in 2 control groups. Mortality: 0 (0), 31.6 (1), 100 (1), 316 (1), 1000 (all birds - 12) ppm. Reduced body weight gain in males at 100 ppm and above. Reduced food consumption and signs of intoxication at 316 ppm and above. All measured parameters reduced at 1000 ppm. No reproductive parameters affected.	05003186 Supplemental (final review pending)
Coturnix quail *Coturnix coturnix*	4-aminopyridene (purity not reported)	NOAEC LOAEC	316 ppm 1000 ppm for sublethal effects	9 pairs of F_1 progeny from chronic feeding study above (8 pairs from control) tested for egg production and hatchability at maturity. All birds from 1000 ppm group had died so no progeny. No effects on egg production and hatchability after reaching maturity.	05003186 Supplemental (final review pending)

Table D-7 Secondary Toxicity of Avitrol to Predatory Birds and Mammals

Test Organism	Test Substance	Endpoint	Value	Comments and Affected Endpoints	MRID Classification
Canine *Canis familiaris-Canis Iatrans* mix and *Canis familiaris*	4-aminopyridine (purity not reported)	Acute Feeding NOAEC	8.24 mg/kg bw	2 weeks after chronic feeding study (below), same male dog fed 10 ground poisoned birds that had been gavaged with 50 mg/kg bw 4-AP. 2 weeks later same male offered 10 birds dosed with 100 mg/kg bw 4-AP. At same time, female dog offered 10 birds dosed with 150 mg/kg bw 4-AP. 2.68 – 8.24 mg/kg 4-AP from birds ingested by dogs without effects.	0004001 0004006 Supplemental (final review pending)
Canine *Canis familiaris-Canis Iatrans* mix and *Canis familiaris*	4-aminopyridine (purity not reported)	Chronic Feeding NOAEC	0.4 mg/ kg bw/ day	1 female beagle-coyote cross & 1 male beagle fed 175 g of mixture containing 75% ground 4-AP poisoned blackbird & 25% dog chow 2x/day for 8 days. Additional dog chow *ad lib*. between feedings. 0.39-0.4 mg/kg/day 4-AP. No effects; however, birds had been fed mixture of 1 part 3.0% 4-AP treated cracked corn with 99 parts untreated cracked corn. Unclear on how many pieces of treated corn birds ate, so unclear on how much dogs received.	0004001 0004006 Supplemental (final review pending)
Rats *Rattus norvegicus*	4-aminopyridine (purity not reported)	Acute Feeding NOAEC	67.5 mg/ kg bw	5 rats fed 1 ground bird dosed with 100-300 mg/kg by gavage. Treated feed mixed with oatmeal and consumed within 3 hours. Mg/kg bw dose of 4-AP from birds ranged from 14.31-67.49. No effects.	0004001 0004006 Supplemental (final review pending)
Rats *Rattus norvegicus*	4-aminopyridine (purity not reported)	Chronic Feeding NOAEC	1.23 mg/ kg bw/ day	10 rats fed *ad lib* mixture containing 75% ground 4-AP poisoned blackbird & 25% oatmeal for 21 days. Mean 1.23 mg/kg/day 4-AP. No effects; however, see comment under chronic feeding dog.	0004001 0004006 Supplemental (final review pending)
Magpies	4-aminopyridine	Chronic	7.74 mg/kg	For 20 days, black-billed magpies fed 2 poisoned	0004001

Table D-7 Secondary Toxicity of Avitrol to Predatory Birds and Mammals

Test Organism	Test Substance	Endpoint	Value	Comments and Affected Endpoints	MRID Classification
Pica pica and *Pica nuttalli*	(purity not reported)	Feeding NOAEC	bw/day	birds/day; another magpie fed 1 bird/day. 1 control fed untreated bird. All consumed except bones, brain and feathers. Other feed available *ad lib*. 3.93-7.74 mg 4-AP from birds/kg bw/day consumed. No observed effects; however, see comment under chronic feeding dog.	0004006 Supplemental (final review pending)
Sparrow hawk *Falco sparverius*	4-aminopyridine (purity not reported)	Chronic Feeding NOAEC	6.05 mg/kg bw/day	For 7, 21, 45 days, 3 sparrow hawks were fed poisoned birds. One control bird. Up to 6.05 mg 4-AP/kg bw/day tested. No observed effects; however, see comment under chronic feeding dog.	0004001 0004006 Supplemental (final review pending)
Sharp-shinned hawk *Accipiter striatus*	4-aminopyridine (purity not reported)	Chronic Feeding NOAEC	6.38 mg/kg bw/day	One hawk received poisoned birds for 21 days. A maximum of 6.38 mg 4-AP from birds/kg bw hawk per day was consumed. No observed effects, including gross necropsy. However, see comment under chronic feeding dog.	0004001 0004006 Supplemental (final review pending)
Red-tailed hawk *Buteo jaraicansis*	4-aminopyridine (purity not reported)	Chronic Feeding NOAEC	1.06 mg/kg bw/day	One hawk received field poisoned birds (4% solution of 4-AP sprayed on husked ripening ears of corn) for 2 weeks. Amounts of 4-AP present/bird unknown but was enough to kill them. Based on LD_{50} of 2.4 mg/kg, authors surmised that hawk received > 0.17 mg 4-AP/bird. No toxicological symptoms.	0004001 0004006 Supplemental (final review pending)

Table D-8 Toxicity of Avitrol to Terrestrial Plants						
Test Organism	**Test Substance**	**Test Type**	**Endpoint**	**Value**	**Affected Endpoint**	**MRID Classification**
Corn (*Zea may*) Pioneer 3956 Hybrid	4-aminopyridene (purity unspecified)	Phytotoxicity to seeds and seedlings	NOAEC	1.0 ppm 10 ppm <0.1 ppm following 2 apps.	Germination (no effect up to 1.0 ppm) Fresh weights of seedlings (no effect up to 10 ppm following single application, slight effect at all concentrations (5-12% reduction) following 2 applications up to 100 ppm	00004124 00004037 Supplemental (final review pending)

Aquatic Effects Characterization

a. Aquatic Animals

(1) Acute Effects

Freshwater Fish and Aquatic Amphibians

The studies with fish were conducted using channel catfish (*Ictalurus punctatus*) and bluegill sunfish (*Lepomis macrochirus*) (MRID 00004083). All studies were 96-hour acute toxicity tests and were conducted at the Fish Control Laboratory, La Crosse, Wisconsin. Groups of ten fish were exposed to varying nominal concentrations (from 0-50 ppm) in four different water hardnesses (10-13, 40-48, 160-180 and 280-320 mg/L as $CaCO_3$) and three different temperatures (12, 17 and 22^oC). The 96-hour LC_{50} for channel catfish ranged from 2.43 ppm in very hard water to 5.80 ppm in standard soft water at 22^oC. In standard water, the test material was significantly less toxic ($P \leq 0.05$) at 22^oC than at either 12 or 17^oC even though mortality occurred more rapidly at 22^oC. At 12^oC, the test material caused similar toxicity in very soft, soft and hard water (LC_{50} of 4.00 ppm) but significantly greater toxicity in very hard water (LC_{50} of 2.43 ppm). The 96-hour LC50 for bluegills varied from 2.82 to 7.56 ppm, depending on temperature and water quality. In soft water, toxicity increased with reductions in temperature with LC_{50} values of 7.56, 5.60 and 4.41 ppm at 22, 17 and 12^oC, respectively. At 12^oC, the LC_{50} values were 3.40, 4.41, 2.82 and 3.20 ppm in very soft, soft, hard and very hard test water. While the data obtained from these studies may not be quantitatively useful for risk assessment purposes, they do provide qualitative evidence of the toxicity of 4AP to fish species.

The avian control chemical, avitrol was developed by the FWS' Denver Wildlife Research Center (DWRC) (ECOTOX Ref. 7412). The primary objectives of this study were to determine the toxicity of avitrol and to evaluate potential negative effects to frog eggs and larvae. A stock solution of the test material was prepared by dissolving it in either water or acetone. The stock solution was then serially diluted to prepare the desired nominal concentrations. The dilution water was then carefully stirred to ensure adequate mixing of the test material without injuring the test organisms, southern leopard frog eggs and tadpoles (*Rana sphenocephala*). The static test was conducted following guidelines outlined in ASTM Committee E-35 on Pesticides (1980) with some minor modification. The frog larvae were tested in spring water reconstituted with minerals to yield a total hardness of about 24 mg/L as $CaCO_3$. Size and volume of the glass jars included 3.78 or 18.9 L with fill volumes of either 3 or 15 L. Tests were conducted temperatures of 12, 16 or 22^oC for 96-hours under static conditions. Temperatures were maintained by immersing test vessels into water baths equipped with water chilling units or heaters. The 96-hour LC/EC_{50} values (and 95% C.I.) were determined using the method of Litchfield and Wilcoxon (1949).

For the tests on frog eggs, leopard frog egg clusters were collected from ponds at the Warm Springs Hatchery within 16 hours after deposition. One egg mass was used for

each test and prior to initiation, each mass was gently teased into approximately equal sizes. Dilution water kept at 16°C and after introduction of the test materials, eggs remained in solution until they hatched or until development ceased. At test termination, the percentages of undeveloped eggs or dead larvae remaining in the gelatinous envelope of embryos which hatched, and of larvae which survived after hatching, were noted. The 96-hour LC_{50}s ranged from 2.3 – 5.9 mg/L with increasing toxicity with increasing temperature. In the egg study, the percentages of undeveloped eggs or dead larvae remaining in the gelatinous envelope of embryos which hatched and of larvae which survived after hatching are summarized as follows: hatchling success was ≤ 5% and larval survival was ≥ 95% at 10 mg/L whereas larval survival was ≤ 5% at 2.0 mg/L. The NOAEC for larval survival was 1.0 mg/L.

Marine/Estuarine Fish

Cowfish (*Lactophyrs tricornis*) and Globe fish (*Chilomycterus sp*) were tested in four, 72-hour acute limit toxicity studies using a single nominal concentration and exposing two fish from each species (four fish total) to each concentration under static renewal conditions (MRID 00004111). The four tests were conducted using nominal concentrations of 4.6 mg/L for the first test, 6.8 mg/L for the second test, 10.2 mg/L for the third test and 15.4 mg/L for the fourth test. A negative control was not used in any of the four tests. Each of the four tests was conducted in separate continuously-aerated aquaria containing two liters of synthetic saltwater. The appropriate amount of test solution was added to each test vessel to achieve the desired concentration and was mixed thoroughly prior to introduction of the fish. Fish were transferred to freshly prepared solutions every 24 hours. At each renewal period, observations of mortality and sub-lethal effects were made and recorded. At test termination, mortality data were used to determine an LC_{50} value using methods of Weil (1952), Thompson (1947) and Thompson and Weil. Necropsies were conducted on all fish that died during the exposure periods. The cowfish had an approximate, average length and weight of 6.0 in and 15 g and the globe fish had an approximate, average length and weight of 6.0 in and 20 g. The fish were acclimated to test conditions for 7 days in a 30 gallon aquarium. No sub-lethal effects were observed in the 4.6 mg/L test (NOAEL). Observed sub-lethal effects at higher concentration levels included hyperkinetic movement (swimming in rapid jerky movements), thrashing, loss of equilibrium and labored respiration; effects typically occurred in the order listed. At the LOAEL of 6.8 mg/L, these effects appeared during the last 24 hours of the exposure period and the two fish that died were found dead at test termination. The two surviving fish appeared normal and healthy six hours after removal from the treated solutions and restoration to the acclimation aquarium. At higher concentrations, the same effects were observed but earlier during the test period. Again, any surviving fish appeared normal and healthy six hours after removal from the treated solutions and restoration to the acclimation aquarium. The combination of mortality data from the four toxicity tests yielded a 72 hour LC_{50} (±SD) of 7.6 (±1.4) mg/L. No gross pathological alterations in the tissues or organs were observed in any of the necropsies performed.

Aquatic Invertebrates

The avian control chemical, avitrol was developed by the FWS' Denver Wildlife Research Center (DWRC) (ECOTOX Ref. 7412). The primary objective of this study was to provide information on potential effects to aquatic organisms from use or accidental application to aquatic habitats. A stock solution of the test material was prepared by dissolving it in either water or acetone. The stock solution was then serially diluted to prepare the desired nominal concentrations. The dilution water was then carefully stirred to ensure adequate mixing of the test material without injuring the test organisms. Test organisms included: adult scud (*Hyalella azteca*), juvenile glass shrimp (*Palaemonetes kadiakensis*), juvenile crayfish (*Procamarus acutus acutus*), damselfly nymph (*Ischnura sp.*), dragonfly nymph (*Didymops sp.*), mayfly nymph (*Isonychia sp.*), caddisfly larvae (*Hydropsyche sp.*), adult backswimmers (*Notonecta sp.* and *Buenoa sp.*) adult river horn snail (*Oxytrema catenaria*), Bukley's filter clam (*Elliptio buckleyi*), adult Asiatic clam (*Corbicula manilensis*) and juvenile water fleas (*Daphnia magna*). Water fleas were obtained from in-house laboratory cultures; glass shrimp and crayfish were cultured in earthen ponds at the Warm Springs National Fishery Hatchery (Georgia). All other invertebrates were obtained from small rivers and streams near Warm Springs and held for at least 3 days prior to exposure. All static tests were conducted following guidelines outlined in ASTM Committee E-35 on Pesticides (1980) with some minor modification. Water fleas were tested in reconstituted water with a hardness of about 40 mg/L as $CaCO_3$; all other species were tested in spring water reconstituted with minerals to yield a total hardness of about 24 mg/L as $CaCO_3$. Size and volume of the test vessels for the tests with water fleas were 600 and 500 mL, respectively. All other species were tested in glass jars with sizes of 3.78 or 18.9 L with fill volumes of either 3 or 15 L. Tests were conducted temperatures of 12, 16 or 22^{o}C for 96-hours under static conditions. Temperatures were maintained by immersing test vessels into water baths equipped with water chilling units or heaters. The 96-hour LC/EC_{50} values (and 95% C.I.) were determined using the method of Litchfield and Wilcoxin (1949). The concentrations tested were not provided in the published report. Only the results were reported. All of the species exposed to 4-AP exhibited ≥50% mortality at one of the tested concentrations after 96-hours of exposure. Juvenile glass shrimp were the most sensitive species with a 96-hour LC_{50} (and 95% C.I.) of 0.37 (0.25-0.56) mg/L. Adult river horn snails and adult Asiatic clams were the most resilient species with LC_{50} values (and 95% C.I.) of 62 (53-73) and 45 (40-50) mg/L, respectively. Surviving snails exhibited signs of 4-AP intoxication at concentrations lower than the LC_{50} (62 mg/L). The observed sub-lethal effects were the inability to right themselves when dislodged and the inability to cling to sidewalls of the test vessel. Results are summarized in the tables at the beginning of this Appendix.

No acute ecotoxicity data are available for marine/estuarine invertebrates.

(2) Chronic Effects

Fish

No chronic ecotoxicity data are available for either freshwater or marine/estuarine fish.

Aquatic Invertebrates

No chronic ecotoxicity data are available for either freshwater or marine/estuarine invertebrates.

(3) Field Studies

There are currently no aquatic field studies available for Avitrol.

b. Aquatic Plants

No ecotoxicity data are available for either freshwater or marine/estuarine plants.

Terrestrial Effects Characterization

a. Terrestrial Animals

(1) Acute Effects

Birds

4-Aminopyridine (4AP), was tested with adult bobwhites (*Colinus virginianus*), mourning doves (*Zenaida macroura*), ring-necked pheasants (*Phasianus colchicus*), coturnix quail (*Cotunrix coturnix*) and starlings (*Sturnus vulgaris*) to show how granivorous birds respond to long-term 4AP exposure (MRID 00004083). Studies were conducted over a five-year period at the Denver Wildlife Research Center and involved three methods of administration; treated bait, feed and gavage. Bobwhites, mourning doves and ring-necked pheasants, in groups of 2 to 7, were fed for 7 to 35 days on 3% 4AP-treated cracked corn baits (average weight of 22 mg) diluted with untreated cracked corn at one or more of the following rates: 1:199 (150 ppm), 1:99 (300 ppm), 1:49 (600 ppm) and 1:29 (1000 ppm). Controls for each species only received cracked corn for the test period. Mourning doves and coturnix, in groups to 6 to 12, were fed a homogeneously mixed diet of Purina Game Bird Breeder Layena with nominal concentrations of 0 (negative control), 31.6, 100, 316 and 1000 ppm for a period of 28 to 40 days. Coturnix received all treatment concentrations while mourning doves received all but the 1000 ppm treatment. Ten starlings, caged individually, were treated with a dose of 1.78 mg/kg 4AP in a propylene glycol solution for 25 days by oral gavage. Ten control starlings only received propylene glycol via gavage. Starlings and doves were captured from wild populations and acclimated to test conditions prior to test initiation; however, the duration of the acclimation period was not reported. Quail and pheasants were bred on site. Food consumption and body weights were determined every 1 to 7 days and observations of signs of intoxication were made daily. The LC_{50} values were determined by the method of Thompson (1947).

Treated bait with dilution ratios of 1:29 and 1:99 caused >50% mortality for bobwhites and mourning doves, respectively. Death in both the bobwhites and mourning doves appeared to be the result of acute intoxication from consuming more than one treated bait at one feeding; single baits are not lethal to either species. All intoxicated birds showed the typical acute poisoning symptoms described by Schafer *et al*. (1973). Erratic mortality was observed at every treatment level in adult ring-necked pheasants; therefore, it was not possible to determine the lowest dilution ratio for 4AP baits that would cause >50% mortality. The observed mortality was not attributed to 4AP treatment, rather to the inability of these birds to subsist for extended periods exclusively on corn. Therefore, the data on ring-necked pheasants were not summarized in the ecological effects characterization section of this risk assessment. Control and treated birds lost 15-30% of their original body weight, and the birds that died exhibited the greatest weight loss. No typical signs of 4AP intoxication were observed. However, in a separate 2-day test with 4-6 month old pheasants, two out of four birds died after a single day of feeding on 1:29 diluted bait and sings of intoxication were observed. Most likely the mortality was a combination of a lower body weight of the adults and a correspondingly higher food intake per unit of body weight.

Treated feed yielded LC_{50} values of 479 and 316 ppm for coturnix and mourning doves, respectively; concentrations of 31.6 and 100 ppm had no observable effects on either species. Coturnix and mourning doves consumed an average of 1.5 and 1.3 LD_{50} doses, respectively, at the 100 ppm treatment level without any observed effects. At the 316 ppm treatment level, 3 of the 387 coturnix died after showing symptoms of acute 4AP poisoning. Those birds surviving exhibited minor symptoms such as hyperactivity and mild body tremors; food consumption and body weight were not affected. Coturnix at the 316 ppm treatment level consumed an average of 5.3 LD_{50} doses per day. At the 316 ppm treatment level, 3 of the 6 doves died after exhibiting 4AP intoxication symptoms throughout the duration of the test; the average consumption at this level was 4.3 LD_{50} doses per day and food consumption was depressed. Coturnix at the 1000 ppm treatment level displayed 4AP poisoning within 5 days of test initiation, food consumption was decreased by 70% and body weight declined rapidly. All toxicity symptoms were severe and lasted anywhere from a few hours to three days; all test birds died within three weeks. Two males and one female became completely paralyzed after severe and prolonged tonic convulsions. The average consumption was 6.6 LD_{50} doses per day.

Starlings treated by gavage did not exhibit any symptoms of 4AP intoxication. The birds were treated for 25 days with 4AP concentrations of 1.78 mg/kg representing 36% of the acute oral LD_{50}, yielding a total consumption of 9 LD_{50} doses over the entire test period.

The authors stated that the results with birds indicate that 4AP does not have cumulative effects in doves or coturnix according to the criteria used in the "Index of Chronicity" (Kenega, 1973). Repeated sub-lethal doses had no greater effects than single sub-lethal doses. All observed toxicity appeared to be caused by the birds ingesting more

test material than could be either excreted or detoxified. In cases where symptoms occurred in mourning doves and coturnix, removal of treated feed resulted in the disappearance of the symptoms.

The acute dietary toxicity of 4-aminopyridine (Avitrol) to 14-d-old mallard ducks (*Anas platyrhynchos*) was assessed over 8 days (MRID 00147985). 4-Aminopyridine was administered to the birds in the diet at nominal concentrations of 0 (negative control), 464, 1,000, 2,150, 4,640 and 10,000 ppm. There was no vehicle control (corn oil in the diet). The 8-day acute dietary LC_{50} was 681 ppm, which categorizes 4-aminopyridine (Avitrol) as moderately toxic to Mallard Ducks (*Anas platyrhynchos*) on an acute dietary toxicity basis. The 8-day NOAEC of 4-aminopyridine based on % body weight gain was <464 ppm. These results are based on the nominal concentrations, as the purity of the test material was not reported and the concentrations that birds were exposed to were not measured. Mortality was observed at all treatment levels, with the three highest treatment levels experiencing complete mortality. Body weight gain was reduced relative to the negative control in the two lower treatment levels. Prior to death, birds experienced depression and lower limb weakness.

4-Aminopyridine was testeed to provide information on the reproductive performance of coturnix quail when fed sub-acute or chronic levels (MRID 05003186). Three separate experiments were conducted: the first consisted of administering single, sub-acute oral doses to males and females and observing reproductive effects; the second test was conducted to determine the effects of chronic dietary exposure on reproduction in breeding pairs; and the third test was conducted to determine the effects of chronic exposure of the parents on reproduction in the F_1 generation. Quails used in the single-dose sub-acute and chronic feeding studies were raised and shipped from the Poultry Science Department, University of California at Davis when they were 4 weeks-old. Quails used in the F_1 generation study were the progeny of the adults in the chronic feeding study. Birds were randomly placed into pairs and were acclimated for 4 weeks under a photoperiod of 8L:16D; following 4 weeks, the photoperiod was adjusted to 16L:8D and records of egg production were kept over the following 7 weeks. Those pairs that produced at least 10 fertile eggs during the last 2-week period were used for the reproduction studies. Fertile pairs were randomly separated into two groups, one for the single-dose sub-acute study (males and females were separated for at least 14 days prior to treatment) and the other group for the chronic feeding study.

In the 4-week oral, single-dose sub-acute study, males and females received a propylene glycol solution containing 0 or 5.62 mg/kg 4AP via gavage, and then paired with untreated mates. Ten males and ten females were treated in the event of mortality; however, only 6 randomly selected pairs were used in each evaluation. In the 8-week chronic feeding study, breeding pairs were fed *ad libitum* for 4 weeks on a standard laboratory diet that was thoroughly blended with concentrations of 0 (negative control), 31.6, 100, 316 and 1000 ppm 4AP. Food consumption, bird weight, egg production, eggshell thinning, hatchability and chick mortality were measured weekly during four, 2-week periods: pretreatment, first treatment period (T1), second treatment period (T2) and post-treatment. Egg production was recorded daily. Each treatment level consisted of

one group of six pair of quail except the control, which consisted of two groups. A second chronic feeding test was conducted incorporating concentrations of 0 and 316 ppm and each treatment level consisting of 12 pairs. The treated feed was provided for three continuous 2-week periods; pretreatment and post-treatment measurements were not made. In the sub-acute and chronic feeding studies, reproductive parameters were not measured on eggs after the death of the male.

The F_1 generation study was conducted by taking chicks hatched during the last two weeks of treatment in the 4-week chronic feeding study and measuring their reproductive performance at maturity. Nine randomly selected pairs of offspring produced by adults fed 31.6, 100 and 316 ppm 4AP and eight pairs produced by adults from the control were tested for egg production and hatchability at maturity. All adult birds from the 1000 ppm 4AP treatment level died within three weeks.

For the four weeks of observation following the single oral dose of 0 (negative control) or 5.62 mg/kg 4AP, egg production ranged from 52.4-69.0% in the negative control, 57.1-71.4% when males were treated with 4AP and 35.7-66.7% when females were treated with 4AP. Egg production was determined as a percent of a 42-egg potential each week (6 hens times 7 days). Hatchability ranged from 51.7-80.0% in the negative control, 60.0-97.1% when males were treated with 4AP and 62.5-92.9% when females were treated with 4AP. Slight to moderate intoxication symptoms (hyperactivity, tremors and minor motor seizures) were observed in the birds treated with 4AP within 1 to 4 hours following administration and two males died within 24 hours. Egg production by treated females was significantly reduced during the third week of the test (35.7%), while hatchability was not affected (86.7%). Hatchability in the treated and untreated groups was depressed during the first week of exposure; however, this was a result of the study design since the birds were not paired with mates until immediately after treatment.

Throughout the duration of the chronic feeding study no mortalities were observed in the negative control, one female died in the 31.6 ppm treatment group, one male died in the 100 ppm treatment group, one male died in the 316 ppm treatment group and complete mortality occurred in the 1000 ppm treatment group. No significant sublethal effects were observed in those birds at the 31.6 ppm treatment level and the average daily intake of 4AP was 4.3 mg/kg. In the 100 ppm treatment group, no reproductive parameters were negatively affected; however, males did not gain weight as they did in the control and 31.6 ppm treatment group. The average daily intake of 4AP was 12.1 mg/kg. Two tests were conducted with the 316 ppm treatment group; in the first test birds were treated for four weeks and in the second test birds were treated for six weeks. Significant reproductive effects were not observed in either test; however, males did not gain weight as in the negative control and 31.6 ppm treatment group. In the first test, food consumption was significantly reduced during T1 relative to the same time period in the negative control and the average daily intake of 4AP was 34.5 and 43.4 mg/kg during T1 and T2, respectively. Many birds showed symptoms of slight acute intoxication (hyperactivity and tremors). All birds in the 1000 ppm treatment group died within three weeks. Severe intoxication symptoms (clonic and tonic convulsions) were observed within three to five days following test initiation. During the T1 period, all

measured parameters were significantly reduced relative the pre-treatment period and the control birds during the T1 period. The average daily intake of 4AP was 51.9 mg/kg.

The F_1 progeny of the birds from the chronic feed study exhibited no deleterious effects when mating after reaching sexual maturity. No significant differences were observed in egg production or hatchability between progeny of treated parental birds and progeny of control parental birds.

These results indicate that 4AP exposure to coturnix quail does not have deleterious impacts on reproduction when exposure is a single sub-acute dose that does not exceed 5.62 mg/kg or when chronic dietary exposure does not exceed 316 ppm daily for 6 weeks. Furthermore, chronic dietary exposure of 316 ppm does not negatively impact the reproductive capacity of the F_1 generation. In the 4-week chronic feeding studies, live chick production was reduced at all treatment levels during the post-treatment period; the reductions were not statistically significant. These reductions were not observed as consistently in the control birds or in the treated birds during the pre-treatment and treatment periods. It is possible that these reductions were the result of some unknown environmental factor or from the removal 4AP from the diet.

4-Aminopyridine was tested in acute oral toxicity tests in Quelea (*Quelea quelea*), house sparrow (*Passer domesticus*) and red-winged blackbird (*Agelaius phoeniceus*, MRID 05003191). The text of both the study and the summary data evaluation record (DER) are unreadable; however, there are some readable tables. Two birds were used at each dose level with a 4-day observation period. No mortality data were provided. Therefore, the results could not be validated. The acute oral LD_{50}'s for quelea, house sparrows and blackbirds were 5.6, 7.5 and 2.4 mg/kg, respectively.

Mammals

An acute oral toxicity study with rats (*Rattus norvegicus*) reports an LD_{50} of 28.7 mg/kg with a very steep dose-response curve (MRID 00004024). 4-Aminopyridine is even more toxic to mongrel dogs when administered by capsule. The LD_{50} is 3.7 mg/kg. Clinical signs were observed, starting at the lowest dose administered (2.0 mg/kg) (MRID 00004024).

(2) Chronic Effects

Birds

This study is summarized in the acute toxicity section (MRID 05003186).

Mammals

No chronic toxicity studies are available for mammals. There are two subchronic toxicity studies conducted with 4-AP hydrochloride with rats (MRID 00004026) and dogs (MRID 00004027) which indicate some neurological symptoms (hyperirritability to

noise and touch in rats at 300 ppm (15 mg/kg bw/day) and salivation and muscular weakness in dogs at 2.0 - 3.25 mg/kg bw/day. There were also some changes in brain weights (increased in rat, decreased in dog) and liver weights (increased in rat). The NOAELs of these two studies were 30 ppm (1.5 mg/kg bw/day) and 1.0 mg/kg bw/day for rats and dogs, respectively.

(3) Secondary Toxicity to Birds and Mammals

To assess the secondary hazards associated with the consumption of 4-aminopyridine-killed blackbirds (*Agelaius phoenicues*), canines (*Canis familiaris-Canis latrans* mix and *Canis familiaris*), rats (*Rattus norvegicus*), magpies (*Pica pica* and *Pica nuttalli*) and sparrow hawks (*Falco sparverius*) were tested under chronic and acute feeding conditions; sharp-shinned hawks (*Accipiter striatus*) and red-tailed hawks (*Buteo jaraicansis*) were tested under chronic feeding conditions (MRID 0004001).

Preparation of blackbirds:

Twenty to forty red-winged blackbirds of mixed age and sex were placed daily in a 6' x 6' x 6' aluminum cage and offered a feed mixture containing 1 part 3.0% 4-aminopyridine-treated cracked corn (average particle weight of 22 g) and 99 parts of untreated cracked corn. No other food was presented, but drinking water was available *ad libitum*. After being exposed to, and ingesting, the treated food the birds were allowed to expire (usually between 15 minutes and 12 hours). Following death, the skin, beak and feet were removed and the carcasses were either refrigerated (33^{o}F) or frozen (0^{o}F) until an aggregate of 10-32 carcasses were collected.

For the canines, the birds were then weighed and chopped in a Hobart food chopper, mixed with an amount of dry dog food approximating 33% of the aggregate carcass weight, and put through an electric meat grinder to reduce bone fragments to a minimal size. The resulting treated feed was refrigerated until use; however, no treated feed was retained for more than 28 hours. For the rats, the birds were then weighed, ground in an electric meat grinder, mixed with an amount of oatmeal approximating 33% of the aggregate carcass weight, mixed and reground in the meat grinder. The resulting material was refrigerated until use; however, no treated feed was retained for more than 4 hours.

For orally dosed blackbirds: groups of ten fasted birds were intubated with propylene glycol solutions of 4-aminopyridine to give individual dosages of 50, 100 and 150 mg/kg (20.8, 41.7 and 62.5 times the acute oral LD_{50} of 4-aminopyridine to redwings which is 2.4 mg/kg). Following death, birds were skinned, debeaked and had the legs removed, weighed, ground to hamburger consistency, reweighed and no traces of propylene glycol were observed in any fecal material ejected prior to death. Death occurred between 5.5 and 7.5 minutes after intubation on average.

For the predators that eat birds: after being exposed to, and ingesting, the treated food the birds were allowed to expire (usually between 15 minutes and 12 hours).

Following death, the birds were frozen (0^{o}F) and held until the day of use and birds were never held for more than 7 days after death.

Canines: Chronic study: One individually-caged adult female beagle-coyote cross (*Canis familiaris-Canis latrans*) and one individually caged adult male beagle (*Canis familiaris*) were fed 175 g of a mixture containing 75% ground 4-aminopyridine-killed red-winged blackbirds and 25% ground dry dog food twice daily for a period of 8 days. Both animals were also offered water and additional dry dog food *ad libitum* between test feedings.

Acute study: Two weeks following the termination of the chronic feeding study, the male beagle was offered 10 ground redwings orally dosed at 50 mg/kg each, which he consumed 2 hours after they were offered; the actual feeding time was only 2-3 minutes. Two weeks later, the same male beagle was offered another 10 ground redwings dosed at 100 mg/kg each, which he again consumed in a 2-3 minute period. Concurrently, the female beagle-coyote mix was offered 10 ground redwings dosed orally at 150 mg/kg each which were consumed similar to those by the male beagle.

Rats: Acute study: Five individually caged male white rats were each offered 1 ground red-winged blackbird that had been previously dosed with propylene glycol intubation with 100, 150, 200, 250 and 300 mg/kg levels of 4-aminopyridine. In order to make the ground redwings more palatable, oatmeal was added to the ground redwings at the rate of 0.33 grams of oatmeal per gram of redwing. The food was consumed by all rats within 3 hours. The acute oral LD_{50} of 4-aminopyridine to rats is 20 mg/kg.

Chronic study: Ten individually-caged white laboratory rates were offered, *ad libitum*, water and a diet consisting of 75% ground red-winged blackbirds killed by ingesting 4-aminopryidine and 25% oatmeal. For one week prior to test initiation, all rats were fed a diet of 75% ground starlings (untreated) and 25% oatmeal to acclimate them to the new kind of feed; acceptance of this and test material was excellent. Feed was prepared daily, feed consumption was noted daily and rat weights were determined weekly.

Magpies: Acute Oral Study: Individually caged adult black-billed magpies and yellow-billed magpies of mixed sex were stomach-tubed with propylene glycol solutions of 4-aminopyridine. Total micro-liter dose administered was at twice the birds' weight in grams, and concentration varied to produce the desired dosage levels. Two black-billed magpies were treated with 1.8 µL and two were treated with 3.2 µL. One yellow-billed magpie was treated with 1.8 µL and another was treated with 3.2 µL.

Chronic Feeding Study: One individually caged black-billed magpie was fed two red-winged blackbirds killed by ingesting 4-aminopyridine daily for 20 days. Another magpie was fed only one redwing each day. A reference magpie was fed one hand-killed redwing for the same amount of time. All birds were provided with water and Purina-Eggers CF "Checkers" *ad libitum* throughout the test. The three magpies consumed all parts of the redwings with the exception of the bones, brain and feathers. On Day 21, the

magpies were sacrificed and gross necropsies were performed on them.

Sharp-Shinned Hawks: One individually caged female sharp-shinned hawk was provided, *ad libitum*, water and red-winged blackbirds killed by ingesting 4-aminopyridine baits daily for 21 days. No reference birds were available for the test. On Day 22, the bird was sacrificed and a gross necropsy was conducted.

Red-Tailed Hawks: For two weeks, one individually caged 450 g sub-adult red-tailed hawk was offered *ad libitum* water and red-winged blackbirds killed in the field by ingestion of a 4% 4-aminopyridine solution sprayed on husked ripening ears of corn. Amounts of 4-aminopyridine in each bird were not known, but must have been >2.4 mg/kg (the LD_{50} of 4-aminopyridine to redwings). Therefore, an average 70 g red-winged blackbird must have ingested >0.17 mg of 4-aminopyridine.

Sparrow Hawks: Acute Oral Toxicity Study: Individually caged female sparrow hawks were stomach tubed and treated with propylene glycol solutions of 4-aminopyridine. One bird each, was treated with solutions of 1.0, 1.8 and 3.2 mg/kg and two birds each were treated with solutions of 4.2, 5.6 and 7.5 mg/kg. Gross necropsies were performed on all birds.

Chronic Feeding Study: For periods of 7, 21 and 45 days, three individually caged sparrow hawks were fed *ad libitum* water and red-winged blackbirds killed by ingesting 4-aminopyridine treated baits. Reference hawks were fed hand-killed starlings or redwings and water *ad libitum*. One treatment bird and one reference bird were tested for each of the three different durations.

At the end of each test, gross necropsies were performed on all treatment and reference birds.

Results

Canines

Chronic Feeding Study:

Both canines consumed approximately the same amount of treated feed (2800 g) over the 8-day feeding period; therefore, both animals consumed approximately the same number of birds per day (5.84). As no adverse effects were observed, it can be assumed that this level of feeding is not hazardous, at least for a period of 1 week. Throughout the test neither dog exhibited symptoms of 4-aminopyridine poisoning or intoxication; therefore, the presence of super toxic avian metabolites of the test material can most likely be disregarded. Both dogs ate the food-redwing mixture readily, and actually preferring it to their normal diet. The acute oral LD_{50} of 4-aminopyridine to beagle dogs is 3.75 mg/kg,

Acute Feeding Study:

This test was conducted to determine the hazards to canines if misuse of the compound resulted in birds consuming many lethal doses before death. Since the canines

were able to consume the equivalent of 0.71 to 2.19 acute oral LD_{50} doses in 2-3 minutes without any deleterious effects, it can be assumed that the test material is either rapidly metabolized or chemically degraded into less toxic metabolites in the avian system.

Rats

Chronic Feeding Study:

The average number of LD_{50} doses consumed by the ten rats was 0.62, with a range of 0.57-0.68. Total number of LD_{50} doses consumed by each rat averaged 1.30, with a range of 1.20-1.43. At test termination (21 Days), all rats were sacrificed and the carcasses were retained for possible residue determinations. No treatment-related mortalities occurred and none of the 10 rats exhibited symptoms of 4-aminopyridine poisoning during the test, indicating that the test material is probably metabolized to super-toxic compounds in the bird, and that its harmful effects in rats, if any, are not cumulative. A total of 232 redwings were killed for this study, resulting in 15,328 grams of rat food containing 153.1 mg of 4-aminopyridine (10 ppm).

Acute Feeding Study:

Rats consumed the equivalent of 0.72 to 3.37 acute oral LD_{50} doses of 4-aminopyridine or its metabolites in a 3 hour period without any observable symptoms of poisoning. Thus, redwings apparently metabolize 4-aminopyridine rapidly to compounds less toxic to rats than the parent compound.

Magpies

Acute Oral Study:

Both of the black-billed magpies treated with 3.2 µL died; one died after 121 minutes and the other died after 116 minutes. Paralysis occurred in both birds after 36 minutes and one bird appeared to be distressed after 53 minutes. The yellow-billed magpie exposed to 3.2 µL was affected after 11 minutes, were paralyzed after 15 minutes, were distressed after 24 minutes and were dead 90 minutes after treatment.

The two black-billed magpies treated with 1.8 µL were affected after 23-30 minutes of treatment, but the effects did not increase in severity and both birds recovered within 1.5-3 hours. The one yellow-billed magpie treated with 1.8 µL was affected after 35 hours, but as with the black-billed magpies, the effects did not increase in severity and the bird full recovered in over 4 hours.

Gross necropsies of all birds did not reveal any significant pathological conditions in any of the birds.

Chronic Feeding Study:

The two magpies offered the 4-aminopyridine killed blackbirds consumed 0.66 mg (3.93 mg/kg) and 1.32 mg (7.54 mg/kg) of test material or its metabolites per day; representing as much as 3.19 LD_{50} doses of 4-aminopyridine per day without any gross pathological effects. Based on these results, it seems that chronic feeding for a 20-day period is not detrimental to an avian predator, the magpie.

Sharp-Shinned Hawk

The hawk consumed a total of 37 redwings, representing 1.68 redwings per day. At no point during the test did the hawk exhibited and symptoms of poisoning and the necropsy showed no gross pathological effects. Furthermore, the hawk gained 47 g of weight over the test period (2.24 g/day) indicating that the 4-aminopyridine killed redwings had no deleterious effect on the hawk.

Red-Tailed Hawk

In 14 days, the hawk consumed 39 redwings (2.8 birds/day) without any toxicological symptoms. This is a minimum of 0.48 of 4-aminopyridine or its metabolites consumed per day, or 1.06 mg/kg/day.

Sparrow Hawks

Acute Oral Toxicity Study:

The birds treated with 1.0, 1.8 and 3.2 mg/kg did not exhibit any noticeable signs of poisoning. The two birds treated with 4.2 mg/kg appeared affected after 20-42 minutes with light body tremors, but fully recovered after 2.24-4.5 hours. One of the females treated with 4.2 mg/kg had been treated with 1.8 mg/kg the previous day; this bird took longer to recover than the bird that had not been previously treated.

One of the birds treated with 5.6 mg/kg experienced increasingly severe tremors and convulsions starting 13 minutes after administration. After 25 minutes, the bird was paralyzed and died after 45 minutes. The other bird treated with 5.6 mg/kg appeared to be affected 18 minutes after exposure, regurgitated after 33 minutes and exhibited moderate body tremors. The bird fully recovered 2 hours and 37 minutes after administration.

Both birds treated with 7.5 mg/kg died within 59-67 minutes of administration. One bird, which had been previously treated with 1.0 mg/kg showed signs of poisoning after 15 minutes, became paralyzed after 30 minutes, appeared distressed after 41 minutes and died 59 minutes after administration of the test material. The other bird, which had been previously treated with 4.2 mg/kg showed signs of poisoning after 12 minutes, became paralyzed after 38 minutes, appeared distressed after 58 minutes and died 67 minutes after administration of the test material.

The reported acute oral LD_{50} of 4-aminopyridie to sparrow hawks is 2.6 (4.2-7.5) mg/kg.

Results from the gross necropsies showed that one bird treated 5.6 mg/kg had two 2mm necrotic foci on the liver.

Chronic Feeding Study:

The treated birds tested for 7, 21 and 45 days consumed an average of 0.98, 1.05 and 1.08 LD_{50} doses daily, respectively. No significant weight loss was noted and birds from all three tests consumed a similar amount of test material over the duration of the

test (5.50-6.05 mg/kg).

No observable symptoms of poisoning were noted and the gross necropsies did not reveal any pathological damage or abnormalities.

b. Terrestrial Plants

The phytotoxicity of Avitrol (4-aminopyridine) was determined on both corn seeds and corn seedlings (*Zea may*, Pioneer 3956 Hybrid; MRID 0004124). To determine the effects on germination, seeds were placed into distilled water for three hours prior to treatment. Following the three hours, seeds were transferred to Petri dishes containing blotter paper. Solutions of the test material were prepared in methanol and applied to the blotter paper at concentrations of 0 (negative control), 0.01, 0.1 and 1.0 ppm. The seeds were allowed to germinate for 2.5 days following treatment, after which % germination and radicle length were determined.

The phytotoxicity to corn seedlings was assessed in two different exposure periods. The first study exposed 3-4 week old seedlings to the test material in various concentrations. The tests were conducted in jars each containing 400 mL of nutrient solution. After 4 or 7 days of exposure, fresh weights of the tops of the plants were determined and used as a measure of phytotoxicity. The first study was conducted as three single-treatment experiments, with three separate concentration ranges. The first experiment consisted of exposing one seedling each to concentrations of 0 (negative control), 0.1, 1.0 and 10 ppm for 4 days; the second experiment consisted of exposing one seedling each to concentrations of 0 (negative control), 0.01 and 0.1 ppm for 4 days; the third experiment consisted of exposing three seedlings each to concentrations of 0 (negative control) and 10 ppm for 7 days.

The second study was conducted as a double-treatment experiment and was similar to the first, except that after two days of exposure, the nutrient solution in each jar was discarded and replaced with fresh solutions and plants were exposed for an additional 2 days. The study consisted of exposing three seedlings each to concentrations of 0 (negative control), 0.1, 1.0, 10 and 100 ppm for 4 days.

Translocation Studies:

Corn seeds, the same variety used in the germination and seedling studies, were germinated and grown in a sandy loam soil for two weeks and were then transferred to jars covered with aluminum foil to exclude light. The tops of the jars were fitted with foil covered lids with holes to allow aeration of the plants and contained nutrient solution. The seedlings remained in the jars for an additional two weeks prior to the addition of the test material. Each culture was changed every 2 days and the plants were grown in an environmental chamber under a 12L:12D photoperiod with an approximate light intensity if 2500 foot-candles. Day temperature was maintained at 27^{o}C while the night temperature was maintained at 21^{o}C. The relative humidity of the chamber was about 40%.

For the translocation tests, ^{14}C-4-aminopyridine, labeled in the alpha position, was used. Twelve seedlings were used and were separated into two exposure regimes. The first consisted of a control and two treatment replicates, each containing three seedlings, with a treatment concentration of 10 ppm; each jar contained 3.25 c of labeled pesticide in 200 mL of solution. The second regime was the same as the first, with the exception that only one plant was used in the control and two treatment replicates.

The amount of labeled test material remaining in the culture was monitored by sampling each solution 1, 3, 5 and 7 days following treatment. After sampling, nutrient solution was added to each culture to maintain the 200 mL volume. After 7 days of exposure, the plants were removed from the solutions. The culture solutions were transferred to new containers and the culture jar, along with the plant roots, were rinsed with water and acetone, and these rinse solutions were retained for ^{14}C determination.

The roots were blotted dry with tissue paper and the plants were sectioned into tops (leaves and stalks) and roots after rinsing with water and acetone. The fresh weights of both the tops and bottoms were recorded.

The tops and roots of 3 treated plants (each one from a separate culture) and 1 untreated plant were dried for five days and macerated in a homogenizer with the aid of a small quantity of finely crushed dry ice. These tissues were retained for combustion.

Following exposure of the test material, germination was not affected at any of the exposure levels. Germination was 90% in the negative control and 0.01, 0.1 and 1.0 ppm treatment levels. Mean root length was 20.1 mm in the negative control and 21.0, 15.2 and 19.3 mm in the 0.01, 0.1 and 1.0 ppm treatment levels, respectively, representing reductions of -4.8, 24.4 and 4.0%, respectively, relative to the negative control.

The fresh weight of plant tops for corn seedlings exposed to single applications of 0 (negative control), 0.1, 1.0 and 10 ppm (with one seedling per treatment level) were 1.6, 2.5, 2.2 and 1.9 g, respectively, after four days of exposure. The fresh weight of plant tops for corn seedlings exposed to single applications of 0 (negative control), 0.01 and 0.1 ppm (with one seedling per treatment level) were 5.0, 5.9 and 7.3 g, respectively, after four days of exposure. The fresh weight of plant tops for corn seedlings exposed to single applications of 0 (negative control), and 10 ppm (with three seedlings per treatment level) were 15.7 and 19.7 g, respectively, after seven days of exposure. The fresh weight of plant tops for corn seedlings exposed to two applications of 0 (negative control), 0.1, 1.0, 10 and 100 ppm (with three seedlings per treatment level) were 7.4, 6.9, 6.4, 6.6 and 6.5 g, respectively, after four days of exposure.

The treated plants that received a single application of the test material did not exhibit reductions in fresh weight relative to the negative control at any treatment level. Conversely, all plants experienced increases in weight gain ranging from 17.9 to 51.2% of the negative control fresh weights. Plants that received two applications of the test

material experienced fresh weight reductions of 5.0 to 12.9% relative to the negative control.

Translocation

During the translocation tests, 4.6% and 13.8% of the ^{14}C-activity was lost from the culture solutions after the first day in solutions containing one and three plants, respectively. Following seven days of exposure, 19.0% and 4.6% of the original ^{14}C-activity was present in the solutions from the single and triple-plant cultures, respectively. In both cultures, the rate of loss increased between the first and third day and leveled off on the fifth day. This increased rate of loss between Days 1 and 3 coincided with an increased rate of transpiration during this same period.

Analysis of the corn tissues revealed that 0.03 and 0.36% of the original ^{14}C-activity remained in the tops and roots, respectively. Only 27% of the original radioactivity was accountable.

The authors state that the presence of more ^{14}C-activity in the single-plant nutrient solutions than the triple-plant nutrient solutions indicates that the presence of more roots in the triple-plant cultures allowed for more absorption of the test material, with 20.9% of the initial ^{14}C-activity being sequestered in the roots and culture jars. Only 4.6% of the initial ^{14}C-activity was detected in the nutrient solution and approximately 0.1% was found in the plant tops.

The observations of the phytotoxicity and translocation studies suggest smaller single doses of Avitrol (4-aminopyridine) can be assimilated by the plants resulting in little to no adverse affects; furthermore, increasing the number of plants in a unit diminishes adverse effects. The low recovery of radioactivity in the culture solutions indicates that a large portion of it was lost as $^{14}CO_2$ from transpiration and nutrient culture metabolism (respiration). Radioactivity was evident in root, lower stem, and leaf tissue nearest the stalk and a general distribution pattern was noted throughout the plant, including the younger tissue.