Appendix A. Ecological Effects Data for Captan

Table A.1. Freshwater Fish Data - Captan Parent

Species	% A.I.	LC50, µg/L (confidence interval)	Measured/ Nominal Flow-through /static	Toxicity Classification	MRID (study year)	Class-ification
Brook Trout	88.4	34 (22 - 52)	Measured, Flow-through 8-day test	Very Highly toxic	00057846 (Hermanutz, 1973)	Supplemental
Fathead Minnow	88.4	65 (59 – 72)	Measured, Flow-through 6-day test	Very Highly toxic	00057846 (Hermanutz, 1973)	Supplemental
Bluegill sunfish	88.4	72 (47 – 111)	Measured, Flow-through 5-day	Very Highly toxic	00057846 (Hermanutz, 1973)	Supplemental
Coho Salmon	90	137 (117-160)	Static	Highly Toxic	(Johnson & Finley, 1980)* 40098001	Supplemental
Coho Salmon	90	56.5 (52.3-61)	Flow-through	Very Highly toxic	(Johnson & Finley, 1980)* 40098001	Supplemental
Chinook Salmon	90	120 (103-140)	Static	Highly Toxic	40098001	Supplemental
Cutthroat trout	90	56.4 (42.2-75.4)	Static	Very Highly toxic	(Johnson & Finley, 1980)* 40098001	Supplemental
Rainbow Trout	90	73.2 (66.6-80.4)	Static	Very Highly toxic	(Johnson & Finley, 1980)* 40098001	Supplemental
Brown Trout	90	80 (63.8– 100)	Static	Very Highly toxic	(Johnson & Finley, 1980)* 40098001	Supplemental
Brown Trout	90	26.2 (21.9-31.3)	Flow-through	Very Highly toxic	40098001	Supplemental
Lake Trout	90	49 (40.1-59.9)	Static	Very Highly toxic	(Johnson & Finley, 1980)* 40098001	Supplemental

Lake Trout	90	63.2 (49.6-80.5)	Static	Very Highly toxic	40098001	Supplemental
Lake Trout	90	51 (39.2-66.2)	Flow-through	Very Highly toxic	40098001	Supplemental
Fathead Minnow	90	200 (168-238)	Static	Highly Toxic	(Johnson & Finley, 1980)* 40098001	Supplemental
Fathead Minnow	90	134 (100-178)	Flow-through	Highly Toxic	40098001	Supplemental
Channel catfish		77.5 (70.5-85.2)	Static	Very Highly toxic	(Johnson & Finley, 1980)* 40098001	Supplemental
Bluegill sunfish	90	141 (119 – 167)	Static	Highly Toxic	(Johnson & Finley, 1980)* 40098001	Supplemental
Yellow Perch	90	120 (97.3-147)	Flow-through	Highly Toxic	(Johnson & Finley, 1980)* 40098001	Supplemental
Bluegill sunfish	90	310 (280 – 340) Slope = 1.17	Static	Highly Toxic	GS0120-042 (1979)	Supplemental
Harlequin Fish (Rasbora heteromorpha)	89	300	Static	Highly Toxic	00034713 Tooby et al. 1975	Supplemental (26 hr test, daily change of test water, no mortality data, test species)

* In Mayer and Ellersieck (MRID 40098001)

* Original source: Johnson, W. W., and M. T. Finley. 1980. Handbook of acute toxicity of chemicals to fish and aquatic invertebates. U.S.F.W.S., Resource. Pub. 137.98 pp.

Table A.2 Freshwater Fish Data - Captan Degradates						
Species	**% A.I.**	**96-hr LC_{50}, µg/L (confidence interval)**	**Measured/ nominal Flow-through /static**	**Toxicity Classification**	**MRID (study year)**	**Satisfies Guideline/ Comments**
Rainbow Trout	96% THPI	> 120,000	Measured, Static Renewal	Practically non-toxic	43869806	Acceptable
Rainbow Trout	96% THPAm	> 126,000	Measured, Static	Practically non-toxic	44738801	Supplemental (used 10 fish, but 30 are required for limit test

A.3. Chronic Toxicity to Freshwater Fish			
Species	**Toxicity**	**Source**	**Effects**
Fathead minnow	NOAEC = 16.5 µg/L LOAEC = 39.5 µg/L	MRID 00057846	Acceptable Reductions in adult and larval survival, growth and overall larval-juvenile development, survival of the juvenile species, a reduction in eggs laid, and an inability for juveniles to reproduce

Table A.4. Aquatic Invertebrate Captan Data						
Species	**% A.I.**	**Toxicity**	**Measured/ nominal Flow-through /static**	**Toxicity Classification**	**MRID (study year)**	**Classification**
Daphnia magna	90	48-hr LC_{50} = 8400 (7060-9960) µg/L Slope= 1.187	Static	Moderately Toxic	GS0120041	Acceptable
Daphnia magna	96% THPI	48-hr LC_{50} >113,000 µg/L	Static	Practically non-toxic	438698-08	Acceptable
Daphnia magna	technical	NOAEC = 560 µg/L LOAEC = 1000 µg/L	Static	--	441488-01	Supplemental (based on nominal concentrations)

Table A.5. Aquatic Plant Captan Data					
Species	**% A.I.**	**EC_{50}, µg/L (confidence interval)**	**Toxicity Classification**	**MRID (study year)**	**Classification**
Scenedesmus subspicatus Green algae (96-hr)	92.7	320	Highly toxic	00137688	Supplemental (based on nominal concentrations)
Selenastrum capricornutum Green Algae (96 hr)	90	1770 (1550-2030)	Moderately Toxic	438698-09	Acceptable
Anabaena flos-aquae Freshwater Algae (96 hours)	99.8	1200 (830-1600)	Moderately Toxic	448065-01	Acceptable
Lemna gibba Duckweed (7 days)	99.8	> 12,700	Slightly Toxic	448065-03	Acceptable
Selenastrum capricornutum Green Algae (72 hours)	96% THPI	> 180,000	Practically non-toxic	438698-10	Supplemental (short test duration)

Note: *Skeletonema costatum* (marine diatom), *Isochrysis galbana*, *Pavlova gyrans, Pavlova lutheria,* and *Dunaliella tertiolecta* (marine algae) are marine species and not applicable to RLF assessment (MRID 40228401).

U.S. EPA. 1986. Acute Toxicity Handbook of Chemicals to Estuarine Organisms., *U.S.EPA, Gulf Breeze,FL* (US EPA MRID 40228401).

Table A. 6. Captan Bird Data						
Species	**LD_{50} mg/kg bw**	**Acute Oral Toxicity (MRID)**	**LC_{50} (mg/kg diet)**	**Subacute Dietary Toxicity (MRID)**	**NOAEC mg/kg diet MRID**	**Affected Endpoints**
Northern bobwhite Quail *Colinus virginianus*	> 2150	00151236 Beavers, 1978	> 2400	GS0120 Fiche/Master ID 00022923 Hill, 1975	1000 (00098295 Fink, 1980)	No affected endpoints
Mallard Duck *Anas platyrhynchos*	> 2000	GS999-001 Hudson, 1984	>5000	GS0120 Fiche/Master ID 00022923 Hill, 1975	1000 (00098296 Fink, 1980)	No affected endpoints

Table A.7. Mammalian Captan Data					
Species	**Test Type**	**LC_{50} (mg/kg diet)**	**NOAEL/ LOAEL (mg/kg diet)**	**Citation (MRID)**	**Comments**
Rat	Acute Oral	> 5000	--	00265785 (1984)	Two males died. One death occurred on day 1 and one on day 12. One female died on day 4. The deaths were treatment related according to necropsy.
Rat	Acute Oral	Male: 5400 (4290-6800) Female: 5500 (4370-6930)	--	ACC# 241805	--
Rat	Acute Oral	9000	--	00054789 (1949)	--
Rat	One generation	--	> 500/ >500	00120315	
Rat	Three Generation	--	250 / 500	00125293 246101 241001	decreases in the mean litter weights of pups and severe sexual organ atrophy in adults and pups, signs of severe changes in liver weights in the adult males as well as abdominal and intestinal atrophy. In females, there were signs of stomach atrophy and esophageal atrophy

Table A. 8. Terrestrial Invertebrate Data				
Species	**Test Type**	**LD_{50} (µg/kg bee)**	**Citation (MRID)**	**Comments**
Apis mellifera Honeybee	Acute Contact	> 10	Fiche/Master ID 05001991 Stevenson, 1978	
Apis mellifera Honeybee	Acute Contact	> 215	Fiche/Master ID 00080871 Atkins, 1972	
Osmia lignaria Bee	72-hr Acute Oral	46.26 (32.75 – 77.44)	Ecotox # 87252 Ladurner et al, 2005	Captan 50WP 48.9% a.i.
	72-hr Acute Contact	269.68 (151.32 – 2841.84)		

Summary of Amphibian Larvae Study

Chemical Name: Captan
PC Code: 081301

ECOTOX Record Number and Citation: 90515. Mouchet, F., Gauthier, L., Mailhes, C., Ferrier, V, and Devaux, A. 2006. Comparative evaluation of genotoxicity of captan in amphibian larvae (*Xenopus laevis* and *Pleurodeles waltl*) using the comet assay and the micronucleus test. Environmental Toxicology 21(3): 264-277.

Purpose of Review: Litigation (California Red-Legged Frog)

Date of Review: October 2007

Brief Summary of Study Findings:
The toxic and genotoxic potentials of captan were evaluated with the micronucleus test (MNT) and the comet assay (CA).

Adult pairs of *Xenopus* and *Pleurodeles* were mated. Viable eggs were maintained until they reached a development stage appropriate for testing (3 weeks for *Xenopus* and 6 weeks for *Pleurodeles*). Experimental conditions generally followed the French Standard AFNOR (French National Organization for Quality Regulation) NF T90-325. Amphibians were exposed to either reconstituted water (RW) to which nutritive salts were added or mineral water (MW). Nominal captan concentrations were: 2000, 1000, 500, 250, 125, 65.5, 31.25, and 15.60 µg/L. Actual concentrations in water were not measured. Negative controls were either RW or MW. Positive controls were benzo[a]pyrene (B[a]P, [50-32-8], purity: 96.0%, Sigma France) at 0.125 mg/L for MNT and methyl methanesulfonate (MMs, [66-27-3], purity: 99%, Sigma France) at 1.56 mg/L for CA. Captan was dissolved in DMSO at a final concentration of 0.05% before addition to water. Media in all flasks was renewed daily.

Acute toxicity was examined for 12 days by visual inspection (death, abnormal behavior, reduced size, diminished food intake. No signs of toxicity or mortality were observed in any of the negative controls (personal communication with F. Mouchet, October 2007).

Captan flasks containing RW became turbid between 12-24 hours after renewal. The study author hypothesized that this turbidity was probably caused by amphibian residues/excretion or by the suspended captan or the degradation products that may interact with mineral ions, which make up a larger proportion of RW than MW.

Results of acute toxicity to *Xenopus* and *Pleurodeles* larvae exposed to captan (µg/L) in mineral water (MW) and reconstituted water (RW) for 12 days

Conc(µg/L)		2000	1000	500	250	125	62.5	31.25	15.60
Xenopus	MW	++(100%)	++(100%)	++(100%)	++(100%)	++(55%)	-	-	-
	RW	++(100%)	++(100%)	++(100%)	+	-	-	-	-
Pleurodeles	MW	++(100%)	++(100%)	++(75%)	++(45%)	-	-	-	-
	RW	++(100%)	++(100%)	++(50%)	+	-	-	-	-

%: percent dead (of 20 larvae); - No toxicity of larvae; + weak toxicity; ++ severe toxicity.

Genotoxicity was only assayed in MW at those concentrations where there was no acute toxicity. At 12 days for MNT and 1, 2, 4, 8, or 12 days for CA a blood sample was taken. Genotoxicity was assessed to the highest concentration that did not lead to signs of acute toxicity of the exposed larvae.

The results of the *Xenopus* MNT showed that a captan concentration of 62.50 µg/L induced a significant genotoxic response. The lowest concentrations (15.60 and 31.25 µg/L) were not genotoxic to *Xenopus* larvae. The results of the *Pleurodeles* MNT showed no genotoxicity regardless of the concentration of captan tested: 125, 62.50, 31.25, or 15.60 µg/L.

Results of the *Xenopus* CA showed that captan had genotoxic effects at all concentrations tested (15.60 µg/L after 8 and 12 days; 31.25 and 62.5 µg/L after 1, 2, 4, and 8 days; and 125 µg/L after 1, 2, and 4 days). The results of the Pleurodeles CA showed genotoxic effects at captan concentrations of 62.5 and 125 µg/L after 1 and 2 days of exposure, whatever the parameter, except with tail DNA after 2 days of exposure to 62.5 µg/L.

LC_{50} and slope (when possible) was estimated by the reviewer using TOXANAL software.

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RESULTS CALCULATED USING THE PROBIT METHOD
ITERATIONS    G              H            GOODNESS OF FIT PROBABILITY
 16            .1202498      1             .8025137
SLOPE    =     4.58749
95 PERCENT CONFIDENCE LIMITS  = 2.996684     AND    6.178296
LC50 =         311.0651
95 PERCENT CONFIDENCE LIMITS = 253.3895 AND  381.8606
```

		LD50, (µg/L) (confid int)	Method
Xenopus	MW	119.4 (62.5, 250)	Binomial
	RW	353.6 (250, 500)	Binomial
Pleurodeles	MW	311.1 (253.4, 381.9)	Probit
	RW	500 (250, 1000)	binomial

Description of Use in Document: Qualitative
Rationale for Use: This is the only known study evaluating the toxicity of captan to amphibians.

Limitations of Study:
1. Detailed raw data not available.
2. LC_{50} and slope (using probit model) not estimable for 3 survival curves as there was only one concentration with partial mortality.
3. Captan concentrations not measured.
4. Turbidity in RW flasks containing captan not definitively explained.

Reviewers: Christine Hartless, Wildlife Biologist (ERB1)

Summary of Wheat Study

Chemical Name: Captan
PC Code: 081301

ECOTOX Record Number and Citation: 91168. Mantecon, J. D. (1989). Persistence of Systemic and Non-Systemic Fungicides in the Control of Seedling Blight of Wheat (Fusarium graminearum). *Tests Agrochem.Cultiv.* 10: 76-77.

Purpose of Review: Litigation (California Red-Legged Frog)

Date of Review: September 2007

Brief Summary of Study Findings:
This study was conducted in a greenhouse at the Experiment Station INTA Balcarce, Buenos Aires Province, Argentina. Highly infected seeds of a durum wheat (Triticum durum Desf.) cv. Buck Patacon were sown in an artificially infested soil. The treatments were arranged in a randomized complete block design with three replications of 100 seeds each. Greenhouse temperatures averaged 20 ± 5 C. Fungicides were applied one day before sowing the seed. Four fungicides (including captan) were evaluated. Captan was applied at a rate of 120 g ai/kg-seed (0.26 lbs ai/cwt) using a wettable powder product from Stauffer Chemicals that was 83% ai.

The measured response variable was number of seedlings present after 7, 14, 21, and 28 days. Data were analyzed for each day by ANOVA and Tukey's test. Only the results from captan and the control are included in the table below.

	Time (days) after sowing			
	7	**14**	**21**	**28**
Captan	67	89	79	69
Control	39	43	28	26

At each time point, there was a statistically significant difference between the mean number of seedlings in the captan and the control groups.

Description of Use in Document: Qualitative

Rationale for Use: One of several seed treatment studies used in lieu of seedling emergence studies.

Limitations of Study:
1. Detailed raw data not available.
2. Only one treatment level evaluated (EC_{25} cannot be determined).
3. Exposure is by seed treatment, rather than by spray on top of soil surface.
4. Watering regime not available.

Reviewers: Christine Hartless, Wildlife Biologist (ERB1)

Summary of Sorghum Study # 91004

Chemical Name: Captan
PC Code: 081301

ECOTOX Record Number and Citation: 91004. Mc Laren, N. W. and Rijkenberg, F. H. J. (1989). Efficacy of Fungicide Seed Dressings in the Control of Pre- and Post-Emergence Damping-Off and Seedling Blight of Sorghum. *S.Afr.J.Plant Soil* 6 : 167-170.

Purpose of Review : Litigation (California Red-Legged Frog)

Date of Review: September 2007

Brief Summary of Study Findings:

This field study was conducted in Potchefstroom, Republic of South Africa in a field in which seedling diseases had been previously recorded. The seed cultivars DC34, DC99, NK283, and PNR8311 were used. A randomized split plot design with five replications was used. Cultivar was the whole plot factor and seed treatment was the sub-plot factor. Captan was applied as a seed dressing at a rate of 135 mg ai/kg-seed (0.30 lbs ai/cwt). Each subplot consisted of three rows, 11 m in length, spaced 1 m apart. After application of 2:3:2 fertilizer (300 kg/ha) seeds were planted to a depth of ± 5 cm and spaced 15 cm apart. A total of 17 fungicide treatments was used, only captan and control results are reported here.

To facilitate recovery of seed from the soil for determination of germination and pre-emergence damping-off, samples of 20 seeds were planted in cocoons, 30 cm in length, folded from single ply cheesecloth. Two cocoons with the relevant seed treatment and cultivar were randomly placed in each subplot row. Cocoons were recovered after 7 days and the percentage germination and per-emergence damping-off were assessed. Pre-emergence damping-off was measured as the percentage germinated seeds in which rotting was so severe that growth had ceased prior to emergence of seedlings from soil.

Twenty-one days after planting the percentage post-emergence damping-off (as a percentage of emerged seedlings) was determined in each sub-plot. Thereafter, 25 seedlings were removed from each sub-plot row and washed to remover adhering soil particles. Visual assessments of the percentage mesocotyl and primary root discoloration were made. Seedlings were also dried and weighed.

For each of the measured parameters, there were no statistically significant differences between the captan and the control group.

	Pre-emergence damping off (%)	Mesocotyl discoloration (%)	Root discoloration (%)	Post-emergence damping off (%)	Seedling mass (g)
Captan	19.4	58.2	19.8	10.5	3.8
Control	18.0	64.9	21.4	11.3	3.5
Least Significant Difference LSD (0.05)	8.2	16.4	7.5	2.3	0.5

Description of Use in Document: Qualitative

Rationale for Use: One of several seed treatment studies used in lieu of seedling emergence studies.

Limitations of Study:
1. Detailed raw data not available.
2. Only one treatment level evaluated (EC_{25} cannot be determined).
3. Exposure is by seed treatment, rather than by spray on top of soil surface.
4. Watering regime not available.

Reviewers: Christine Hartless, Wildlife Biologist (ERB1)

Summary of Sorghum Study # 90836

Chemical Name: Captan
PC Code: 081301

ECOTOX Record Number and Citation: 90836. Davis, M. A. and Bockus, W. W. (2001). Evidence for a *Pythium* sp. as a Chronic Yield Reducer in a Continuous Grain Sorghum Field. *Plant Dis.* 85: 780-784.

Purpose of Review (DP Barcode or Litigation): Litigation (California Red-Legged Frog)

Date of Review: September 2007

Brief Summary of Study Findings:

Field experiments
Two field experiments were conducted (planting dates of 11 May 1995 and 7 June 1995) in which there were three treatment groups (control, captan, and metalaxyl). A high vigor commercial hybrid seed (germination rate > 90%, Cargill 618Y) was planted. Prior to planting, seed was treated. A glass canning jar (1 liter) was "seasoned" by adding 2.5 ml water, the correct amount of chemical, and 100 g of seed. Jar was shaken until all liquid was absorbed by seed. This seed was discarded; procedure was repeated to produce treated seed for experiments. Treated seed was placed in paper bags to dry before sowing. Captan 400D at 3.0 fl oz/cwt (73g ai/kg-seed or 0.16 lbs ai/cwt) was used. Stand counts (plants/m^2) were taken on 12 and 23 June, vigor ratings (scale of 1 to 5) were taken on 3 July (boot and growing point differentiation growth stages), and grain yields (kg/acre) were measured on 17 and 20 October. There was a statistically significant increase or no difference in the captan treated seed responses relative to the control seeds in all measured parameters for both experiments.

		11 May 1995	7 June 1995
Stand			
	Control	3.8 c	6.0 b
	captan	7.3 a	7.9 a
	Metalaxyl	6.3 b	7.7 a
Vigor			
	Control	2.3 b	4.2 a
	captan	2.9 a	4.0 a
	Metalaxyl	3.1 a	3.6 a
Grain yield			
	Control	2592 b	5651 b
	captan	2754 b	6302 ab
	Metalaxyl	3947 a	6742 a

Values within a column and parameter followed by a common letter are not significantly different according to analysis of variance followed by least significant difference (P = 0.05).

Greenhouse experiment

Seed (Cargill 618Y) was treated or not treated with captan or metalaxyl at 0.16 lbs ai/cwt using the same method as described above. The experiment was arranged in a randomized complete block design using 10 plastic tubes 2.5 cm in diameter by 15 cm long. Each treatment had four replications. Soil was collected from the field experiment site above and left nontreatd or autoclaved at 121C for 2 hrs and placed I the tubes. One seed was sown per tube and plants were maintained in a greenhouse at 15-27 C Plant counts (out of 10 seeds planted) and shoot fresh weight per plant were recorded after 28 days. . There was a statistically significant increase or no difference in the captan treated seed responses relative to the control seeds in all measured parameters for either naturally infested soil or autoclaved soil.

		Experiment 1		**Experiment 2**	
Seed trt	**Soil trt**	**Stand**	**Fresh shoot wt**	**Stand**	**Fresh shoot wt**
Nontreated	Autoclaved	5.8 a	0.83 a	7.8 a	1.03 a
Captan	Autoclaved	7.5 a	0.85 a	8.0 a	1.18 a
Metalaxyl	Autoclaved	7.0 a	0.87 a	8.0 a	1.15 a
Nontreated	Nonautoclaved	3.0 b	0.57 b	5.5 b	0.52 b
Captan	Nonautoclaved	7.0 a	0.60 b	7.8 a	0.69 b
Metalaxyl	Nonautoclaved	7.8 a	0.90 a	8.0 a	1.07 a

Values within a column followed by a common letter are not significantly different according to analysis of variance followed by least significant difference (P = 0.05).

Description of Use in Document: Qualitative

Rationale for Use: One of several seed treatment studies used in lieu of seedling emergence studies.

Limitations of Study:
1. Detailed raw data not available.
2. Only one treatment level evaluated (EC_{25} cannot be determined).
3. Exposure is by seed treatment, rather than by spray on top of soil surface.

Reviewers: Christine Hartless, Wildlife Biologist (ERB1)

Summary of Lupine Study

Chemical Name: Captan
PC Code: 081301

ECOTOX Record Number and Citation: 91007. Fahim, M. M., Osman, A. R., Sahab, A. F., and El-Kader, M. M. A. (1983). Agricultural Practices and Fungicide Treatments for the Control of Fusarium Wilt of Lupine. *Egypt.J.Phytopathol.* 15: 35-46.

Purpose of Review (DP Barcode or Litigation): Litigation (California Red-Legged Frog)

Date of Review: September 2007

Brief Summary of Study Findings:

In vivo experiments were carried out in unsterilized 25-cm diameter clay pots containing clay sand mixture (1:1, w/w), referred to as loamy soil. The seeds were treated with the tested fungicides by shaking them in polyethylene bags until an even dressing was observed. Captan was applied at 0.50 lbs ai/cwt as the enduse product Orthocide (75% captan, recommended rate of 3 g Orthocide/kg-seed). Each treatment had five replicates. A total of eight fungicides and the control were evaluated in the experiment; only the captan results are summarized below.

Soil infestation was conducted by mixing cultures of *Fusarium oxysporum* with the soil at a rate of 5%, w/w. The inoculum was a 2-week-old growth of a virulent isolate, obtained from Alquam, Giza Governorate, on barley/sand (3:1, w/w) medium at 30 C. visual observations were made during the growth season. Macroscopic checks were also carried out at maturity. Seeds were air-dried for several days.

At the end of growing season, average weight of 100 seeds in the treated group was the same or greater than in the control. Percent occurrence of diseased plants was less in treated group than in control group.

		Diseased plants, %			**Avg wt of 100 seeds, g.**
		Pre-emergence	**Post-emergence**	**total**	
Captan	Infested	0	10	10	19.0
	Uninfested	0	7.5	8	20.2
Control	Infested	12.5	68.9	73	14.7
	Uninfested	7.5	19.1	25	16.7
Least Significant Difference (LSD) at P=0.05					
Main effect of fungicide		3.5	6.2	-	3.3
Main effect of infestation		1.6	2.9	-	1.5
Interaction (fungicide x infestation)		4.9	8.6	-	4.7

Description of Use in Document: Qualitative

Rationale for Use: One of several seed treatment studies used in lieu of seedling emergence studies.

Limitations of Study:
1. Detailed raw data not available.
2. Only one treatment level evaluated (EC_{25} cannot be determined).
3. Exposure is by seed treatment, rather than by spray on top of soil surface.
4. Rainfall/watering regime not available.

Reviewers: Christine Hartless, Wildlife Biologist (ERB1)

Summary of Blueberry Study

Chemical Name: Captan
PC Code: 081301

ECOTOX Record Number and Citation: 63909. Polavarapu, S. (2000). Evaluation of Phytotoxicity of Diazinon and Captan Formulations on Highbush Blueberries. *Horttechnology* 10: 308-314.

Purpose of Review (DP Barcode or Litigation): Litigation (California Red-Legged Frog)

Date of Review: September 2007

Brief Summary of Study Findings:

Experiments were conducted during the 1997 and 1998 growing seasons at Rutgers University Blueberry and Cranberry Research and Extension Center, Chatsworth, NJ, on highbush blueberries planted in 1994. Bushes were 4-5 yrs old, approx 5 ft tall, and spaced 9 x 4 ft apart on light sandy organic matter soil wth pH of 4.5. Two formulations of diazinon (Diazinon AG600 and Diazanon 50W) and of captan (Captec 4L and Captan 80WP) as well as an adjuvant, LI-700 were evaluated. Results pertaining to the adjuvant will not be reported here. All experiments described below had a negative control group. Application rates (author stated maximum labeled rates were used) are below:

formulation	Rate/acre	lbs ai/acre
Diazinon AG600	22.5 fl oz	NA
Diazanon 50W	2 lb	NA
Captec 4L	3.12 lb	2.43 lbs ai/acre
Captan 80WP	2.5 qt	2.5 lbs ai/acre

NA – not applicable, reviewer did not calculate as only captan is under review in this summary.

Treatments were arranged in a randomized complete block design. Treatments within a block were separated by at least 4 bushes and blocks were arranged 50-133 ft apart.

Pesticides were applied with a CO_2 pressurized backpack sprayer equipped with a hollowcone nozzle calibrated to deliver 30 gal/acre. At each evaluation, samples of foliage and fruit were collected in polyethylene bags and transported to lab for phytotoxicity evaluations. A fruit or foliage cluster was determined to have phytotoxicity even if only one fruit or leaf was injured.

Phytotoxicity injury occurred within 24 to 36 hrs after application of pesticides. Phytotoxicity on berries ranged from deep purple blotches to circular depressions, especially where residues accumulated. In the most severe cases, fruit had 2 to 3 mm diameter circular depressions filled with apparent pesticide residue. Phytotoxicity on leaves was typically brownish purple spots on the underside of the leaf surface. The

degree of phytotoxicity severity caused by the mixtures of captan and diazinon was much greater than the phytotoxicity when captan or diazinon was applied alone.

Data were analyzed using ANOVA and Duncan's multiple range test (P=0.05). Data were transformed before analysis using square root (number of clusters with phytotoxicity, number of berries, and berry weight) or arcsin (percent phytotoxicity) transformations.

Experiment 1

- Conducted in 1997, treated on 11 June 1997
- 5 single bush reps per treatment, variety Ellicot
- single treatment was Diazinon AG600 and Captec 4L
- 5 fruit and 5 foliage clusters collected from each side of each bush - 10 days after treatment
- the combined treatment had a significantly greater proportion of berries exhibiting phytotoxicity and lighter weight berries; although, the number of berries per 10 clusters was not different than the control.

	Berries with phytotoxicity (%)		Number of berries/10 clusters	Wt of 100 berries (g)
treatment	green	Blue		
Diazinon AG600 + Captec 4L	99.6±0.4 a	97.7±1.7 a	103±6.6 a	108.4±7.2 a
Untreated	0.0±0 b	1.5±1.0 b	99.2±5 a	145.5±11.2 b

For each response variable, treatment means followed by different letters are significantly different at P=0.05.

Listed response is mean ± standard error

Experiment 2

- Conducted in 1997, treated on 12 June 1997
- Three replications, each consisting of 6 bushes in a row, variety Bluecrop
- Treatments were combinations of the 4 listed pesticides.
- First evaluation 7 days after trt, 10 clusters from 3 randomly selected bushes within each replication
- Second evaluation 13 July with 25 fruit clusters per rep (during harvest)
- For all responses, the single pesticide applications were not significantly different from the control. Responses with no significant differences (means not listed in summary, are available in paper) were number of berries per 30 clusters 7 days after treatment, blue berries with phytotoxicity/25 clusters (%) at harvest, all berries with phytotoxicity/25 clusters (%) at harvest, and number of berries/25 clusters at harvest.

	Clusters with phytotoxicity (no/30 clusters) 7 d after treatment		Green berries with phytotoxicity/25 clusters (%) at harvest
	fruit	leaf	
Diazinon AG600	0.0±0 c	0.0±0 d	0.7±0.7 b
Captec 4L	0.0±0 c	0.3±0.3 d	1.9±0.5 ab
Diazinon AG600 + Captec 4L	9.3±1.9 a	22.7±1.2 a	5.2±1.4 a
Diazinon AG600 + Captan 80WP	7.7±1.3 a	16.0±2.0 b	1.1±0.5 b
Diazinon 50W + Captec 4L	3.0±0 b	3.0±1.0 c	0.4±0.4 b
control	0.0 ±0 c	0.0±0 d	0.6±0.6 b

For each response variable, treatment means followed by different letters are significantly different at P=0.05, Duncan's multiple range test.
Listed response is mean ± standard error

Experiment 3

- Conducted in 1997, treatment applied on 25 June 1997
- Three replications, each consisting of three bushes, variety Ellicott
- Treatments were combinations of the 4 listed pesticides.
- 20 fruit and leaf clusters per rep (10 each from two randomly selected bushes) sampled 8 d after treatment.
- In addition to responses reported below, percent phytoxicity/20 clusters was also analyzed, results were similar to the number of clusters (reported below). There were no significant differences in the number of berries per 20 clusters among treatments.
- Relative to control, captan alone or with diazinon resulted in no significant change or an increase in the observed phytotoxicity in fruit and leaves.

	Clusters with phytotoxicity (no/20 clusters) 8 d after treatment	
	fruit	leaf
Diazinon AG600	0.0±0 c	4.0±1.5 c
Diazinon 50W	0.0±0 c	0.3±0.3 d
Captec 4L	0.3±0.3 bc	14.0±1.5 b
Captan 80WP	0.7±0.3 bc	0.3±0.3 d
Diazinon AG600 + Captec 4L	4.0±2.5 a	20.0±0 a
Diazinon AG600 + Captan 80WP	1.0±1.0 bc	15.3±0.9 b
Diazinon 50W + Captec 4L	1.0±0.6 bc	4.7±1.7 c
control	0.0±0 c	0.0±0 e

For each response variable, treatment means followed by different letters are significantly different at P=0.05, Duncan's multiple range test.
Listed response is mean ± standard error

Experiment 4

- Conducted in 1998, treatments applied on 18 May. For some trts, diazinon applied first, followed by captan 8 hrs later.
- 4 reps, each consisted of 6 bushes in a single row, variety Weymouth

- 30 fruit and leaf clusters sampled from each rep 9 days after trt.
- In addition to responses reported below, percent phytoxicity/30 clusters was also analyzed, results were similar to the number of clusters (reported below).
- Relative to control, captan alone or with diazinon resulted in no significant change or an increase in the observed phytotoxicity in fruit and leaves. Applying captan 8 hrs after diazinon did demonstrate a significant reduction in phytotoxicity relative to applying both simultaneously.

	Clusters with phytotoxicity (no/30 clusters) 9 d after treatment	
	fruit	**leaf**
Diazinon AG600	0.0±0 e	0.0±0 c
Diazinon 50W	0.3±0.3 de	0.3±0.3 c
Captec 4L	1.8±0.5 c	8.0±1.5 b
Diazinon AG600 + Captec 4L	25.8±2.5 a	14.5±2.0 a
Diazinon 50W + Captec 4L	13.5±0.6 b	7.2±0.9 b
Diazinon AG600 first + Captec 4L 8 hrs later	2.8±1.1 c	7.0±1.1 b
Diazinon 50W first + Captec 4L 8 hrs later	1.5±0.6 cd	5.0±1.5 b
control	0.0±0 e	0.0±0.0 c

For each response variable, treatment means followed by different letters are significantly different at P=0.05, Duncan's multiple range test.
Listed response is mean ± standard error

Experiment 5

- Conducted in 1998, treatments applied on 26 May. For some trts, chemicals were applied with an 8 h interval between them.
- 4 reps, each consisted of 6 bushes in a single row, variety Bluecrop
- 30 fruit and leaf clusters sampled from each rep 8 days after trt.
- Relative to control, captan alone resulted in no significant change or an increase in the observed phytotoxicity in fruit and leaves. Using an 8 hr interval between pesticide applications (with either captan or diazinon first) resulted in a significant reduction in phytotoxicity relative to applying both simultaneously.

	Phytotoxicity /30 clusters (%) 8 d after treatment	
	fruit	**Leaf**
Captan 80WP	0.2±0.2 b	1.4±0.5 b
Diazinon AG600 + Captan 80WP	18.1±3.1 a	9.0±1.3 a
Captec 4L first + Diazinon AG600 8 hrs later	2.2±1.8 b	3.5±1.1 b
Captan 80WP first + Diazinon AG600 8 hrs later	1.5±1.0 b	1.8±0.8 b
Captan 80WP first + Diazinon 50W 8 hrs later	0.1±0.1 b	2.3±1.3 b
control	0.0±1 b	0.0±0 c

For each response variable, treatment means followed by different letters are significantly different at P=0.05, Duncan's multiple range test.
Listed response is mean ± standard error

Experiment 6

- Evaluated effect of repeated applications of captan and diazinon applied together
- Conducted in 1998, treatments applied on 22 May, 26 June, 29 July.
- Variety Ellicott was used

- Samples collected 5 to 8 days after treatment.
- Only one treatment (Diazinon AG600 + Captec 4L, applied at same time) that caused most severe phytotoxicity plus control were used.
- Statistical analysis indicated a time*treatment interaction – a greater percentage of fruit and leaves showed phytotoxicity after 22 May application (immediately following petal fall) than after the other two application dates.

Description of Use in Document: Qualitative

Rationale for Use: Foliar spray study used in lieu of vegetative vigor studies.

Limitations of Study:
1. Detailed raw data not available.
2. Only one treatment level evaluated (EC_{25} cannot be determined).
4. Watering regime not available.
5. Impact on growth of plants not measured.
6. Plants were established, not young seedlings.

Reviewers: Christine Hartless, Wildlife Biologist (ERB1)

Summary of Bee Study

Chemical Name: Captan
PC Code: 081301

ECOTOX Record Number and Citation: 87252. Ladurner, E., Bosch, J., Kemp, W. P., and Maini, S. (2005). Assessing Delayed and Acute Toxicity of Five Formulated Fungicides to *Osmia lignaria* Say and *Apis mellifera*. *Apidologie* 36: 449-460.

Purpose of Review (DP Barcode or Litigation): Litigation (California Red-Legged Frog)

Date of Review: October 2007

Brief Summary of Study Findings:

Contact and oral toxicity of five formulated pesticides were evaluated in this study. Only the results for captan (Captan 50WP, 49% ai) will be reported here.

In May 2002, wintering *O. lignaria* females, reared at the Bee Biology and Systematics Laboratory, Logan, Utah, were incubated at 25 C until emergence from cocoons. Unfed females were transferred to a screened flight cage to all them to deposit meconium. Females were then starved overnight and exposed to a specific fungicide treatment the next morning, approximately 24 h after emergence. In June 2002, *A. mellifera* foragers of different ages from a healthy, queen-right colony were captured in a clear plastic jar as they left the hive in the morning. All bees were chilled for a maximum of 30 minutes at 4 C prior to treatment.

In the contact toxicity tests, 1 µL of test solution was applied to the dorsal surface of the thorax with a 50 µL-micro syringe. Test solution was prepared by dissolving fungicide in acetone and purified distilled water (50% v/v) to obtain desired concentrations; fresh test solution was used for all tests.

In the oral toxicity tests known amounts of the fungicide were dissolved in a feeding solution (25% v/v sucrose in purified distilled water) to obtain desired concentrations. *O. lignaria* and *A. mellifera* were fed 10 µL of the test solution using the flower method devised by Ladurner et al (2003). The test solution was pipetted into a plastic ampoule and inserted into the calyx of a flower (cherry for *O. lignaria* and morning glory for *A. mellifera*). Flowers and bees were individually housed in holding cages (waxed cardboard cups, 8 cm diameter x 5 cm height) with a wire mesh screen lid. Flowers and bees in holding cages were kept in an incubator (22 C for *O. lignaria* and 25 C for *A. mellifera*) under artificial light (two 15W Cool White fluorescent tubes 15cm above holding cages) for one hour.

For the contact test, control bees were dosed with the mixture of acetone and purified distilled water (50% v/v). For the oral test, control bees were fed the feeding solution (25% v/v sucrose in purified distilled water).

TEST 1
Three sets of ten bees each were evaluated for delayed toxicity in the form of a single dose (122.5 µg ai/bee) for both oral and contact tests. After exposure, each set of 10 bees was transferred to a holding cage (same as described for oral test) with an artificial feeder. The feeder was a 5 mL-LDPE sample vial containing a sucrose solution (25% v/v sucrose in water) with a soaked cigarette filter inserted through the end of the vial. Fresh solution was provided every 24 hrs. Holding cages for *A. mellifera* were also provided with a piece of wax foundation comb. Holding cages were kept in an incubator (*O. lignaria* – temperature=22 C, relative humidity=60-80%, L:D=12:12hr; *A. mellifera* – temperature=25 C, relative humidity=60-80%, L:D-0:24 hr). Survival was recorded every 24 hrs for 7 days.

In oral exposure trials, 97.7% of *A. mellifera* and 88.2% of *A. mellifera* consumed all the test solution in one hour. Control survival was 100% in the *O. lignaria* studies and was 75-80% in the *A. mellifera* studies. Captan resulted minimal mortality for *A. mellifera* and higher mortalty rates for *O. lignaria.*

For *A. mellifera*, survival was not significantly reduced relative to control at the end of 7 days (Wilcoxon test) in either the oral or contact tests. For *O. lignaria*, survival was significantly reduced relative to control at the end of 7 days: in the contact test, survival was approximately 50%; and in the oral test, survival was approximately 35% on day 1 and approximately 0% by day 3.

TEST 2 – Methods of administration and bee maintenance were the same as described above. Only *O. lignaria* bees were used for captan, as there was minimal mortality for *A. mellifera* in the first test.

This test was designed to provide an estimate of an LD50. Five doses were administered; however, the test concentrations were not provided. Probit analysis was used for LD50 estimation.

	24 hr	48 hr	72 hr	7 days
Contact	NA	NA	269.68 (151.32, 2841.84)	95.26 (79.83, 134.59)
oral	NA	100.45 (63.75, 245.23)	46.26 (32.75, 77.44)	10.87 (5.40, 19.28)

Units are in µg ai/bee
NA – not available (LD50 was > than highest dose)
95% confidence interval in parentheses

Reference:
Ladurner, E., Bosch, J., Maini, S., Kemp, W.P. 2003. A method to feed individual bees (Hymenoptera: Apiformes) known amounts of pesticides. Apidologie 34: 597-602.

Description of Use in Document: Quantitative

Rationale for Use: This study provides a definitive toxicity endpoint for bees.

Limitations of Study:
1. Detailed raw data not available.
2. Dose concentrations not provided for second test.

Reviewers: Christine Hartless, Wildlife Biologist (ERB1)