# **Appendix J: Summary of Available Ecotoxicity Information for Iprodione TGAI and Formulated Products**

# **Open Literature Review Summary**

# Chemical Name: Vinclozolin and Iprodione

CAS No: 113201(V) and 109802 (I)

**ECOTOX Record Number and Citation:** 38526 Riviere, J.L., J. Bach. And G. Grolleau. 1983. Effect of Pyrethroid Insecticides and N-(3,5-dichlorophenyl) Dicarboximide Fungicides on Microsomal Drug-metabolizing Enzymes in the Japanese Quail (*Coturnix coturnix*). Bulletin of Environmental Contamination and Toxicology. 31, 479-485.

# Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-07-09

**Summary of Study Findings:** The study examines the effects of pyrethroids and dicarboximides on liver enzyme production in Japanese quail (*Coturnix coturnix*). The measurement of these enzymes is used as an indicator of metabolic activity and/or suppression by the tested chemicals which is in turn applied to the metabolic elimination rates of other toxicants.

The animals used for this study were female Japanese quail (approximately 6 wks of age). They were commercially purchased and maintain for at least one week in the lab before treatment began. They were kept in wire-floored cages that were thermostatically regulated to an undefined temperature and given a 20:4 L:D rate. The quail were fed a nutritionally balanced diet obtained from the CNRZ. They were given free access to food and tap water and were not starved before being sacrificed.

The vinclozolin used in the experiment was 97.8% pure; the iprodione was >95% pure. The animals received a diet of 2000 ppm for seven days. After 7 days of treatment, the quail were sacrificed and their enzyme levels were analyzed. The figure below shows the complete results of this analysis. Vinclozolin treated birds showed a significant difference in liver weight (p<0.05), hepatosomatic index (p<0.05), cytochrome p-450 activity (p<0.01), NADPH-cytochrome c reductase (p<0.01), aniline hyroxylase (p<0.05), aldrin epoxidase (p<0.01) and 7-ethoxyresorufin dealkylase (p<0.01) (**Table 2**). Iprodione treatment resulted in statistically significant differences in cytochrome-P450 (p<0.01), NADPH-cytochrome c reductase (p<0.01), aldrin epoxidase (p<0.05), 7-ethoxycourmarin dealkylase (p<0.01) and 7-ethoxyresorufin dealkylase (p<0.01) (**Table 2**).

In the vinclozolin-treated birds, liver weights were on average 22% greater and the HIS was on average 20% greater. Cytochrome P450 was roughly 3.6 times greater in vinclozolin-treated birds. Activity of 7-ethoxyresorufin dealkylase was 12 times greater than controls.

In the iprodione-treated birds, liver weights were not significantly different than controls; however, cytocrhome P450 activity was roughly 4 times greater than controls. Activity of 7-ethoxyresorufin dealkylase was 12 times greater than controls.

	Control	Iprodione	Vinclozolin	Procymidone
Body weight (g)	244 ± 20	237 ± 16	247 ± 16	235 ± 16
Liver weight (g)	6.8 ± 1.0	7.7 ± 1.1	8.3 ± 1.5 <sup>×</sup>	7.1 ± 0.8
Ratio liver weight/body weight	0.0279	0.0325	= 0.0336×	0.0302
Microsomal proteins (mg/g)	13.3 ± 1.0	13.9 ± 1.8	14.8 ± 2.7	12.4 ± 1.7
Cytochrome P-450 (nmol/mg)	0.20 ± 0.06	0.79 ± 0.21 <sup>%%</sup>	0.71 ± 0.20 ××	0.23 ± 0.06
NADPH-cytochrome c reductase (nmol/mg x min)	107 ± 19	158 ± 23 <sup>***</sup>	154 ± 30***	106 ± 18
Aniline hydroxylase (nmol/mg x min)	0.71 ± 0.17	1.41 ± 0.33 <sup>××</sup>	1.10 ± 0.28 <sup>%</sup>	0.68 ± 0.19
Aldrin epoxidase (nmol/mg x min)	0.14 ± 0.03	0.23 ± 0.07 <sup>×</sup>	0.46 ± 0.06 <sup>%%</sup>	0.17 ± 0.02
7-Ethoxycoumarin dealkylase (nmol/mg x min)	1.80 ± 0.44	2.90 ± 0.64 ××	1.81 ± 0.51	1.32 ± 0.41
7-Ethoxyresorufin dealkylase (nmol/mg x min)	0.10 ± 0.06	1.21 ± 0.31 <sup>××</sup>	$1.20 \pm 0.46$	0.46 ± 0.21 %
7-Ethoxycoumarin dealkylase + metyrapone, 100 μM (%)	54 ± 2	58 ± 3	66 ± 4	60 ± 4
7-Ethoxycoumarin dealkylase + 7,8-benzoflavone, 10 µM (%)	69 ± 2	72 ± 6	72 ± 13	68 ± 4

TABLE 2. Effect of N-(3,5-dichlorophenyl) dicarboximide fungicides on hepatic microsomal enzymes in female Japanese quail

a Mean ± SD (6 animals)

" Significantly different, P < 0.05 ; "" significantly different, P < 0.01

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study provides a link between the actual response to a toxicant and the end result of that response. It quantifies the effects of vinclozolin and iprodione dosing on enzyme production. Other than the effects on enzyme activity for both vinclozolin and iprodione and on liver weight for vinclozolin alone, the study does not mention any other adverse effects on the birds after a 7-day dietary exposure.

Limitations of Study: There is no information on the bird's backgrounds prior to the study.

The study does not report the number of birds treated; however, **Table 2** suggests that the data are based on 6 females. Methods does not state whether dietary concentrations were verified and does not indicate whether the diets were refreshed during the 7-day study. The study does not mention whether birds showed any signs of toxicity and/or whether food consumption was an issue. Finally, the enzymatic response is never actually linked to the response of the entire organism, it is only implied.

#### Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 68414 Pekár, S. 2002. Susceptibility of the spider *Theridion impressum* to 17 pesticides. Journal of Pest Science. 75, 51-55.

#### Purpose of Review (DP Barcode or Litigation): Litigation

#### Date of Review: 8-31-09

**Summary of Study Findings:** The study examines the acute effects of iprodione (Rovral FLO), among other pesticides, on the spider *Theridion impressum*.

Immature *T. impressum* were collected from vegetation around the Research Institute of Crop Production in Prague. They were then placed individually in Petri dishes one day prior to the start of the experiment. Each dish had a fine sand substrate and a mesh cover. They were kept at  $22 \pm 1^{\circ}$ C with 55% relative humidity and a photo period of 14:10 L:D. Each dish was sprayed with water every morning and evening. The spiders were not fed during the study. The chemical was diluted in water to achieve the desired a.i. concentration (not stated) according to the recommendations of Kuzma, 1997<sup>1</sup> and can be seen in **Table 1**. This dilution was applied using a Potter tower with a dosage of two ml per spider/dish. Following treatment with solution, dishes were placed in a well ventilated area so that they were dry within 10 minutes. The control group was only sprayed with water, and was not dosed with any sort of solution from a Potter tower; 30 individuals were used in each treatment group. Mortality was assessed each morning for four days. The study did not find any significant increase in mortality in the group exposed to iprodione over the control group.

Туре	Tradename	Common name	Dose [400 l]	Characteristics
I	Aztec 140 EW	Triazamate	0.5 1	contact action, systemic selective
I	Decis 50 WG	Deltamethrin	0.15 l	contact & stomach action non-systemic non-selective
I	Novodor FC	B. thuringiensis s.p. tenebrionis	41	stomach action, non-systemic, selective
I	Nurelle D	Cypermethrin+chlorpyrofos	0.6	contact & stomach action non-systemic non-selective
I	Pirimor 25 WG	Pirimicarb	0.4 1	contact & stomach action, non-systemic, non-selective
Ι	Vaztak 10 EC	a-cypermethrin	0.11	contact & stomach action, non-systemic, selective
Α	Actellic 50EC	Pirimiphos-methyl	1.5 1	contact action non-systemic non-systemic, non-selective
А	Cascade 5 EC	Flufenoxuron	0.6 1	contact & stomach action non-systemic non selective
А	Mavrik B	$\tau$ -fluvalinate+thiometon	0.21	contact & stomach action, systemic, non-selective
Α	Talstar 10 EC	Bifenthrin	0.1 1	contact & stomach action, non-systemic, non-selective
F	Delan 700WG	Dithianon	0.281	contact de stoffacti action, non-systemic, non-selective
F	Fundazol 50 WP	Benomyl	1 kg	
F	Rovral FLO	Iprodione	21	
F	Syllit 65	Dodine	0.41	
H	Butices SW 50 SC	Mar. 11		
H Ku	zma, S. 1997. Seznam	restrovanych prostredku na ocran	u rostlin (	A list of registered pesticides for plant protection).
н	Prague: Minist	ry of Agriculture of Czech Republ	ic. 185 pr	
	0		11	
A—aca	anciae, r – rungiciae, r	i – neroicide, i – insecticide		

Table 1. List of tested pesticides. Names and characteristics (shown only for insecticides and acaricides) are according to TOMLIN (1994). Recommended doses are after KUŽMA (1997).

# Description of Use in Document (QUAL, QUAN, INV): Invalid

**Rationale for Use:** The study does not report the percentage of active ingredient nor does it state the nominal treatment rate used in the study. It states that 2L of a 400L "dose" was used; however, it is unclear what these numbers are intended to represent. At "recommended concentrations" of formulated produce (Rovral) used in the study, iprodione is not acutely toxic to spiders.

**Limitations of Study:** The study uses wild-caught subjects. It does not specify the percent a.i. of the compound used, or the percent a.i. of the solution tested. As such, exposure cannot be determined from the information provided in this study.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 69254 Gray, Earl L (Jr), C. Wolf, C. Lambright, P. Mann, M. Price, R. L.Cooper, amd K.Ostby. 1999. Administration of potentially antiandrogenic pesticides (procymidone, linuron, iprodione, chlozolinate, p,p'-DDE and ketoconazole) and toxic substances (dibutyl- and diethylhexyl phthalate, PCB 169 and ethane dimethane sulphonate) during sexual differentiation produces diverse profiles of reproductive malformations in the male rat. Toxicology and Industrial Health 15, 94-118.

# Purpose of Review (DP Barcode or Litigation): Litigation

# Date of Review: 7-30-09

**Summary of Study Findings:** The study examines the reproductive effects of 10 chemicals with known and/or suspected anti-androgenic properties. Among other chemicals, the study examined iprodione, procymidone and chlozolinate, all of which are dicarboximide fungicides. The study found that chlozolinate and iprodione did not produce any signs of maternal or fetal endocrine toxicity at 100 mg/kg/day; more specifically, iprodione treatment had no demasculinizing or feminizing effects on male pups exposed to 100 mg/kg/day from gestational day 14 to post-natal day 3 (of lactation).

The iprodione used in the study was not in a formulated product and was >99% pure.

The animals used in the study were pregnant Long Evans (LE) hooded and Sprague-Dawley (SD) rats (*Rattus norvegicus*) purchased from the Charles River Breeding Laboratory in Raleigh, NC. They were placed in individual cages and fed on a diet of Purina Chow 5008 before lactation and Purina Rat Chow 5001 with tap water provided *ad libitum*. The photoperiod was 14:10 L/D and temperature ranged from 20-24°C and relative humidity was kept between 40 and 50%.

Although the dose method is not specifically stated (implied by gavage in corn oil) the dose amount was 100 mg/kg/day. This dose level was adjusted daily based on the rats' weights and was applied from gestational day 14 until post-natal day 3 (as stated above).

Males born from the group were allowed to grow until about 5 months of age, at which time they were sacrificed by decapitation "within 15s of removal from their home cage in a separate room during the dark phase of the animal's daily cycle." They were examined for abnormalities associated with hermaphrodism and/or de-masculination. The ventral surface of each male was shaved and examined for abnormalities (retained nipples, cleft phallus, vaginal pouch, hypospadias) and were examined internally for ectopic/atrophic testes; agenesis of the gubernaculums, epididymides, sex accessory glands, ventral prostate, bladder. Weights measured included body, pituitary, adrenal, kidney, liver, ventral prostate, seminal vesicle, testes and epididymis

According to the study, chlozolinate and iprodione did not demasculinize or feminize male pups after exposure to 100 mg/kg/day from gestational day 14 to post-natal day 3.

Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study provides qualitative information on the absence of transgenerational effects from iprodione at 100 mg/kg/day during pregnancy in male rats.

**Limitations of Study:** The data from the iprodione study are not reported in the study. The dosing method for the mothers in the study is not specifically stated. Although the study examined both reproductive and growth parameters in the offspring of treated rats, none of the body/organ weight data are discussed and it would have to be presumed that iprodione treatment had no effect. Also, the study does not provide any information on whether iprodione affected the treated female rats.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 71382 De Nardo, E. A. B. and P. S. Grewal. 2003. Compatibility of *Steinernema feltiae* (Nematoda: Steinernematidae) with Pesticides and Plant Growth Regulators Used in Glasshouse Plant Production. Biocontrol Sciences and Technology. 13.4, 441-448.

# Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-31-09

**Summary of Study Findings:** The study examines the effects of iprodione on the beneficial nematode *Steinernema feltiae*. It found that at the concentration tested there were no significant effects.

Infective juvenile *S. feltiae* (UK strain) were cultured from the last instar *Galleria mellonella* larvae. They were kept at 4°C in tap water at a density of 1500 IJ's/ml. They were used within six weeks of harvest. Nematode viability was assessed before use. Only groups with >95% viability were used. Iprodione was tested at the highest recommend label concentration (Chipco 26GT 23.3% a.i., 2.4g/L). Solutions were prepared to double this concentration, and then combined in a 1:1 ratio with the nematode containing stock (somehow containing 1500 individuals). Water was used as a control treatment. Each chemical was tested in four replicates. Following exposure, viability of each group of juveniles was assessed at 4, 24 and 72 hours. This was done by removing 50µl samples from each tube and observing each under a stereomicroscope. Nematodes that did not move after prodding were considered dead. At least 100 individuals were counted for each treatment and the control. Infectivity was assessed by introducing a last instar *G. mellonella* larvae to a Petri dish containing a treated nematode. Mortality of the larvae after incubation at 22°C was used to determine infectivity.

Statistics were corrected for control mortality using Abbott's correction. A one-way ANOVA was run. Mean separation was done using Tukey's procedures ( $\alpha$ =.05).

None of the fungicides tested caused a significant effect (p>0.05) on infectivity of the surviving S. feltiae compared to the untreated (water) control. Iprodione had no effect on either viability or infectivity on nematodes in juvenile *S. feltiae* treated with iprodione (**Table 2**).

Provide Theory			% change 1 control aft	n nematode sur er exposure to different duration	vival over the chemicals for ons	<sup>26</sup> change in nematode infectivity <sup>4</sup> over the control after exposure
Pesticide type	Irade Name	Technical Name	4h	24h	72h	to chemicals for 72 h
Biofungicides	Mycostop	Streptomyces griseociridis Strain K 61-	0.00	i 0.13	1.00	0.34
	Rootshield	Trichoderma harzianum	-0.25	+0.50	+0.25	-9.85
Chemical fungicides	Chipco 26GT Fungo Flo 4.5F Heritage Medallion Prostar 70 WP Subdue Maxx 21.3 ME Terraclor 400 F Terraguard 50W Terrazole WP	Iprodione Thiophanate methyl Azoxystrobin Fludioxonil Flutiokanil Mefenoxam PCNB Triflumizole Etridiazole	$\begin{array}{c} -0.13 \\ 0.00 \\ 0.00 \\ -0.40 \\ +0.62^{\star} \\ -0.25 \\ -0.13 \\ +0.12 \\ +0.72^{\star} \end{array}$	$\begin{array}{c} -0.13 \\ -0.63 \\ 0.50 \\ +0.25 \\ +1.12^{\bullet} \\ -0.38 \\ -0.50 \\ -0.25 \\ +10.62^{\bullet} \end{array}$	0.00 -2.63 -2.75 +3.75 * -3.00 * -1.62 -2.63 +15.00*	$\begin{array}{r} -2.61 \\ -3.20 \\ +0.33 \\ +0.48 \\ +2.9 \\ +2.1 \\ +1.47 \\ +3.58 \\ -14.15^{\bullet} \end{array}$
Bioinsecticides	Conserve SC Gnatrol	Spinosad Bacillus thuringiensis subsp. israelensis	+0.50 +2.13*	+2.00 +9.12*	+7.88* +17.5*	0.64 +9.61
Chemical insecticides	Adept IGR Precision 25WP Orthene 97 PE	Diflubenzuron Fenoxycarb Acephate	+0.53 +1.38 +0.25	+2.00* +0.63 +3.37*	+7.62* +2.00 +9.62*	-2.58 -6.29* +4.14
Herbicide	Envoy	Clethodim	+0.25	-1.62	+1.88	+0.33
Plant growth regulators	A-Rest Bonzi Sumagic	Ancymidol Paclobutrazol Uniconazole P	-0.25 -0.37 +0.38	+1.38* +0.25	2.62* 0.37 0.37	-6.03 +2.09
Water (Control)		Children (	0.58	1.44	2.96	53.70

TABLE 2. Percent change in survival and infectivity of Steinernema feltiae after incubation in different treatments for different periods.

<sup>1</sup> Per cent mortality of last instar *Galleria mellonella* after 5 days by the nematodes exposed to pesticides for 72 hrs. • Values significantly different ( $P \le 0.05$ ) from the control (Tukey's multiple range test)

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative

Rationale for Use: The study provides qualitative information that at the treatment concentration used, iprodione did not have a statistically significant effect on nematode survival and/or infectivity under the conditions tested.

Limitations of Study: This is essentially an efficacy study that provides some information on the effects of iprodione on terrestrial invertebrates.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 71382 De Nardo, E. A. B. and P. S. Grewal. 2003. Compatibility of *Steinernema feltiae* (Nematoda: Steinernematidae) with Pesticides and Plant Growth Regulators Used in Glasshouse Plant Production. Biocontrol Sciences and Technology. 13.4, 441-448.

# Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-31-09

**Summary of Study Findings:** The study examines the effects of iprodione on the beneficial nematode *Steinernema feltiae*. It found that at the concentration tested there were no significant effects.

Infective juvenile *S. feltiae* (UK strain) were cultured from the last instar *Galleria mellonella* larvae. They were kept at 4°C in tap water at a density of 1500 IJ's/ml. They were used within six weeks of harvest. Nematode viability was assessed before use. Only groups with >95% viability were used. Iprodione was tested at the highest recommend label concentration (Chipco 26GT 23.3% a.i., 2.4g/L). Solutions were prepared to double this concentration, and then combined in a 1:1 ratio with the nematode containing stock (somehow containing 1500 individuals). Water was used as a control treatment. Each chemical was tested in four replicates. Following exposure, viability of each group of juveniles was assessed at 4, 24 and 72 hours. This was done by removing 50µl samples from each tube and observing each under a stereomicroscope. Nematodes that did not move after prodding were considered dead. At least 100 individuals were counted for each treatment and the control. Infectivity was assessed by introducing a last instar *G. mellonella* larvae to a Petri dish containing a treated nematode. Mortality of the larvae after incubation at 22°C was used to determine infectivity.

Statistics were corrected for control mortality using Abbott's correction. A one-way ANOVA was run. Mean separation was done using Tukey's procedures ( $\alpha$ =.05).

None of the fungicides tested caused a significant effect (p>0.05) on infectivity of the surviving S. feltiae compared to the untreated (water) control. Iprodione had no effect on either viability or infectivity on nematodes in juvenile *S. feltiae* treated with iprodione (**Table 2**).

Provide Theory			% change 1 control aft	n nematode sur er exposure to different duration	vival over the chemicals for ons	<sup>26</sup> change in nematode infectivity <sup>4</sup> over the control after exposure
Pesticide type	Irade Name	Technical Name	4h	24h	72h	to chemicals for 72 h
Biofungicides	Mycostop	Streptomyces griseociridis Strain K 61-	0.00	i 0.13	1.00	0.34
	Rootshield	Trichoderma harzianum	-0.25	+0.50	+0.25	-9.85
Chemical fungicides	Chipco 26GT Fungo Flo 4.5F Heritage Medallion Prostar 70 WP Subdue Maxx 21.3 ME Terraclor 400 F Terraguard 50W Terrazole WP	Iprodione Thiophanate methyl Azoxystrobin Fludioxonil Flutiokanil Mefenoxam PCNB Triflumizole Etridiazole	$\begin{array}{c} -0.13 \\ 0.00 \\ 0.00 \\ -0.40 \\ +0.62^{\star} \\ -0.25 \\ -0.13 \\ +0.12 \\ +0.72^{\star} \end{array}$	$\begin{array}{c} -0.13 \\ -0.63 \\ 0.50 \\ +0.25 \\ +1.12^{\bullet} \\ -0.38 \\ -0.50 \\ -0.25 \\ +10.62^{\bullet} \end{array}$	0.00 -2.63 -2.75 +3.75 * -3.00 * -1.62 -2.63 +15.00*	$\begin{array}{r} -2.61 \\ -3.20 \\ +0.33 \\ +0.48 \\ +2.9 \\ +2.1 \\ +1.47 \\ +3.58 \\ -14.15^{\bullet} \end{array}$
Bioinsecticides	Conserve SC Gnatrol	Spinosad Bacillus thuringiensis subsp. israelensis	+0.50 +2.13*	+2.00 +9.12*	+7.88* +17.5*	0.64 +9.61
Chemical insecticides	Adept IGR Precision 25WP Orthene 97 PE	Diflubenzuron Fenoxycarb Acephate	+0.53 +1.38 +0.25	+2.00* +0.63 +3.37*	+7.62* +2.00 +9.62*	-2.58 -6.29* +4.14
Herbicide	Envoy	Clethodim	+0.25	-1.62	+1.88	+0.33
Plant growth regulators	A-Rest Bonzi Sumagic	Ancymidol Paclobutrazol Uniconazole-P	-0.25 -0.37 +0.38	+1.38* +0.25	2.62* 0.37 0.37	-6.03 +2.09
Water (Control)		Children (	0.58	1.44	2.96	53.70

TABLE 2. Percent change in survival and infectivity of Steinernema feltiae after incubation in different treatments for different periods.

<sup>1</sup> Per cent mortality of last instar *Galleria mellonella* after 5 days by the nematodes exposed to pesticides for 72 hrs. • Values significantly different ( $P \le 0.05$ ) from the control (Tukey's multiple range test)

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative

Rationale for Use: The study provides qualitative information that at the treatment concentration used, iprodione did not have a statistically significant effect on nematode survival and/or infectivity under the conditions tested.

Limitations of Study: This is essentially an efficacy study that provides some information on the effects of iprodione on terrestrial invertebrates.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 74745 Morale, S.G., and B. P. Kurundkar. 1989. Effect of some pesticides on root-knot of brinjal [eggplant] caused by *Meloidogyne incognita*. Indian Journal of Plant Pathology. 7.2, 164-166.

## Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 9-1-09

**Summary of Study Findings:** The study examines the effects of iprodione on root-not nematode (*Meloidogyne incognita*) infestation in eggplants (*Solanum melongena*). The study finds that iprodione treatment did not reduce the number of galls per root system, but did result in increases in other growth characteristics.

Eggplant seedlings were raised in sterile soil for four weeks. After this time, they were transferred to 22.5 cm (dimension not given) pots containing a 1:1 sterilized soil/compost mixture. Plants were allowed to establish themselves for four days before they were inoculated with 2000 freshly hatched *M. incognita* juveniles. Iprodione (Rovral 50 WP) was applied to the plants at a "0.1%" concentration. Forty-five days after this inoculation the roots of the plants were observed and scored visually for root knots. A 0-10 scale was used with 1 being equal to 1-10% galling of the root system and 10 being equal to 91-100% galling of the root system. Fresh and dry plant weights were also recorded. Drying was done in an oven. Other nematode biometrics, including the number of mature females, egg masses and eggs were recorded based on a one gram (fresh) root sample from each system.

Results indicated that root disease was not reduced by treatment with iprodione (**Table 1**). Iprodione was also not found to reduce the number of egg masses per plant. According to the report's text, iprodione was found to increase the leaf area per plant, dry root weight and dry shoot weight (**Table 1**); however, **Table 1** did not provide any indication that these measurement parameters were significantly different than controls.

	Mean per plant at 45 days of perticidal application										
Pesticides	Percentage of	Root-	Galls	Height	Leaves	Leaf	Root dry	Shoot dry	Mature	Egg	Eggs
	total root system galled	knot index	(No)	(cm)	(No)	(cms)	(g)	(g)	(No)	(No)	(No)
(Lannate 20 EC)	20.50	3	205.00	17.33	15.56	572.00	0.88	4.03	165.00	165.00	22275.00
Benfurocarb (Oncol 40 EC)	7.33 (15.56)	1	166.66	23.00	17.00	621.33	0.93	3.87	70.66	114.86	15480.00
(Plantvax 20 EC)	7.00 (15.31)	١	158.33	16.33	12.66	288.33	0.15	1.26	17.66	11.33	12760.00
Coxadyxil 8%	15.33 (23.00)	2	203.33	10.33	9.00	144.00	0.38	1.77	163.33	153.33	25300,90
Phorate O (Thimet 10g)	1.33 (6.53)	1	50.00	16.00	12.00	666.00	0.89	3.37	29.33	41.33	5580.00
(Puradan 50 SP)	9.33 (17.76)	1	130.33	15.66	9.33	541.64	1.01	2.96	117.33	118 00	15930.00
(Bavistin 50 WP)	25.00 (29.99)	3	490.00	20.00	10.00	420.00	0.60	3.06	330.00	330,00	54450 00
Ipridione (Rovrol 50 WP)	30.00 (33.20)	3	935.33	18.00	11.33	\$24.00	0.83	3.21	839.33	839.33	138490.00
(Topsin M70, 70 WP)	28.66 ) (32.36)	3	1041.66	16.66	11.00	500.33	0.87	3.03	1205.00	1073.00	177155.00
Dithionon (Delan 75 WP)	15.00 (22.78)	2	368.00	15.00	13.66	434.33	0.65	2.86	384.00	384.00	633360.00
Control	80.00 (63.42)	8	727.33	14.66	10.33	282.00	0.55	2,28	992.00	880.00	246400 00
S.E.	0.98		16.03	2.35	1.58	50.04	0.05	0.30	14.75	15.80	2888.07
C.D. at 5%	2,29		47.38	N.S.	4.66	147,60	0.16	0.90	43.52	46.63	8519.90

Table 1. Effect of pesticides on root-knot. vegetative growth of bringing and nematode multiplication.

Figures in parentheses are arc sine values.

## Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study provides qualitative information that at the concentration tested, iprodione significantly affected growth (leaf area per plant, shoot and root weight) of eggplant seedlings.

**Limitations of Study:** Dosing is unclear (specified only as 0.2%) and the percent a.i. of the formulation used is not stated. Also, the number of trials and/or replicates is not stated which leads to consistency questions. The method used to determine root characteristics also seems to be subjective.

## Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 74876 Gange, A.C., V. K. Brown, and L. M. Farmer. 1992. Effects of pesticides on the germination of weed seeds: implications for manipulative experiments. Journal of Applied Ecology. 29(2), 303-310

# Purpose of Review (DP Barcode or Litigation): Litigation

## Date of Review: 9-2-09

**Summary of Study Findings:** The study examines the effects of iprodione on seed germination in 20 weed species of various groups (forb, annual grass, perennial grass etc.). It found that iprodione reduced the germination rate of one perennial forb species, *i.e*, . English plantain, *Plantago lanceolata* 

Seeds for the study were collected from pesticide free plants at Silwood Park, Ascto, Berkshire, United Kingdom. The following species were used for testing: *Conyza Canadensis, Polygonum persicaria, Sonchus oleraceus, Sperfula arvenisis, Stellaria media, Tripleurospermum inodorum, Veronica persica, Vicia sativa, Cirsium arvense, Glechoma hederacea, Laminum album, Plantago lanceolata, Plantago major, Stachys palustris, Trifolium repens, Medicago lupulina, Poa annua, Agrostis stolonifera*, and *Holcus lanatus*. All seeds were chilled at 4°C for four months to simulate the winter season and provide the chilling required by some species. One hundred seeds of each species were used for each treatment. They were placed in groups of 10 on a germination disc in a Petri dish. The iprodione used for this experiment was formulated in Rovral (10% w/w GR). It was mixed with distilled water to achieve the concentration used in field experiments by Brown and Grange, 1989<sup>1</sup> (not specified). The amount a.i. applied to each growth disc ended up being 6.4 mg. Control groups were treated with only distilled water. Groups were dosed, sealed and kept at 20°C. They were observed every three days for four weeks. The number of germinated seeds was recorded at each observation.

The number of germinated seeds was considered using a three-factor ANOVA. Means were compared using the Tukey test.

The only plant species whose germination was found to be significantly (P<0.01) inhibited by iprodione was the perennial forb, the English plantain *P. lanceolata*. It showed an almost total inhibition of germination when exposed to iprodione (**Figure 2**).



Fig. 2. Effects of three posticides on seed germination. (a) The annual grass, Poa annual posts:  $F_{dimensione} = 21.6$ , P < 0.001;  $F_{absorption} = 5.5$ , P < 0.05;  $F_{produce} = 0.72$ , P > 0.05; (b) The perennial forb, Planago Intervalue,  $x \sim xx$ ;  $F_{dimensione} = 0.8$ , P > 0.05;  $F_{chargetiles} = 9.4$ , P < 0.01;  $F_{produce} = 44.9$ , P < 0.001. (c) The perennial grass, Agr < 0.05; Notations = 7.3, P < 0.05;  $F_{chargetiles} = 0.2$ , P > 0.05;  $F_{produce} = 1.4$ , P < 0.001;  $F_{produce} = 0.1$ , P > 0.05. Conventions as in Fig. 1.

# Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study provides qualitative information that although iprodione did not affect seed germination in many of the species on which it was tested, it did significantly affect seed germination of a perennial forb at a treatment rate that was considered representative of a field application rate.

**Limitations of Study:** Much of what the study examines are chemical mixtures. It does not explore the effects of a concentration gradient on germination.

Primary Reviewer: TJ Graven, Biologist

<sup>1</sup>Brown, V.K. Gange, A.C. 1989. Differential effects of above and below groundinsect herbivory during early plant succession. Oikos. 54, 67-76

## Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 77594 Dernoeden, P.H., L. R. Krusberg, and S. Sardanelli. 1990. Fungicide Effects on *Acremonium* Endophyte, Plant-Parasitic Nematodes, and Thatch in Kentucky Bluegrass and Perennial Ryegrass. Plant Disease. 74(11), 879-881

## Purpose of Review (DP Barcode or Litigation): Litigation

## Date of Review: 9-2-09

**Summary of Study Findings:** The study examines the effects of the fungicide iprodione on nematode infection in several grass species. It found that iprodione did not affect nematode infections.

Four Kentucky bluegrass cultivars were seeded at a rate of 98 kg seed/ha and two perennial ryegrass cultivars were seeded at a rate 293 kg seed/ha. Just prior to seeding fertilizer 10-3-3 was applied to supply 75 kg nitrogen/ha. Study area was maintained at 6 - 8 cm and clippings were not removed. The site was treated once a year in fall with a mix of 2,4-D +MCPP +dicamba to control broadleaf weeds. Fungicides were applied to the same plots midmonth form April to September duirng 1983 - 1987 for a total of 6 applications per year for 5 years. Iprodione was applied at a rate of 3.1 kg a.i./ha; according to the study, "generally" no rain or irrigation followed within 24 hrs of application. Cultivar plots were 3 x 6 m an fungicide subplots were 1 x 3 m. Three thatch measurements per plat were averaged for each replicate for the statistical analysis. Three 2-cm wide x 6 - 8 cm deep plugs were taken randomly from each plot for pH measurements. Soil was sampled for plant-parasitic nematodes in September 1986 and 1987 using twelve 2-cm wide and 12-cm deep plugs from each plot.

According to the study, none of the fungicides evaluated in the study showed significant nematicidal activity.

## **Description of Use in Document (QUAL, QUAN, INV):**

**Rationale for Use:** Under the conditions tested, iprodione did not affect nematode levels in either Kentucky bluegrass or ryegrass cultivars over a two-year period.

**Limitations of Study:** The study site was treated yearly with 2,4-D, MCPP and dicamba to control broadleaf weeds. It was treated twice with oxadiazon to control grass weeds. The application of these chemicals resulted in mixtures that render the study of limited utility for assessment purposes; however, controls were presumably treated with the same chemicals. Potential chemical interactions though could not be ruled out. Even with such potential interactions though, none of the fungicides appear to have had an effect on soil nematode levels.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 87252 Ladurner, E., J. Bosch, W. P. Kemp, and S. Maini.. 2005. Assessing delayed and acute toxicity of five formulated fungicides to *Osmia lignaria* Say and *Apis mellifera*. Apidologie. 36

# Purpose of Review (DP Barcode or Litigation): Litigation

# Date of Review: 7-28-09

**Summary of Study Findings:** The study focuses on the effects of certain fungicides, *i.e.*, neem oil (Trilogy<sup>®</sup>), iprodione (Rovral<sup>®</sup>), propiconazole (Orbit<sup>TM</sup>), benomyl (Benlate<sup>®</sup>), and captan (Captan<sup>®</sup> 50WP) on two bee species (*Osmia lingaria* and *Apis mellifera*). The fungicides were all used in their commercial formulated state.

*O. lignaria* females used in the study were obtained from the Bee Biology and Systematic Laboratory in Logan Utah. They were incubated at  $25^{\circ}$  C until they emerged from their cocoons. Following emergence they were transferred unfed to a 40x30x30 cm flight cage. They were then kept overnight without food and exposed to a fungicide treatment the next morning (approximately 24 hours after emergence).

*Apis mellifera* foragers of varied age were captured from a queen healthy colony as they left the hive in the morning. They were then transported to the laboratory. The condition of their confinement once at the lab is not described

Toxicity was tested using two dosing methods, contact and oral.

For contact tests, bees were chilled at  $4^{\circ}$ C until they stopped moving, but not more than 30 minutes. Test solution (1µL) was then applied to the thorax of the bee using a micro-syringe. The test solution was prepared by dissolving the given amount of fungicide in acetone and distilled water (50% volume/volume). This solution, without the addition of any fungicide, was used as the control for the contact toxicity test. The amount of fungicide dissolved in each treatment's solution is displayed in the **Table 1**.

**Table I.** Fungicide doses administered to *A. mellifera* and *O. lignaria* in contact and oral toxicity tests, and highest recommended field rates of the five fungicides (see Tab. II). The solutions used were at the highest concentration that could be dissolved in the solvent solutions.

	Contact administration	Oral administration	Highest recommended field rate
Fungicide	µg a.i./bee	μg a.i./bee	µg a.i./ha
Neem oil	196.4	196.4	12230
Iprodione	125.0	125.0	1120
Propiconazole	104.0	65.0	125
Benomyl	125.0	125.0	1121
Captan	122.5	122.5	2192

For oral toxicity testing, a quantity of fungicide necessary to achieve a desired concentration (listed above) was dissolved in a solution of sucrose in distilled water (25% v/v). This solution,

without the addition of any fungicide, was used as the control for the oral toxicity test. Individuals were then fed 10  $\mu$ l of a given solution using the method devised by Ladurner *et al*<sup>1</sup>.

After dosing (contact or oral) groups of 10 bees were moved to a holding cage (waxed cardboard ice cream cups) containing an artificial feeder, i.e., 5ml low density polyethylene (LDPE) vial containing a sucrose solution (25% v/v) and a soaked cigarette filter inserted trough the lid of the vial. Fresh solution was provided every 24 hr. The cages used for *A. mellifera* were supplied with a piece of wax foundation comb. The holding cages were kept in an incubator with the following conditions: *O. lignaria*= 22°C, 60-80% relative humidity, 12 hours light, 12 hours dark. *A. mellifera*= 25°C, 60-80% relative humidity, no light. Dimethoate (30.5%) was used as a positive control. To determine whether the fungicides had delayed toxicity effects at high doses, mortality was recorded every 24 hr for seven days. **Table II** displays the results of a comparison of the control and experimental mortalities.

Table II. Comparison of survival in treated and control O. lignaria and A. mellifera after contact and oral administration of single high doses (see Tab. I) of the five fungicides (Wilcoxon Test: df = 1).

		O. lign	aria	A. mellifera				
	Contact adm	ninistration*	Oral admi	inistration	Contact adr	ninistration	Oral admi	nistration
Product	χ <sup>2</sup>	Р	χ <sup>2</sup>	P	χ <sup>2</sup>	Р	χ <sup>2</sup>	Р
Neem oil	1	/	2.0339	0.1538	10.6990	0.0011	0.1513	0.6973
Iprodione	/	/	1.0000	0.3173	1.6973	0.1926	1.4011	0.2365
Propiconazole	1.0000	0.3173	53.4435	< 0.0001	1.2791	0.2581	29.8306	<0.0001
Benomyl	1	. /	1.0000	0.3173	0.3240	0.5692	3.3600	0.0668
Captan	24.9622	(j)<0.0001	52.8843	<0.0001	0.1145 م	0.7351	لم 2.3500	So.1253

\* Survival of O. lignaria after contact administration of neem oil, iprodione, benomyl and in the control with the dosing vehicle was 100%.

Survival was analyzed using the LIFE TEST Procedure<sup>2,3</sup>. *A. mellifera* mortality was adjusted using Abbot's formula<sup>4</sup> to account for natural mortality. Since there were no *O. lignaria* deaths in the control groups, no mortality correction was necessary.

According to the study, for both bee species, delayed toxicity survival rates after oral and contact exposure to single high dose of benomyl and iprodione were comparable to those in the control with the dosing vehicle.

The analysis showed significant difference in the mortality rates of controlled bees and those treated with iprodione.



acetone in water).

Description of the state

zole (104.0 μg a.i./bec) and a.i./bee). Survival after and captan (122.5 μg contact (125.0 μg ai/bee), iprodione (125.0 μg ai/bee), benomyl (125.0 μg ai/bee) and the control with the dosing vehicle (50% v/v acctone in water) was 100%





## **Description of Use in Document (QUAL, QUAN, INV):** Quantitative

(125.0 µg a.i./bee), and captan (122.5 µg a.i./bee). Survival in the control with the dosing vehicle

(25% v/v sucrose in water) was 100%

Rationale for Use: The study provides a good line of evidence for the effects of delayed iprodione (as well as other) mortality. Its methods are based on established techniques and there seems to be little room for the effects of confounding variables. The source and percent a./i. of the chemicals used is stated.

Limitations of Study: The source of the bees, specifically A. mellifera, could be a problem. Also, the doses used seem unnecessarily high relative to the maximum allowed dose.

#### Primary Reviewer: TJ Graven, Biologist.

#### Secondary Reviewer (required if study results are used quantitatively):

<sup>&</sup>lt;sup>1</sup>Ladurner E., Bosch J., Maini S., Kemp W.P. 2003b. A method to feed individual bees (*Hymenoptera, Apiformes*) known amounts of pesticides. Apidologie 34, 597-602

SAS Institute Inc. 1989. SAS/STAT User's guide. Version 6. Cary, NC

<sup>&</sup>lt;sup>3</sup> Allison, P.D. 1999. Logistic regression using the SAS System: Theory and application, SAS Institute Inc., Cary, NC

<sup>&</sup>lt;sup>4</sup> Abbot, W.S. 1925. A method of computing the effectiveness of an insecticide. J. Econ.Entomol. 18, 265-267.

## Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 89784 James, David G. 1989. Effect of pesticides on survival of *Amblyseius victoriensis* (Womersley), an important predatory mite in southern New South Wales peach orchards. Plant Protection Quarterly. 4(4): 141 – 143..

# Purpose of Review (DP Barcode or Litigation): Litigation

# Date of Review: 9-2-09

**Summary of Study Findings:** This study examines the effects of iprodione on the mite *Amblyseius victoriensis* in peach trees over a 28-day period. It finds that the number of mites and mite eggs were both significantly reduced by iprodione exposure.

The experiment was performed in a 10-year old peach (*Prunus persica*) orchard known to support a population of predacious mites, *A. victorensis*. Each tree was treated as an experimental unit. Experimental design was randomized and three replicates were used. Buffer trees were used to prevent cross-contamination, although the number and/or size of the buffer zone is not disclosed. Treatments were applied using a truck mounted spray unit with a handheld attachment. The solution was applied until run-off (40 L per tree). The weather on application days was calm and sunny with temperatures between 23 and 30°C. Iprodione applied in this experiment was in the form of Rovral (50% wp) and was applied at a rate of 75g product per 100L water [30 g/tree]. This concentration is described as the "recommended" use rate for orchards. Control trees were not treated with anything.

Fifty leaves were collected from each tree were 1-2 days prior to treatment. Fifty additional leaves were collected 2, 14 and 28 days after treatment. Leaves were collected at chest height with half coming from the outer canopy and half coming from the inner branches. Leaf selection was random within each location. After collection, leaves were stored in paper bags at 5°C for up to 48 hours before they were microscopically examined. The number of infested leaves, number of eggs per leaf and number of motile individuals per leaf were all recorded. Data were analyzed using ANOVA and LSD procedures.

Iprodione was found to significantly reduce the *A. victoriensis* populations compared to the control group over each epoch. Appropriately, it also significantly reduced the number of *A. victoriensis* eggs during the study. These results can be seen in **Tables 2** and **3** below. At a treatment rate of roughly 30 g/tree, iprodione treatment resulted in a significantly lower (p<0.05) mean number of mites at 2 dat (77% decrease), 14 dat (93% decrease) and 28 dat (99% decrease). The mean number of mite eggs were also significantly reduced by 2 dat (43% reduction), 14 dat (97% reduction) and 28 dat (100% reduction). The study concluded that iprodione, along with other pesticides in the study, had significant deleterious effects on predator mite survival

Pesticide	Pre-	Post - treat	Post - treatment (d)				
	treatment	2	14	28	rating		
clofentezine	0.55	0.30	0.53°	0.43ª	L		
chlorothalonil	0,39	0.31	0.14 <sup>™ 00</sup> *	0.31	L		
dithianon	0.27	0.08	0.07	0.13 <sup>b</sup>	м		
carbendazim	0.46	0.27°	0.11 <sup>be</sup>	0.10 <sup>bc</sup>	м		
hexythiazox	0.55	0.1160	0.17 <sup>hc</sup>	0.09∞	м		
endosulfan	0.50	0.03 <sup>6c</sup>	0.11 <sup>bc</sup>	0.09∝	м		
propargite	0.69	0.26¢	0.05**	0.01*	M		
iprodione	1.01°	0.23	0.07	0.01∞	M		
zineb	1.04°	0.14 <sup>bc</sup>	0. <b>08</b> ∞	0.03 <sup>bc</sup>	м		
benomyl	0.42	0.12 <sup>be</sup>	0.05*	0.01 <sup>∞</sup>	M		
phosmei	0.61	0.02 <sup>bc</sup>	0.03 <sup>bc</sup>	0.02∝	M		
dicofol	0.51	0.08⊳	0.01≈	Ope	н		
mancozeb	0.51	0.01 <sup>∞</sup> 003	0.01	0∞	Ĥ		
maldison	0.64	0.64	0.00	Ope	ਸ		
aziophos-methyl	0.59	0 <sub>ec</sub>	Opc	0 -	н		
carbaryl	0.33	0 <sub>pc</sub>	0∞	0.00	H		
pirimicarb	0.51	0×	0∞	Opc	н Н		
oxythioquinox	0.51	0 <sup>6c</sup>	Ope	0	ਸ		
UNTREATED	0.41	0.43	0.30	0.28			

Table 2. Mean number of motile A. victoriensis per leaf before and after treatmen with pesticides. Treatments are ranked in approximate order of least effect against A. victoriensis.

a Significantly greater than value for untreated trees on same date (P < 0.05)

b Significantly less than value for untreated trees on same date (P < 0.05)

c significantly less than pre-treatment value (P<0.05)

Toxicity Ratings: L = Low (no significant effect on survival),

M = Medium (permits some survival)

H = High (No survival)

Table 3. Mean number of eggs of <u>A. victoriensis</u> per leaf before and after treatment with pesticides. Treatments are ranked in approximate order of least effect against <u>A. victoriensis</u>.

ost

Pesticide	Pre-	Post-treat	ment (d)	
	treatment	2	14	28
clofentezine	0.13	0.17	0.31a	0.04
chlorothalonil	0.08	0.11	0.05	0.03 /
propargite	0.10	0.10	0	0.01
carbendazim	0.03	0.03	Ó	0.03
endosulfan	0.11	0.03	0.04	0.03
dithianon	0.10	0.03	0.01	05
hexythiazox	0.17	0.01	04	0.024
iprodione	0.37	0.21	0.01	0.02
zincb	0.33	0.03	0.01	ů,
benomyl	0.10	0.10	0	0.
dicofol	0.27	0.02	0.01	0°
phosmet	0.15	0.01	0.02	Õ <sup>ь</sup>
mancozeb	0.21	0.17	0 004	0 <sup>b</sup>
maldison	0.14	0.03	0.01	0
azinphos-methyl	0.34	0.07	0.01	0°
carbaryl	0.19	0.03	0.015	õ≫
pirimicarb	0.17	0.01	0.01	0°
oxythioquinox	0.11	05	0	0 <sup>b</sup>
UNTREATED	0.03	0.17	0.03	0.04

a Significantly greater than value for untreated trees on same date (P<0.05)

b Significantly less than value for untreated trees on same date (P < 0.05)

c Significantly less than pre-treatment value (P<0.05)

# Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study provides qualitative information regarding the effects of iprodione on a non-target species of mites. Iprodione is used on peach trees to control brown rot and there was concern regarding the potential effects of the fungicide on the predatory mite A. victoriensis given their importance in controlling phytophagous mites. According to the report, the exposure concentration was consistent with label recommended rates. At a treatment rate of roughly 30 g/tree, iprodione treatment resulted in a significantly lower (p<0.05) mean number of mites at 2 dat (77% decrease), 14 dat (93% decrease) and 28 dat (99% decrease). The mean number of mite eggs were also significantly reduced by 2 dat (43% reduction), 14 dat (97% reduction) and 28 dat (100% reduction).

**Limitations of Study:** The background of the test site is not disclosed, nor is the width of the buffer zone used between treatments. Residue levels on the peach tree leaves are not quantified; therefore, exposure levels are unknown.

## Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 90495 Yi, W., S. E. Law, and H. Y. Wetzstein. 2003. An *In Vitro* Study of Fungicide Effects on Pollen Germination and Tube Growth in Almond. HortScience. 38(6) 1086-1088.

## Purpose of Review (DP Barcode or Litigation): Litigation

## Date of Review: 9-4-09

**Summary of Study Findings:** The study examines the effects of iproidone and other fungicides on tubule germination and tubule morphology in almond (*Prunis dulcis*) pollen. Iprodione exposure at rates equivalent to 1 - 100% of the recommended field rate inhibited pollen germination and at treatment rates of 10 and 100% of recommended field rates significantly reduced pollen grain tube growth.

The iprodione used for this experiment was in the formulated product Rovral<sup>®</sup> (50% a.i.).

Pollen was gathered from almond trees in Bakersfield, CA. After collection it was stored at  $-20^{\circ}$ C until use. The germination medium was solution of 12% sucrose (w/v), 0.062% CaNO<sub>3</sub> (w/v), and 0.024% boric acid (w/v). The remainder of the solution is not disclosed, but is presumed to be water. For each assay, 200 µl of medium were used. The medium was combined with iprodione in three concentrations. These concentrations were based on the "recommended field rate" (RFR) and were 100% RFR, 10% RFR and 1% RFR. The recommended field rate defined as one pound per acre. Each treatment was replicated five times. Approximately 400 grains of pollen were added to each solution. The mixtures were then incubated at 27°C in the dark for 2.5 hours. After incubation, 20µl of HistoChoice fixative was added to stop growth and preserve morphology. Pollen was considered germinated if their tubules extended further than the diameter of the grain. Length and morphology were measured and observed in 50 randomly selected grains.

Results were statistically analyzed using Duncan's multiple range test and the general linear model (Proc GLM) procedure in SAS.

At 100% of the recommended field rate none of the pollen treated with iprodione germinated as opposed to 100% of the controls. At 10% and 1% of the recommended field rate 11.2 and 62.9%, respectively, of the iprodione-treated pollen germinated. As such, all of the iprodione treatments significantly (p=0.05) affected pollen germination (**Tables 2**). Pollen tube length was also significantly affected by iprodione treatment at both the 100% and 10% of recommended field rate treatments (**Table 3**). At the 100% RFR, no pollen germinated and as such there were no tubules; in the 10% RFR, the length of tubules was roughly 60% less than controls (**Table 3**).

	Fungicide concn (% of RFR)					
Fungicides	100	10	1			
No-fungicide control	100 a <sup>7</sup>	100 a	100 a >			
Propiconazole	0.0 Ь	8.0 cd	103.9 a			
Benomyl	X		96.3 a			
Myclobutanil	0.0 Ъ	58.3 Ъ	76.4 b			
Iprodione	0.0 Ь	11.2 c	62.9 c			
Thiophanate-methyl			59.4 cd			
Maneb	0.0 b	0.0 d	50.7 d			
Cyprodinil	0.0 ь	0.0 d	30.2 e			
Ziram	0.0 Ь	b 0.0	18.8 e			
Azoxystrobin	0.0 Ь	0.0 d	0.6 f			
Captan	0.0 b	0.0 d	0.2 f			

Table 2. Germination of almond pollen in presence of selected fungicides.<sup>z</sup>

Germination percentages shown are relative to the control, which is expressed as 100%. Actual pollen germination in the no-fungicide control was 53.7%. RFR = recommended field mate.

Mean values within a column followed by the same letter are not significantly different at P = 0.05, Duncan's multiple range test.

Not counted because of interference of particulate materials from the fungicide.

Table 3. Tube growth of	almond pollen	germinated in	medium containing
selected fungicides.	-	•	•

	Tube length (µm)*					
Fungicide	100% RFR	10% RFR	1% RFR			
No-fungicide control	504 a	504 a <sup>y</sup>	504 a			
Propiconazole	0 Ь	93 d	422 в			
Benomyl	·		453 a			
Myclobutanil	0 Ъ	349 Ъ	459 a			
Iprodione	06	202 c	442 a			
Thiophanate-methyl	·		403 a			
Maneb	0 b	0.e	165 b			
Cyprodinil	0 b	0 e	163 b			
Ziram	0 b	0 e	107 c			
Azoxystrobin	0 b	0 e	50 d			
Captan	0 b	0e	53 d 1			

<sup>3</sup>Mean length of pollen tubes germinated in medium with fungicide concentration of 100%, 10%, or 1% recommended field rate (RFR). Length was measured 2.5 h after inoculation.

Mean values within a column followed by the same letter are not significantly different at P = 0.05, Duncan's multiple range test.

Not measured because of interference of particulate materials from the fungicide.

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The background of the trees from which the pollen was collected was not disclosed. The study provides qualitative information that at rates equivalent to 1 to 100% of recommended rates, iprodione inhibited pollen grain germination and at 10 to 100% of the recommended field rate significantly reduced pollen grain tube length

**Limitations of Study:** This is an in vitro study and as such does not establish whether iprodione treatment reduces pollination success in intact plants.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 91954 Benson, D.M. 1992. Fungicides as Foliar Sprays or Rooting Cube Soaks in Propagation of Poinsettia. HortScience 27 (9), 1006-1008.

# Purpose of Review (DP Barcode or Litigation): Litigation

## Date of Review: 9-8-09

**Summary of Study Findings:** The study examined the effects of iprodione on root formation and growth of poinsettia (*Euphorbia pulcherrima*) plants. It tested two different application methods (spraying and rooting cube soaking) and found that plant height and root initiation were affected by iprodione spraying while only root initiation was effected by rooting cube soaking.

The iprodione used for this experiment was in the formulated product  $Chipco^{\text{(8)}} 26019 50W$  (50% a.i.). It was tested at a rate of 1.2 g/L.

Data were analyzed using ANOVA techniques and PROC GLC linear contrast comparisons were used to assess any differences in findings.

For the rooting cube soaking portion of the experiment strips of five cubes (one strip) were soaked in 200 ml (240 mg iprodione) of a solution containing the appropriate concentration of fungicide. Control strips were soaked in untreated water. Each strip was allowed to absorb its liquid completely before cuttings from a stock poinsettia population were stuck into each cube. For spray-application testing it is assumed, but not specified, that cuttings were placed in rooting cubes soaked only in water. It is stated that each cutting was sprayed to runoff and that runoff occurred at approximately 60 ml per strip (72 mg iprodione). The sprayed cuttings were allowed to dry before they were placed under the misting apparatus.

The cuttings were moved to a mist bench where they were misted two minutes of every hour from 7am to 7 pm for the first day and then two minutes of every three hours after 7 pm on the first day. Temperatures averaged  $21.4^{\circ}$ C during the experiment. It is never stated how many replicates were run during the experiment, but at days 14, 21 and 28 after planting two replicates of five cuttings (one strip) for each treatment along with one control strip were selected for root ratings.

Rooting was scored using a numerical rating system on a 1-7 scale with one being the lowest level of development and seven being the highest. Roots were also counted and measured. No significant differences were found between any treated group and their respective control groups earlier than 28 days. On day 28 root counts were significantly lower in both the iprodione-soaked and sprayed treatment groups when compared to the control (**Table 1**). There was also a significant difference in the root counts of sprayed cuttings versus those exposed in soaked cubes. According to the study, root counts for cuttings in cubes treated with iprodione were significantly lower (p=0.05) than for the untreated controls.

Rooted cuttings were then transplanted and allowed to grow for 58 days in undisclosed conditions. The plant heights were measured at after 30 and 58 days. At 30 days both sprayed and soaked plants were significantly shorter than controls while at 58 days only sprayed plants

were significantly shorter (**Table 2**). According to the study, after rooted cuttings were transplanted and grown for 30 days, those treated with iprodione as a spray were not as tall as the untreated controls and even 58 days after transplanting, iprodione spray treated plants remained significantly (p=0.05) shorter than the control plants.

poinsettia cuttings treated with fung	gicides applied as	foliar sprays or roo	bling cube soaks	
Source of variation	df	MS	F value	Pr > F
	Root rating at	14 days		
Replicative (Rep)	1	0.0077	0.03	0.8530
Treatment (Trt)	12	0.7974	3.57	0.0002
Rep × Trt	12	0.2077	0.93	0.5193
Linear contrasts				
Sprays vs. soaks	1	0.4083	1.97	0.1862
Flutolanil spray vs. fluto. soak	1	1.8000	8.07	0.0123
Flutolanil soak vs. control	1	3.2000	15.4	0.0020
	Root count at	14 days	0.02	0 3363
Rep	1	0.3769	0.93	0.3362
Trt	12	0.9333	1.68	0.0827
Rep × Trt	12	0.0709	1.00	0.0027
Linear contrasts		0.6750	1.00	0 3377
Sprays vs. soaks	1	0.6750	5.08	0.0308
Flutolanil spray vs. fluto. soak	1	4.0500	8.94	0.0113
Flutolanil soak vs. control	I De est motione est	21 days	0.71	••••
	Root rating at	21 aays	6 11	0.0150
Rep	12	2.4925	3 78	0.0001
Trt	12	0.8756	2.15	0.0197
Rep × 1rt	12	0.0700		
Linear contrasts	1	1 6333	1 87	0.1971
Sprays vs. soaks	1	5.0000	5.71	0.0342
Metalaxyl spray vs. metal soak	1	4.0500	4.63	0.0526
Iprodione soak vs. control	Root count at	21 days		
Pap	1	6.469	0.04	0.8360
Rep Trt	12	763.1	5.08	0.0001
$Rep \times Trt$	12	485.2	3.23	0.0006
Linear contrasts				
Sprave ve soaks	1	23.4	0.05	0.8298
Iprodione soak vs. control	1	1248.2	2.57	0.1347
Benomyl soak vs. control	1	952.2	1.96	0.1866
	Root rating a	t 28 days		
Bep	1	1.731	2.42	0.1229
Trt	12	2.677	3.74	0.0001
$\text{Rep} \times \text{Trt}$	12	2.597	3.63	0.0001
Linear contrasts				
Sprays vs. soaks	1	2.408	0.93	0.3546
	Root count a	t 28 days		
Ren	1	0.1923	0.00	0.9774
Trt	12	2603.8	10.9	0.0001
Rep × Trt	12	538.2	2.26	0.0140
Linear contrasts				
Sprays vs. soaks	1	291.4	0.54	0.4760
Benomyl spray vs. control	1	2668.1	4.96	0.0459
Benomyl soak vs. control	1	3302.5	6.14	0.0291
Chlorothalonil spray vs. control	1	4203.2	13.2	0.0130
Iprodione spray vs. control	1	5848 2	10.9	0.0064
iprodione soak vs. control	1	3040.2	10.7	3.0031

Table 1. Analysis of variance results for root rating and root count over three sampling dates for poincettia cuttings treated with fungicides applied as foliar sprays or rooting cube soaks.<sup>z</sup>

<sup>2</sup>Linear contrasts for spray vs. soak application of fungicides are reported along with contrasts for significant individual fungicide effects and for two nonsignificant contrasts with low root count compared to the control.

Treatment	Plant ht (cm) <sup>z</sup>						
	30 0	lays	58 days				
	Spray	Soak	Spray	Soak			
Benomyl	12.2 bc	13.3 abc	23.2 abc	22.6 abc			
Chlorethelenil	13 3 abc	12.2 bc	22.7 abc	20.8 cd			
Chlorothalonn	14.5 2	14.7 a	23.6 abc	23.9 ab			
Flutolanii	14.5 a 11 7 c	14.5 a	19.4 d	23.5 abc			
Iprodione	11.7 C	14.3 u 14.2 a	21.5 bcd	23.9 ab			
Metalaxyl	12.4 UC	14.2 0	22.5 abc	25.3 a			
Metalaxyi + benomyl Untreated control	13.5 ab	.5 a	22.5 400 23.2	2 abc			

Table 2. Effect of several fungicides applied as a foliar spray or as a rooting cube soak to 'V-14 Glory' poinsettia cuttings on subsequent plant height 30 and 58 days after transplanting of rootec cuttings

<sup>2</sup>Mean separation for plant heights within a sample date by Waller-Duncan k ratio: k = 100, P = 0.05. See text for fungicide rates used.

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study compares the effects of iprodione using different dosing methods. The most significant finding of this study is that relative to controls, plant height was significantly affected (p<0.05) (30 and 58 days post-treatment) when iprodione was applied as a foliar spray.

**Limitations of Study:** Not all aspects of the study are well explained and some of the measurements are subjective rankings of appearance.

**Chemical Name:** Vinclozolin (V) and Iprodione (I)

**CAS No:** 113201 (V) and 109802 (I)

**ECOTOX Record Number and Citation:** 96140 Olien, W.C., R. W. Miller Jr., C. J. Graham, E. R. Taylor Jr., M. E., Hardin. 1995. Effects of combined applications of ammonium thiosulphate and fungicides on fruit load and blossom blight and their phytotoxicity to peach trees. Journal of Horticultural Science 70, 847-854.

# Purpose of Review (DP Barcode or Litigation): Litigation

## Date of Review: 8-11-09

**Summary of Study Findings:** This study examines the synergistic effects of fungicides and the fertilizer ammonium thiosulphate (ATS) on peach trees (*Prunus persica*).

The test orchard was located at the Musser Fruit Research Station in the South Carolina Agricultural Experiment Station, Clemson University. The trees were planted in 1985 and thinned to a spacing of 4x4.5 m (555 trees per ha) in 1992. They were trained to a central leader form 2-m basal diameter and 2.5-m high. Trunk cross sectional area was  $108 \pm 25$  cm<sup>2</sup> and was not significantly different between replications or treatments (P > 0.05).

Twenty treatments including controls were assigned using a randomized complete block designs. Each treatment was a single tree and each was replicated four times for a total of 80 trees. The test trees were pre-treated with brown rot (*Monilinia fructicola*) by spraying trees with a mixture of cultured spores, water and Trugitol<sup>®</sup> F surfactant. Trees were sprayed until runoff to ensure exposure. After spraying, twelve 9-cm diameter Petri dishes containing sterile PDA were exposed at random trees. They were then incubated at 22 °C for 24 hours. Every exposed plate grew *M. fructicola* indicating a successful inoculation. Various fungicides were then applied "either alone or with ATS (2% formulation v/v)" at the rates recommended by the South Carolina Spray Guide during full bloom. Vinclozolin was applied in Ronilan<sup>®</sup> DF (2.4 g/L)at a rate of 1.58 kg a.i./ha. Iprodione was applied as Rovral® 4F (2.5 mL/L) at a rate of 1.58 kg a.i./ha. The controls for this experiment were water and 2% ATS solution alone. Mixtures were applied using a hand sprayer and until runoff to ensure uniform coverage. During spraying a 2.4 x 3.0 m plastic shield was used to prevent drift to adjacent trees. During spraying, winds were present at 4.5 km/h.

Growth and disease were rated 30 days after the bloom. Fruit number was graded using a 0-3 scale, with 3 being a heavy crop and 0 representing no crop. Final fruit numbers were also obtained at harvest and expressed in a "per tree" and "per CSA" form. Blossom burn was rated on a similar 0-3 scale with 0 being no burn and 3 representing severe burn.

ANOVA was performed using Proc GLM in the SAS statistical software program.

Results showed that pesticides were, in fact, effective in controlling *M. fructicola* blossom blight cankers per tree as a measure of efficacy where both vinclozolin and iprodione significantly reduced the number of cankers relative to controls. Blossom burn ratings for vinclozolin and iprodione did not differ statistically (p>0.05) from controls; slight blossom burn was observed in all of the treatments including controls. The study also found that fruit load in trees treated with both ATS and fungicide was not significantly different from the effects seen with fungicide

alone. Blossom burn was significantly increased over the effects of water alone by ATS x fungicide treatments. However, ATS alone increased blossom burn more than did any of the mixtures. These results can be seen in the graph below.



Fig. 2

Phytotoxicity of nine fungicides to peach blossoms when applied alone or simultaneously with ATS (scale: 0 = no damage, 3 = severe). Means with lower case letters in common are not significantly different at P = 0.05. Treatments: A-water control; B-benomyl; C-chlorothalonil; D-captan; E-triforine; F-propiconazole; G-vinclozolin; H-iprodione; I-sulphur; J-thiophanate-methyl (full details of the fungicides and rates of application are given in Table I).

No fungicide treatment when applied without ATS increased burn damage to one year old shoots whereas ATS alone increased shoot burn over the water control



FIG. 3

Phytotoxicity of nine fungicides to shoots of peach trees when applied alone or simultaneously with ATS. Means with lower case letters in common are not significantly different at P = 0.05. Treatments: A-water control; B-benomyl; C-chlorothalonil; D-captan; E-triforine; F-propiconazol; G-vinclozolin; H-iprodione; I-sulphur; J-thiophanatemethyl (full details of the fungicides and rates of application are given in Table

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** At the application rate tested, neither vinclozolin nor iprodione appeared to affect the number of burned shoots per tree. In combination with ATS, neither vinclozolin nor iprodione differed from controls in the number of burned shoots per tree.

Limitations of Study: The study examines a relatively specific endpoint and it is difficult to gauge the overall phytotoxicity potential of either vinclozolin or iprodione from this study. The study is essentially measures efficacy relative to plant damage from brown rot fungus. Frost damaged 10-20% of the flowers; it's unclear how this may have impacted the study.

#### Chemical Name: Iprodione

#### CAS No: 109801

**ECOTOX Record Number and Citation:** 101692 Gullino, M. L., G. Lento, and A. Garibaldi. 1984. Control of *Rhizoctonia solani* of Vegetables with New Fungicides.

#### Purpose of Review (DP Barcode or Litigation): Litigation

#### Date of Review: 9-9-09

**Summary of Study Findings:** The study examines the effects of iprodione on the web blight (damping off) fungus *Rhizoctonia solani* in basil (*Ocymum basilicum*) and beans (*Phaseolus vulgaris*). It finds that increasing amounts of iprodione decrease the effects of fungus.

Both plants were grown in 2500 cm<sup>3</sup> containers. Beans were grown in a 75:25 mixture of steam sterilized soil and Perlite while basil was grown in a 50:50 mixture of sterilized soil and peat. Plants were kept at 25°C during the day and 22° during the night. Photoperiod was 15:9 L:D. Plants were inoculated using infected wheat kernels. The iprodione used in this experiment was in a formulation listed as "EXP 1861" and had 25% a.i. Seeds were treated in a drench method with the following concentrations of iprodione (g/m<sup>2</sup>): 0.125, 0.25, 0.5, 1, 4. The total volume of solution used was 100 ml/pot. The effects on the fungus as well as phytotoxicity were observed. Plant toxicity data can be seen in **Table 1** below. While it is stated that toxicity was seen at high concentrations and in young plants, there was no statistical analysis performed.

Table 1 - Influence of soil drenching with different dosages of several fungicides on seed emergence and on mean fresh weight of bean plants (data collected 10 days after emergence).

Fungicide	Doşage (g/m <sup>°</sup> a.i.)	Emergence (%)	Mean fresh weight (g)	
Benomy 1	4	92 a*	2.18 a	
"	2	89 a	2.15 a	
"	1	93 a	2.44 a	
lutoluanil	4	92 a	2.39 a	
"	2	85 ab	2.41 a	
	1	92 a	2.48 a	
urmetamide	4	75 b	2.66 a	
	2	83 ab	2.20 a	
н х	1	92 a	2.36 a	
prodione	4	100 a	2.48 a	
	2	92 a	2.18 a	
"	1	94 a	2.44 a	
lepronil	4	86 ab	2.20 a	
	2	94 a	2.23 a	
	1	89 a	2.27 a	
encycuron	4	94 a	2.31 a	
	2	86 ab	2.26 a	
	1	89 a	2.96 a	
olchlofos-methyl	4	89 a	2.21 a	
	2	94 a	2.23 a	
	1	92 a	2.39 a	
ontrol	-	94 a	2.70 a	

\* Mean separation in columns by Duncan's multiple range test, 5% level

# Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** Although very few details are provided on the study methodology, under the conditions tested, iprodione soil drench treatments at  $1 - 4 \text{ g/m}^2$  did not appear to significantly affect percent emergence or basil fresh weight.

**Limitations of Study:** The study focuses more on iprodione's effectiveness as a fungicide than its effects on plants. The study provides little information on the methods and results and only provides qualitative information that soil drench seed treatments under the conditions and treatment concentrations used, did not appear to affect the few parameters measured in the study.

## Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 104765 Hautier, L., J-P. Jansen, N. Mabon, and B. Schiffers. 2005. Selectivity Lists of Pesticides to Beneficial Arthropods for IPM Programs in Carrot-First Results. Comm. Appl. Biol. Sci 70(4), 547-557

## Purpose of Review (DP Barcode or Litigation): Litigation

## Date of Review: 9-10-09

**Summary of Study Findings:** The study examines the effects of iprodione and other pesticides on beneficial arthropods associated with carrots. It found that iprodione was not toxic to the test subjects.

Iprodione used in this study was in the formulated product Rovral WG (50% a.i.). It was applied using a pneumatic atomizer at 200 l/ha for glass treatments and 400 l/ha for sand and soil treatments. It is also stated that iprodione was applied at the recommended rate of 750 g a.i/ha. The actual volume or amount of pesticide applied is never stated.

The species used for testing were as follows: adult parasitic wasp *Aphidius rhopalosiphi*, adult carabid beetle *Bembidion lamprosm*, adult rove beetle *Aleochara blilineata*, larval ladybird beetle *Adalia bipunctata*, and larval hoverfly *Episyrphus balteatus*. The backgrounds of the individuals used for the study are not disclosed.

According to the study, the toxicity of chemicals to beneficial arthropods were assessed according to SETAC guidelines (Barrett *et al.* 1994) and according to the methodology developed by Copin *et al.* 2001. by using glass, sand or soil substrates. The substrates were analyzed to ensure that at least 85% of the dosed chemical persisted. tested on glass substrate were exposed for 48 hours while those tested on other substrates were exposed for two weeks. Any dosage changes are not noted.

The study found no significant effects from iprodione exposure in any species on any substrate.

**Table 1.** Results of toxicity tests, corrected mortality (CM) or parasitism reduction (PR) (%). A: results on inert substrate (glass or sand); B: results in semi-controled conditions (plants or soil); -: no or weak pesticide exposition; ED: ecotoxicological data; §: not yet completely tested.

	active ingredients	formulation												
	- *		a.i. concentration (%)	g a.i./ha	A therefore the	A. IIIupauspri	e himmedate	d. Upwikidia	C holtootus	L, valicatus	A, biineata		B. lamoors	
					Α	В	A	В	A	В	A	В	Α	В
	carbofuran	Curater	5	0.0625	-	•	•	-	-	•.,	ED		ED	
	carbosulfan	Sheriff 1 Gr	1	0.0625		•	-	-		-	100	ş	5	6
	chlorpyriphos-ethyl	Dursban 5G	5	0.2		-	-	-	-		ED		ED	
-ä	deltamethrin	Decis 2.5EC	2.5	10	100	75	100	100	75	77	100	ş	72	ş
33	diazinon	Disonal	60	510	-	-	-	-	-	-	ED		ED	-
æ	dimethoate	Hermoetrox EC	50	250	100	100	100	100	100	100	ED		ED	
⊒,	λ-cyhalothrin	Karate Zeon CS	10	10	100	1	100	100	0		100	ş	100	ş
	pirimicarb	Pirimor WG	50	200	100	12	21		80	94	ED		ED	-
	pirimicarb+λ-cyhalothrin	Okapi EC	10+0.5	150+7.5	100	3	100	100	100	100	100	ş	96	§
	azoxystrobin	Ortiva SC	25	250	63	7	21		14		1		4	
	difenoconazole	Geyser EC	25	125	0		3		21		0		20	
<u>8</u>	dithlanon	Ditho WG	70	1260	35	24	17		0		0		0	
ġ	iprodione	Rovral WG	50	750	6		30		10		0		0	
Ĕ	myclobutanil	Systhane 24EC	20	- 60	4		0				0		4	
4	sulfur	Horizon EW	25	250	92	5	96	32	10		ED		0	
	tebuconazole	Hermovit WG	80	4000	17		45	11	7		0		0	
	chlorpropham	Chloor IPC EC	40	2400	-	-					100	ş	ő	6
	clomazone	Centium 360 CS	36	90	-	-		-	-	-	0	-	14	-
	cycloxydime	Focus Plus EC	10	600	-	-		-	-		ED		0	
	fluazitop-p-butvl	Fusilade EC	25	500	-			-	-		ED		4	
<b>9</b> 2	glufosinate-ammonium	Basta S SL	20	600	-	-	-		-		ED		ş	ş
ġ	dvohosate	Roundup energy SG	68	2176						-	ED		ŝ	ŝ
ê	linuron	Linuron 500 SC	50	500	-	-				-	16		10	-
Ъб	metoxuron	Dosanex WP80		3600		-		-		-	2		ş	ş
	paraguat	Gramoxone SL	20	1000		-	-			-	1		ŝ	§
	paraguat+diguat	Prigione SL	12+8	600+400	•	-	-	-	-	-	18		ş	5
	guizalolop-ethyl D	Targa Prestige EC	5	150	٠	-		-			2		30	
	tepraloxydim	Aramo EC	5	100				-	-	-	0		0	

# Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study suggests that under the conditions tested, iprodione was not particularly toxic to beneficial insects and the chemical was assigned a "green category (harmless)" since corrected mortality was less than or equal to 30%.

**Limitations of Study:** Although the study provides a reference for how "corrected mortality" is caculated, the study itself does not elaborate. The presumption from the the information provided in the study is that iprodione, applied at a rate equivalent to a field application of 750 g ai./ha resulted in less than 50% mortality on the insect species tested. There is very little information provided on the actual study results and there is no mention of controls.

**Chemical Name:** Vinclozolin (V) and Iprodione (I)

CAS Nos: 113201 (V) and 109801 (I)

**ECOTOX Record Number and Citation:** 105131 Rouchard, J., C. Moons and J. A. Meyer. 1984. Effects of Pesticide Treatments on the Carotenoid Pigments of Lettuce. Journal of Agricultural Food Chemistry. 32 (6) 1241-1245.

#### Purpose of Review (DP Barcode or Litigation): Litigation

#### Date of Review: 8-04-09

**Summary of Study Findings:** The study examines the effects of various herbicides and pesticides on the carotenoid pigment content of lettuce (*Lactuca sativa*). It finds no difference between the values found in lettuce treated with vinclozolin and lettuce in the control treatment.

The lettuce culture used for the study was "made" at the Research Station for Vegetables St. Kateijne-Waver, Belgium. The plants were grown in a greenhouse. They were transplanted to a field when they reached the six-leaf growth stage. At transplantation, they were arranged in a grid with 30 cm between each plant on each side. All of the plots received "the common and usual fertilization treatments" described by Rouchaud *et al* (1982). The treatments were arranged in a randomized block design and were repeated four times each. In the case of vinclozolin, plants were dosed at the 12-leaf stage with an emulsion containing 10 g Ronilan<sup>®</sup> (vinclozolin 50 % a./i. treated at 10 g Ronilan<sup>®</sup>/acre) or Rovral<sup>®</sup> (iprodione 50 g % treated at 50 g Rovral<sup>®</sup>/acre). Four harvests were made at 14, 20, 26 and 32 days post-treatment. The replicate groups were homogenized and prepared for spectrometry analysis. Visual observations were also made to score the color of each plant and weights of plants were also recorded.

The results of the analysis found that vinclozolin caused no significant difference in carotenoid content across any of the sampling periods when compared with the control group (**Table 11**). Iprodione caused significant (p<0.1) in total carotene, -cryptoxanthin, lutein, violaxanthin and neoxanthin content across all of the sampling periods (**Table 11**). Fresh weights of vinclozolin-treated plants were significantly different (p<0.1) than controls 14 and 26 days post-treatment, but were not significantly different by Day 32 post-treatment (**Table 1**). Vinclozolin-treated plants were 47%, 12%, 13% and 5% heavier than controls on sampling days 14, 20, 26 and 32, respectively. For iprodione-treated plants, weights were significantly different (p<0.1) throughout the sampling period (**Table 1**). In general, iprodione-treated plants weighed 61, 24, 21 and 14% more than controls at 14, 20, 26 and 32 days post-treatment, respectively.

pesticide	unitary fresh weights of the lettuces, g, <sup>d</sup> for harvest date						
treatment	5/20	5/26	6/1	6/7			
control	$191 \pm 16$	340 ± 14	483 ± 20	626 ± 22			
propyzamide	$191 \pm 15^{\circ}$	291 ± 12 <sup>b</sup>	453 ± 20°	508 ± 22°			
chlorpropham	$171 \pm 14^{\circ}$	$302 \pm 13^{\circ}$	$391 \pm 16^{a}$	487 ± 18°			
propyzamide plus chlorpropham	$265 \pm 23^{a}$	$400 \pm 16^{\circ}$	542 ± 18⁰	687 ± 32ª			
benomyl	$207 \pm 19^{\circ}$	$308 \pm 14^{\circ}$	$420 \pm 17^{a}$	$539 \pm 18^{\circ}$			
iprodione	$307 \pm 26^{a}$	421 ± 23 <sup>a</sup>	$583 \pm 25^{a}$	$716 \pm 25^{a}$			
vinclozolin	$281 \pm 18^{a}$	$379 \pm 16^{\circ}$	$547 \pm 21^{a}$	658 ± 27°			

Table I. Unitary Mean Fresh Weights of the Lettuces

<sup>a-c</sup> Significantly different from the control at the 1% (a) and 5% (b) level or nonsignificant (c), respectively. <sup>d</sup> Means  $\pm$  SD of the weights of 20 lettuces. <sup>e</sup> Month and day, year 1983.

Table II. Carotenoid Content of the Fresh Lettuce

	harvest date.	carotenoid content, $\mu g/100$ g of fresh lettuce <sup>d</sup>							
pesticide treatment	month and day (year 1983)	total carotene	β-crypto- xanthin	lutein	violaxanthin	neoxanthin			
control	5/20	$2523 \pm 111$	$172 \pm 10$	$2573 \pm 121$	1111 ± 49	$433 \pm 21$			
	5/26	2308 ± 97	$160 \pm 9$	$2185 \pm 92$	$1188 \pm 52$	460 ± 23			
	6/1	$1585 \pm 70$	$112 \pm 7$	$1553 \pm 68$	750 ± 32	375 ± 20			
	6/7	$755 \pm 35$	60 ± 4	$856 \pm 40$	$392 \pm 18$	$202 \pm 11$			
propyzamide	5/20	$2913 \pm 131^{\circ}$	$198 \pm 12^{\circ}$	$2924 \pm 123^{\circ}$	$1312 \pm 56^{\circ}$	498 ± 26°			
	5/26	$2648 \pm 117^{\circ}$	189 ± 11°	$2579 \pm 113^{\circ}$	1406 🗭 66°	$537 \pm 27^{\circ}$			
	6/1	$1826 \pm 77^{\circ}$	134 ± 8°	$1900 \pm 89^{a}$	918 ± 40 <sup>a</sup>	$452 \pm 24^{\circ}$			
	6/7	914 ± 40°	$74 \pm 5^{\circ}$	$1051 \pm 47^{\circ}$	$468 \pm 20^{\circ}$	$264 \pm 13^{\circ}$			
chlorpropham	5/20	2959 ± 139 <sup>a</sup>	$191 \pm 12^{c}$	$3015 \pm 127^{\circ}$	1279 ± 55°	490 ± 25°			
	5/26	2707 ± 119 <sup>e</sup>	$189 \pm 11^{a}$	$2608 \pm 115^{\circ}$	1384 ± 61 <sup>e</sup>	$543 \pm 29^{\circ}$			
	6/1	$1863 \pm 78^{\circ}$	137 ± 8°	$1910 \pm 86^{\circ}$	$919 \pm 43^{\circ}$	$449 \pm 22^{a}$			
	6/7	964 ± 42°	$70 \pm 4^{a}$	$1063 \pm 50^{a}$	$503 \pm 21^{a}$	236 ± 13 <sup>a</sup>			
propyzamide plus chlorpropham	5/20	$2890 \pm 130^{a}$	$186 \pm 12^{c}$	$3015 \pm 142^{\circ}$	1335 ± 56 <sup>a</sup>	$482 \pm 24^{\circ}$			
	5/26	2737 ± 120 <sup>e</sup>	$197 \pm 12^{a}$	2708 ± 119°	$1446 \pm 68^{a}$	$560 \pm 27^{\circ}$			
	6/1	1845 ± 77°	130 ± 8°	1872 ± 79°	$901 \pm 40^{a}$	$445 \pm 24^{\circ}$			
	6/7	1018 ± 48 <sup>a</sup>	$69 \pm 4^{\circ}$	$1122 \pm 49^{\circ}$	515 ± 22 <sup>a</sup>	$257 \pm 14^{\circ}$			
benomyl	5/20	$2544 \pm 114^{\circ}$	$171 \pm 10^{\circ}$	$2461 \pm 108^{\circ}$	$1149 \pm 48^{\circ}$	492 ± 24 <sup>a</sup>			
	5/26	2518 ± 111 <sup>6</sup>	157 ± 9°	$2145 \pm 97^{\circ}$	1167 ± 51°	451 ± 23°			
	6/1	$1492 \pm 63^{\circ}$	$108 \pm 7^{\circ}$	$1510 \pm 71^{\circ}$	$703 \pm 33^{\circ}$	$391 \pm 21^{\circ}$			
	6/7	597 ± 26 <sup>a</sup>	$62 \pm 4^{\circ}$	874 ± 37°	400 ± 17°	$208 \pm 11^{\circ}$			
iprodione	5/20	2811 ± 118°	$190 \pm 11^{\circ}$	$2995 \pm 135^{\circ}$	$1250 \pm 59^{a}$	$488 \pm 26^{a}$			
	5/26	2612 ± 110 <sup>a</sup>	$189 \pm 11^{\circ}$	$2585 \pm 109^{\circ}$	$1409 \pm 59^{\circ}$	$549 \pm 26^{a}$			
	6/1	1849 ± 81ª	135 ± 9ª	1819 ± 85 <sup>a</sup>	925 ± 40 <sup>a</sup>	$441 \pm 23^{a}$			
	6/7	$1023 \pm 48^{\circ}$	$75 \pm 5^{\circ}$	$1099 \pm 48^{a}$	$523 \pm 23^{\circ}$	$236 \pm 12^{a}$			
vinclozolin	5/20	$2518 \pm 118^{\circ}$	$170 \pm 11^{\circ}$	$2474 \pm 111^{\circ}$	$1103 \pm 52^{\circ}$	$509 \pm 25^{\circ}$			
	5/26	$2229 \pm 98^{\circ}$	$162 \pm 10^{\circ}$	$2258 \pm 106^{\circ}$	$1207 \pm 53^{\circ}$	$428 \pm 21^{\circ}$			
	6/1	1469 ± 62°	120 ± 7°	$1561 \pm 69^{\circ}$	$763 \pm 33^{\circ}$	$371 \pm 20^{\circ}$			
	6/7	756 ± 34°	$65 \pm 4^{\circ}$	$884 \pm 37^{\circ}$	$389 \pm 16^{\circ}$	$225 \pm 12^{b}$			

<sup>a-c</sup> As in Table I. <sup>d</sup> Means  $\pm$  SD of four replications.

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** Although the study has limitations, it provides qualitative evidence that both iprodione and vinclozolin have effects on terrestrial plants. Treatments with vinclozolin and iprodione significantly affected (increased) growth over the period of time sampled. While vinclozolin treatment did not affect pigment content of lettuce leaves, iprodione treatment appeared to significantly increase pigment content in leaves relative to controls. Since plant
weight was significantly different in iprodione-treated lettuce plants, this may account for the increase in pigment content although the pigment content is corrected for weight in the study.

**Limitations of Study:** It is not clear from the study methods whether the data are expressed in terms of active ingredient of formulation. Only one test concentration was used for iprodione and vinclozolin. Pigment content has not been linked to assessment endpoints of impaired growth, survival or reproduction

### Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 108977 Rankin, G. O., V. J. Teets, D. W. Nicoll, and P. I. Brown. 1989. Comparative Acute Renal Effects of Three N-(2,3-Dichlorophenyl) Carboximide Fungicides: N-(3,5-Dichlorophenyl) Succinimide, Vinclozolin and Iprodione. Toxicology 56, 253-272.

# Purpose of Review (DP Barcode or Litigation): Litigation

# Date of Review: 9-14-09

**Summary of Study Findings:** The study examines the nephrotoxic properties of three fungicides, including iprodione, in male rats. It attempts to find a correlation between fungicidal activity and nephrotoxicity. No renal effectes were found to result from iprodione exposure.

Male Fischer 344 rats (*Rattus norvegicus*) were purchased from Hilltop Lab Animals, Inc. They were kept in individually in stainless steel metabolism cages during the control and post-treatment periods. At all other times the rats were kept in standard plastic animals cages with no more than six animals per cage. Conditions during the test were as follows; temperature was between 21 and 23° C with 40-55% relative humidity and a photoperiod of 6:18 L:D. Rats were allowed acclimatize for one week prior to being transferred to individual metabolism cage. They were kept in these individual cages for an additional day of acclimatization before one day of monitoring to establish baseline conditions.

Rats were treated interperitoneally in groups of four with single doses ranging from 0.4 and 1.0 mmol/kg (114 - 286 mg/kg bw) dissolved in sesame oil. A control group was given and injection of sesame oil at 2.5 ml/kg. Renal function was monitored at 24 and 48 h by measuring urine samples for protein, glucose, ketones and blood. Body weight, food intake and water intake were also measured at the same intervals. The rats were killed at 48 h. Left kidneys were removed and chemically analyzed using techniques described by Rankin *et al*<sup>1</sup> and Yang *et al*<sup>2</sup>. Right kidneys were removed, weighed and cut longitudinally. The cross-section was fixed in a solution and examined histologically. Blood was drawn from rats three days prior to the move to individual cages and again just before termination.

Statistics were analyzed using a one-way ANOVA and/or Student's t-test. The 0.05 level was used for significance.

Rats treated with iprodione showed few effects from exposure. Rats showed a significant reduction in urine volume at 48 hours when compared to the pair-fed control group. There was no significant increase in proteinuria , glucosuria or hematuria as all were not observed in iprodione treated rats. Ketonuria was not found in any treatment group. No increase in kidney weight or morphological damage was observed in rats exposed to iprodione. However, according to **Table 1** of the report, both food and water intake were significantly different (lower; p<0.05)) for rats treated with 1 mmol/kg vinclozolin and iprodione on Day 1 and 2 compared to Day 0. For vinclozolin food and water intake were also significantly different (p<0.05) on Day 1 and Day 2 following treatment.

<sup>1</sup>Rankin, G.O. Cressey-Veneziano, K. Brown, P.I. 1984. Onset of and recovery from acute N-(3,5- dichlorophenyl)succinimideinduced nephrotoxicity in Sprague-Dawley rats. Toxicology 30, 205.

<sup>2</sup>Yang, D.J. Lo, H.H. Teets, V.L. Brown, P.I. Rankin, G.O. 1987. Nenhrotoxicity of N-(3.5-dihalonhenvl)succinimides in Fischer

Compound	Dose	Food intake	Water intake (ml)				
	(mmol/kg)	Day 0	Day 1	Day 2	Day 0	Day 1	Day 2
IPDO	0.4	$12.4 \pm 1.2$ $12.3 \pm 1.3$	$11.1 \pm 1.9$ 38 + 1.4°	$13.1 \pm 1.1$ $38.6 \pm 2.4^{\circ}$	$24 \pm 2$ 21 \pm 3	$22 \pm 3$ 8 ± 2 <sup>e</sup>	$22 \pm 2$ $22 \pm 2$
VCLZ	0.4 1.0	$12.3 \pm 1.0$ $14.3 \pm 1.0$ $15.3 \pm 1.2$	$11.6 \pm 1.2^{\circ}$ $10.6 \pm 1.0^{\circ}$	2)11.9 ± 1.4° 11.7 ± 1.6°	26 ± 3 27 ± 1	19 ± 3° 21 ± 1°	21 ± 3° 20 ± 1°

EFFECT OF IPDO OR VCLZ ADMINISTRATION ON FOOD AND WATER INTAKE

• Rats were administered IPDO or VCLZ and food and water intake determined at 24-h intervals post-injection (Day 1 and Day 2). Day 0 values were obtained on the control day prior to treatments.

<sup>b</sup> Values are means  $\pm$  S.E. for N = 4 rats/group.

• Significantly different (P < 0.05) from the corresponding Day 0 value with a group.

**Figure 2** of the report indicates that for iprodione, controls had a significantly lower urine volume at 24 hours post-treatment. In the vinclozolin group, controls had significantly lower urine output at both 24 and 48 hrs post-treatment. Similar patterns also existed for the iprodione and vinclozolin-treated rates at both treatment levels. Because of the apparent solvent (sesame oil), the treatment effects related to urinary output are suspect.



#### Description of Use in Document (QUAL, QUAN, INV): Invalid

**Rationale for Use:** The study did not demonstrate any correlation between fungicidal activity and nephrotoxicity in rats under the conditions tested. There is some illustration of (the lack of) whole organism expression coupled with organ condition.

**Limitations of Study:** The formulation of the chemical used is never given although the studied units are mmol/kg. Most of the focus in the study is on *in vitro* data. The concentrations used are not justified. There was an apparent solvent effect on urinary output and it is uncertain how this effect may have influenced the study results. As such, the study is considered scientifically unsound.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 109021 Gadeva, P., and B. Dimitrov. 2008. Genotoxic effects of the pesticides Rubigan, Omite and Rovral in root-meristem cells of *Crepis capillaris* L. Mutation Research 652, 191-197.

# Purpose of Review (DP Barcode or Litigation): Litigation

### Date of Review: 9-14-09

**Summary of Study Findings:** The study examines the genotoxicity of several pesticides including iprodione in a terrestrial plant. It found that iprodione may act as a aneugen, *i.e.*, affects cell division and the mitotic spindle apparatus resulting in the loss or gain of whole chromosomes inducing an aneuploidy, but iprodione does not impact meristem growth or cell proliferation.

Plants used in the experiment were smooth hawk's beard *Crepis capillaris* obtained from the Institute of Botany, Sofia, Bulgaria. Their background care is not detailed outside of the statement that they were raised "in the green-house."

The iprodione containing chemical used in this experiment was Rovral 25 Flo containing 250 g a.i/L . Dose concentrations were determined by "doses used in agricultural practice." They were 0.1, 0.2, 0.4 and 0.8% a.i. Two controls were used for the experiment. The negative control was distilled water and the positive was ethyleneimine (0.05%). Treatment solutions were prepared by dissolving the appropriate amount of formulated chemical in water. 1.5-2 mm primary root meristems were treated with one of the listed chemical concentrations for 2h. The method of treatment is not detailed. Following treatment the roots were washed in tap water for 1 hr and allowed to recover in a thermostat at 24°C for 4, 16 and 24 hrs. At each interval samples were taken and fixed in a 3:1 alcohol-acetic acid solution for chromosomal and micronucleus analysis. Two hours prior to sampling, half of the would-be sampled material was treated with a 0.05% colchicine solution to facilitate chromosomal analysis. Both chromatid and chromosome aberrations were analyzed. The experiment was replicated four times.

Aberrations were scored using Sigma Plot9 with Sigma Stat Integration. A one way ANOVA was then performed. If a statistically significant (<0.05) result was found, a Holm-Sidak multiple comparison was performed against the negative control. The power of the test statistic was  $\geq$ 0.08. Regression analysis was then done to find a dose-response relationship.

Iprodione treatment resulted in no significant differences in aberrations during metaphase, anaphase or telophase. It also did not result in any statistically different results in SCE per metaphase. There was a statistically significant increase in micronucleus formation that peaked at 16 hrs (**Table 4**).

#### Table 4

Frequency of micronuclei in root-meristem cells of *C. capillaris* after treatment with the pesticides Rubigan, Omite and Rovral

Pesticides	Concentration	Recovery time (h)	Cells with	micronuclei
	(-)	()	Number	%mean ± S.E.M.
Rubigan	0.025	4	4	$0.10 \pm 0.04$
		16	4	$0.10 \pm 0.04$
		24	12	$0.30 \pm 0.09$
	0.05	4	5	$0.13 \pm 0.06$
		16	13	$0.28 \pm 0.08$
		24	15	$0.38 \pm 0.10$
	0.1	4	4	$0.10 \pm 0.05$
		16	6	$0.15 \pm 0.06$
		24	5	$0.12 \pm 0.02$
	0.2	4	7	$0.18 \pm 0.04$
		16	6	$0.15 \pm 0.06$
		24	6	$0.10 \pm 0.04$
Control <sup>*</sup>		24	4	0.10 ± 0.05
EIP	0.05	24	540	13.50 ± 0.25***
Omite	0.05	4	30	$0.75 \pm 0.12$
		16	38	$0.95 \pm 0.16$
		24	66	$1.65 \pm 0.24^{**}$
	0.1	4	38	$0.95 \pm 0.32$
		16	59	$1.48 \pm 0.31^{*}$
		24	69	1.72 ± 0.24**
	0.2	4	54	1.35 ± 0.18**
		16	87	2.19 ± 0.22***
		24	98	2.45 ± 0.23***
	0.4	4	85	2.12 ± 0.23***
		16	112	2.80 ± 0.23***
		24	125	3.12 ± 0.28***
Control <sup>a</sup>	-	24	23	$0.58 \pm 0.08$
EIP	0.05	24	485	12.12 ± 0.11***
Rovral	0.1	4	45	$1.12 \pm 0.21$
		16	64	$1.60 \pm 0.45$
		24	98	2.45 ± 0.52**
	0.2	4	105	$2.62 \pm 0.38^{**}$
		16	148	3.70 ± 0.50***
		24	213	5.32 ± 0.45***
	0.4	4	129	3.22 ± 1.26***
		16	177	4.42 ± 1.45***
		24	242	6.05 ± 0.30***
	0.8	4	226	5.65 ± 0.34***
		16	246	6.15 ± 0.28***
		24	288	7.20 ± 0.08***
Control <sup>*</sup>	-	24	28	$0.70\pm0.08$
EIP	0.05	24	604	$15.10 \pm 0.19^{+++}$

4000 cells per fixation time were scored. \*P<0.05; \*\*P<0.01; \*\*\*P<0.001, with respect to negative control.

<sup>a</sup> Negative control.

<sup>b</sup> Positive control (EI).

\*\*\* P< 0.001 with respect to negative control and pesticides.

### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study provides *in vitro* data on terrestrial plants, specifically cell proliferation in terrestrial plants. The two highest test concentrations of Rovral resulted in a complete destruction of the mitotic spindle. The authors conclude that Rovral is capable of inducing numerical chromosomal aberrations and may be considered as a potential aneuploidogen.

**Limitations of Study:** Experimental methods are not very clear. Much of the data gathered are *in vitro* and doesn't translate to any sort of "whole organism" endpoint.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 109022 Huntzinger, C.I., R. R. James, J. Bosch, and W. P. Kemp. 2008. Fungicide Tests on Adult Alfalfa Leafcutting Bees (Hymenoptera: Megachilidae). Journal of Economic Entomology 101(4), 1088-1094

# Purpose of Review (DP Barcode or Litigation): Litigation

# Date of Review: 9-16-09

**Summary of Study Findings:** The study examines the effects of iprodione and other fungicides on adult leafcutter bee (*Megachile rotundata*) via three different exposure methods. The study finds that contact and oral dosing reduce bee survival while topical exposure does not.

Iprodione used in the experiment was in the formulated product Rovral<sup>®</sup> 50 WP (50% a.i.).

Bees used in the study were purchased from a commercial supplier as nesting cells. They were stored at  $4^{\circ}$ C until they were needed. Cells were then placed in a wooden emergence box inside an incubator at 29° C. Bees were incubated in the dark. After 19 days of incubation, a light was placed at the end of an exit tube to draw bees into a receiving container. Bees emerging in the first 24hr were separated by sex and randomly divided into groups of 10. Bees used for contact and topical tests were moved into feeding containers made from paper ice cream cups, Petri dishes and a feeding tube. They were fed a 1:1 sucrose:water solution. Treatment for the orally dosed bees was the same except the solution they were fed contained a Rovral concentration of 5, 30, 50 or 100 g a.i./liter. The water used in all of the feeding solutions was sterilized using reverse osmosis.

For topical tests, bee groups were held at 25°C for 20 min to reduce activity. One microliter of at either 400 or 600 g a.i./L was applied to the dorsal thorax of each bee. Replicates were performed on different days using a "complete random design." Subjects were then returned to 29°C with a 12:12 photoperiod. According to the methods, 1  $\mu$ L of the fungicide solution (400 or 600 g a.i./L) was applied to the thorax; therefore 0.4 or 0.6 mg was applied.

In contact tests, a piece of Whatman No.1 filter paper was trimmed to completely cover the bottom of the bees' enclosure. One ml of solution containing 0.3, 3.0 or 30 g a.i./L was applied evenly over the filter paper and allowed to dry for 2 hrs before bees were allowed contact with it. As such, 0.3, 3 or 30 mg a.i. was applied to each filter paper Mortality was assessed every 24 hrs.

Bees tested for oral toxicity were not fed prior to exposure to the sucrose solution described above. Bees (10 in each treatment group) were exposed to 5, 30, 50 or  $100 \,\mu\text{g/}\mu\text{L}$ .

Each exposure method had accompanying control groups. One control received no treatment of any kind, while the other received a treatment similar to the exposed bees but without any fungicide. In the case of the orally-treated bees, this meant that the former group was not fed at all. This group was compared to the treated groups to see if there may have been an aversion to food resulting from treatment.

Each dose of each treatment was run at least twice on different days with new batches of solution and new bee cohorts to achieve more reliable statistics.

Survivorship was analyzed using the Proc Life Test with the mortality rate of each bee included in the analysis. Survivorship curves were compared to those of corresponding water controls using the Kaplan-Meier method.

No significant effect was seen in male or female bees exposed topically (thoracic application) to iprodione when compared to the water control to 0.4 or 0.6 mg/bee (**Figure 4**) over the 20-day study period. Based on Figure 4 though, there appears to be a differential response between males (**Figure 4A**) and females (**Figure 4B**); it is unclear why no results are provided for females in the highest (0.6 mg/bee) treatment. Additionally, control survival among males declined to roughly 60% by day 5 and suggests that study conditions were not ideal.



Contact treatment showed a significant reduction in survival in males at 30 mg a.i ( $\chi^2$ =5.48.27, df=1, P<0.0192) (**Figure 5**) over the 20-day study period. The Chi-squared value presented here is identical to that listed in the report, and is assumed to be a typographical error. However, control survival declined throughout the study period and suggests that study conditions were not ideal.



In oral toxicity testing, there was a significant difference in the lowest dose given to males and females. Males were minimally dosed at 5.0  $\mu$ g a.i./ $\mu$ L while females were minimally dosed at

3.0 or 30 µg a.i./µL according to the report. The report contradicts itself with regard to the female dosage since the methods section did not indicate that the females were provided a different dose than the males. Also, the doses for females discussed in the result text do not agree with what is depicted in **Figure 9**. Regardless, the lowest dose resulted in significant reductions in survival rates ( $\chi^2$ = 41.0793, df=1, P< 0.0001 for males and  $\chi^2$ =12.3992, df=1, P<0.0004 for females). All higher dosages similarly reduced survival rates (**Figures 9A and 9B**). Although not discussed in the study, there appears to be a differential sensitivity between male and female bees.



Fig. 9. Effect of Rovral (g [AI]/liter sucrose solution) fed to adult *M. rotundata* on percent survival in males (A) and females (B).

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative.

**Rationale for Use:** The study provides qualitative information that bees exposed orally to concentrations as low as 5.0  $\mu$ g a.i./ $\mu$ L exhibit statistically significant mortality relative to controls over a 20-day exposure period.

**Limitations of Study:** Of the different methods of application tested, only the oral toxicity test exhibited reasonable control mortality. There is uncertainty regarding actual treatment levels, since the exposure concentrations discussed in the results section for female bees differ from that described in the methods.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 109595 St. Clair, S. B., and J. P. Lynch. 2005. Base cation stimulation of mycorrhization and photosynthesis of sugar maple on acid soils are coupled by foliar nutrient dynamics. New Phytologist 165, 581-590.

### Purpose of Review (DP Barcode or Litigation): Litigation

### Date of Review: 9-21-09

**Summary of Study Findings:** The study examines the effects of iprodione on mycorrhizal associations with sugar maple (*Acer saccharum*) and then correlates that association with photosynthetic production.

Iprodione used in this experiment came from the formulated product Rovral. The exact formulation is not given. It was applied to test plants at a rate of  $2.0 \text{ g/m}^2$ . The chemical was dissolved in 500 ml deionized water and applied at 5-wk intervals.

Second year bare-root sugar and red maple (*Acer saccharum* and *Acer subrum*) seedlings from Penn Nursery, Spring Hills, PA were used for the study. They were individually grown in 11.37 L plastic pots filled with forest soil collected from Susquehannock State Forest in Potter County, PA. Plants were raised in a greenhouse at  $(40^{\circ}49^{\circ}N, 77^{\circ}49^{\circ}W)$  in the following conditions: daytime temperature  $28\pm5^{\circ}$ C, relative humidity  $62\pm6\%$ . They were watered two-three times weekly using an automated drip of approximately 500ml deionized water.

Two experimental sections were run in successive years. The first year, experiments tested the effects of different soil conditions. The soil conditions tested were soil, soils + Calcium and soil + Calcium and Magnesium. For the Ca treatment, laboratory grade CaO was mixed into the soils. The Ca+Mg treatment was created by placing coarse limestone (53.5% CaCO<sub>3</sub>, 42% MgCO<sub>3</sub>) on the surface of soil that had already been treated with CaO. The second years' trees were grown in soil prepared in the same manner, but were treated with iprodione as described above. The effects of varied conditions was measured by observing photosynthetic properties, leaf nutrition and arbuscular mycorrhizal colonization. The plants were allowed to grow from May 11-September 5, 2002 and June 7- September 3, 2003.

For foliar element analysis, leaves collected from trees were dried at  $65^{\circ}$ C for 72 hours and then pulverized using glass beads and a shaker. They were then ashed at  $550^{\circ}$ C and dissolved in a solution. The results for treated and non-treated years can be seen below in **Tables 2** and **3**. Iprodione treatment significantly (p<0.05) affected calcium uptake; fungicide-treated seedlings accumulated less foliar Ca than control seeds (**Table 3**).

Table 2 Mean foliar element concentrations of sugar maple (Acer saccharum) and red maple (Acer rubrum) seedlings grown on an acidic, nonglaciated forest soil (soil) collected at SSF#1 (Kolb & McCorrnick, 1993) amended with base cations (soil + Ca or soil + Ca + Mg) during the summer of 2002

	Ν	Р	К	Ca	Mg	Mn	Fe	Cu	Zn	AI
Sugar maple										
Soil	17720±417	1840 ± 305	4160 ± 420	12080 ± 491a	840 ± 67a	6277 ± 298a	104 ± 13a	$5.0 \pm 0.95$	26.0 ± 2.9a	61.8 ± 8.2a
Soil + Ca	17720 ± 2037	1820 ± 193	4380 ± 378	16560 ± 1896b	1100 ± 202a	2766 ± 788b	56.6±14b	4.8±0.74	17.2 ± 2.2b	19.2 ± 1.4b
Soil + Ca + Mg	16300 ± 561	2400 ± 216	3480 ± 213	16240 ± 1113b	2600 ± 291b	954 ± 166c	49.6±7b	3.8±0.66	14.0 ± 1.7b	14.2 ± 0.74b
Red maple										
Soil	14220 ± 660a	$1040 \pm 51a$	4160 ± 222 a	10360 ± 1037a	1100 ± 84a	4802 ± 745a	39.4 ± 4.5a	3.8±0.66	57.4 ± 7.1a	28.4 ± 4.2a
Soil + Ca	11740 ± 850b	1240 ± 233a	3240 ± 191 b	14620 ± 1669ab	1380 ± 208a	1139 ± 158b	28.6 ± 3.0ab	6.4 ± 2.0	28.4 ± 5.5b	8.6 ± 0.82b
Soil + Ca + Mg	11425 ± 526b	1900 ± 280b	3125 ± 298b	16125 ± 1492b	4225 ± 394b	674 ± 126b	25.7 ± 3.6b	6.3 ± 1.0	$24.0\pm5.0b$	7.0 ± 1.2b

Foliar element concentrations are µg g<sup>-1</sup> d. wt.

Data are means  $\pm$  1 SE (*n* = 5). Different superscript letters in each column category indicate statistically significant differences among the treatments at the  $\alpha \le 0.05$  level.

Table 3 Mean foliar element concentrations of sugar maple (Acer saccharum) seedlings grown on an acidic, nonglaciated forest soil (soil) collected at SSF#1 (Kolb & McCormick, 1993) as influenced by CaO and fungicide treatments in the 2003 experiment

	Ν	Р	K	Ca	Mg	Mn	Fe	Cu	Zn	AI
Soil Soil + funøicide	18240 ± 810 19980 ± 790	2960 ± 390 1950 ± 288	4860 ± 295 4933 ± 287	10840 ± 684a 8316 ± 818b	1209 ± 123 1116 ± 162	6532 ± 385 5246 ± 725	89 ± 12 144 ± 34	7.6±1.6 7.0±1.6	44 ± 4.0a 46 ± 4.8a	108 ± 6.5a 95 ± 9.6a
Soil + Ca Soil + Ca + fungicide	19580 ± 1580 18700 ± 960	3060 ± 666 3057 ± 557	4520 ± 220 4385 ± 290	20480 ± 1586c 17471 ± 1125c	1140 ± 81 1300 ± 97	5072 ± 788 4583 ± 632	83 ± 25 121 ± 29	5.2 ±0.97 5.0 ± 0.84	21 ±1.1b 19 ±0.85b	33 ± 3.4b 31 ± 1.6b

Foliar element concentrations are µg g<sup>-1</sup> d. wt.

Data are means  $\pm 1$  SE (n = 5). Different superscript letters in each column category indicate statistically significant differences among the treatments at the  $\alpha \le 0.05$  level.

Following all other experiments, the roots of seedlings were removed from the soil and rinsed. Feeder roots were collected from the upper, mid and lower portions of the root system. The selected roots were then boiled in 10% KOH for 15 minutes and rinsed with deionized water. They were then placed in a 5% bleach solution for two minutes and rinsed again with deionized water. Next the roots were placed in a 5% HCl solution for one minute and stained in a Trypan blue solution. These processes were carried out to aid in indentifying and counting mycorrhizal colonization. **Tables 4** and **5** show the colonization data from the treated and untreated experiment portions. Iprodione was found to significantly reduce (P=0.03) arbuscular mycorrhizal colonization.

Gas exchange and chlorophyll fluorescence (photosynthetic measurements) were made using a Li-Cor 6400 gas exchange system and a 6400-40 leaf chamber fluorometer. A light intensity of 1500  $\mu$ mol photons/m<sup>2</sup>/s was used for the leaf chamber. Measurements were taken at ambient temperature and humidity. Leaves were sealed in the 2 cm<sup>2</sup> proximally to the major sinus to avoid the primary veins on either side. Electron transport rate was determined using the following equation:

$$ETR = \Phi_{PSII} \times PFDa \times 0.5$$

Carbon dioxide exchange rates were taken but their methods are not disclosed. It was found that fungicide treatment decreased both CER and ETR in Ca amended seedlings, but did not do so in control seedlings.

The study then attempts to draw connections between the three observed characteristics, but cannot establish any linear relationships.

The study uses ANOVA and Fischer's Protected Least Significant Difference tests to find significance.

Table 4Coefficients of determination indicating the relationshipbetween foliar nutrition with arbuscular mycorrhiza (AM) rootcolonization,  $CO_2$  exchange rate (CER) and electron transport rate(ETR) of sugar maple (Acer saccharum) and red maple (Acer rubrum)seedlings in the 2002 experiment

	AM colonization	CER	ETR
Sugar maple			
N	< 0.01	0.03	0.25
Р	0.33*	+0.39*	0.05
К	0.06	< 0.01	0.16
Ca	+0.31*	0.06	0.05
Mg	+0.32*	+0.34*	0.02
Mn	-0.58***	-0.38*	0.09
Fe	-0.55**	0.11	0.08
AI	-0.70***	-0.42*	0.15
Red maple			
N	0.10	< 0.01	0.08
Ρ	0.15	0.03	< 0.01
К	0.12	< 0.01	0.09
Ca	0.20	0.08	< 0.01
Mg	+0.36*	0.03	< 0.01
Mn	-0.34*	0.06	< 0.01
Fe	0.24	0.13	0.01
AI	-0.30*	0.09	0.02

n= 14. Significance designated as: \*P  $\leq$  0.05, \*\*P  $\leq$  0.01,

 $***P \le 0.001.$ 

Table 5 Coefficients of determination showing the relationship between foliar nutrition and arbuscular mycorrhiza (AM) root colonization, CO<sub>2</sub> exchange rate (CER) and electron transport rate (ETR) in sugar maple seedlings, as influenced by fungicide treatments in the 2003 experiment

	AM colonization	AM colonization		CER		
	–Fungicide	+Fungicide	–Fungicide	+Fungicide	–Fungicide	+Fungicide
N	0.04	0.04	0.11	0.06	0.03	0.19
Р	0.02	0.08	0.14	0.03	< 0.01	0.08
К	0.20	0.14	< 0.01	0.06	0.02	< 0.01
Ca	0.28	< 0.01	0.15	< 0.01	+0.63**	< 0.01
Mg	0.08	0.10	0.02	0.18	0.15	0.06
Mn	0.24	0.04	-0.47*	< 0.01	0.18	0.08
Fe	0.04	0.09	0.07	< 0.01	< 0.01	< 0.01
AI	-0.53*	0.08	-0.56*	< 0.01	-0.68**	0.19

n = 10. Significance designated as: \* $P \le 0.05$ , \*\* $P \le 0.01$ .

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative.

**Rationale for Use:** Shows a link between fungicides and electron transport rates in plants; calcium was the only element [monitored in the study] to be significantly (p<0.05) affected by iprodione treatment with iprodione-treated seedlings accumulating less foliar calcium than controls.

**Limitations of Study:** The percent a.i applied is not disclosed and as such, exposure cannot be gauged. Additionally, the total number of applications is not stated in the study. The study also relied on differing lengths of growing season and only a single treatment level was used. The study has less to do with fungicides and is more focused on soil nutrients and their effect on mycorrhiza root colonization.

### Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 109597 Goettel, M.S., K. W. Richards, and B. G. Schaalje. 1991. Bioassay of selected fungicides for control of chalkbrood in alfalfa leafcutter bees, *Megachile rotundata*. Apidologie. 22. p 509-522.

### Purpose of Review (DP Barcode or Litigation): Litigation

### Date of Review: 7-27-09

**Summary of Study Findings:** The study examines the effects of prophylactic fungicide (Rovral 50 WP) application in leafcutter bees (*Megachile rotundata*). The fungicide was incorporated into the natural provisions of the bee larvae and the effects of the fungicide on growth, mortality and the incidence of chalkbrood were determined.

Eggs were obtained from a wild population by manually extracting them from their cells within a hive. *Ascosphaera aggregate* spores were obtained by harvest from a Canadian hive known to have been infected by the fungus. After collection, the spores were then passed through a fine plastic screen to remove excess material, sealed in a test tube, and refrigerated at 4° C until they were needed.

Spore viability was determined by placing approximately one-million spores in 0.1 ml Sabouraud dextrose broth with 2% yeast extract, streptomycin (50  $\mu$ g/ml) and penicillin (25 IU/ml) in a 16 ml sterile test tube. The tube was then filled with CO<sub>2</sub> and incubated at 30°C for 24 h. Germination rates were determined to be greater than 90%. Spore inoculum was prepared in a buffered isotonic saline solution just before it was used. Spore counts were set at one million spores per 2  $\mu$ l using an improved Neubauer hemocytometer.

The fungicides tested in this study were Benlate 50 WP, Rovral 50 WP (50% iprodione), Ascodidin, DFMO, and a 20% enilconazole solution. All of the fungicides but enilconazole were diluted in sterile distilled water. Enilconazole was diluted in 70% enthanol. The fungicide solutions were prepared so that a 2  $\mu$ l portion would create concentrations in each treatment ranging from 1 to1000 covering each order of magnitude.

The fungicide solutions were applied by injecting 2 ul directly beneath the surface of the provision on the side of the egg. Each fungicide/dose combination was applied to 50 cells/treatment. The controls were dosed with either water, 70% ethanol, or nothing. Cells were kept in three groups of 25 per 96 cell microtitration plate. Empty cells in the plate were filled with water to create an adequately humid environment.

After incubating at  $30^{\circ}$  C for 24 h in continuous light, the eggs were exposed to the spore solution. It was applied in the same manner as the fungicide, but was placed on the opposite side of the egg as the fungicide. Only half of the eggs received the spore solution while the other half received buffered isotonic saline solution without any spores.

The cells were then placed back in the incubator and observed daily. Life stages described by Whitfield *et al* were used to determine age if deaths occurred and eggs not enclosing within 72 hours were considered to be dead. Four weeks after the last larva had finished spinning its

cocoon all specimens were stored at 4°C. Each cell was cut open and its contents were weighed and examined.

Four trials of this experiment were conducted. Weighted ANOVA and contrasts of treatments and their controls were performed on untransformed, arcsine and logistic transformed percent mortality. Mortality was divided into four categories (sporulating chalkboard, part-sporulating chalkboard, non-sporulating chalkboard, and non-chalkboard). The mortality for the control groups is displayed below.

Diagnosis	Challenged <sup>b</sup>			Not challenged		
	BISS °	+Water d	+Ethanol *	BISS	+Water	+Ethanol
Chalkbrood						
Sporulating	$34 \pm 8.4$	26 ± 11.7	28 ± 8.1	0	1 ± 1.2	1 ± 1.6
Part-sporulating	3±1.1	3± 1.9	1 ± 1.1	1 ± 1.1	0	1 ± 1.0
Non-sporulating	6±1.2	9± 1.9	7 ± 4.3	$2 \pm 2.2$	$5 \pm 1.9$	3±1.7
Total	$43 \pm 7.1$	38± 6.5	$36 \pm 6.5$	$3 \pm 2.1$	$6 \pm 3.0$	6 ± 2.6
Non-chalkbrood	8±2.8	9± 1.9	17 ± 4.8	7 ± 2.7	$10 \pm 3.6$	$10 \pm 2.2$
Total	$51 \pm 5.9$	47 ± 10.4	53 ± 9.3	$10 \pm 2.1$	$16 \pm 5.8$	$16 \pm 2.8$

Table I. Percent mortality of <i>Megachile rotundata</i> larvae used as controls
--

<sup>a</sup> Values are means  $\pm$  SE of 4 replicates each consisting of 25 field-collected eggs reared at 30 °C each in their own cell and provisions. <sup>b</sup> Challenged with 1 x 10<sup>6</sup> ascospores of *Ascosphaera aggregata* in 2 µl buffered isotonic salt solution (BISS) per cell 24 h after field collection. <sup>c</sup> Treated with 2 µl BISS per cell 24 h after field collection. <sup>d</sup> Treated with 2 µl BISS for 24 h thereafter. <sup>e</sup> Treated with 2 µl 70% ethanol per cell on day of collection followed by 2 µl BISS for 24 h thereafter.

Neither the addition of alcohol nor the addition of water had an effect on mortality levels. Individuals challenged by spores showed a much higher mortality rate than those not challenged. Likewise, the presence of alcohol or water did not affect growth of the larvae. Growth data can be seen below. However, according to **Table II**, percent completed cocoons and mean days to death were significantly different (p<0.05) in unchallenged nonchalkbrood controls treated with water relative to those using buffered isotonic salt solution (BISS) used to prepare the spores or ethanol. Ethanol was used to prepare enilconazole. None of the brood treated with BISS completed cocoon while 37% of the water controls completed cocoons. Non-chalkbrood brood that were unchallenged also took significantly less time to die in the water treatment as opposed to BISS or ethanol controls. As such, there was a significant solvent effect.

Parameter		Challenged <sup>b</sup>		N	ot challenged	
	BISS °	+Water <sup>d</sup>	+Ethanol º	BISS	+Water	+Ethanol
%Defecated						
Chalkbrood	100	90±10	100	100	100	100
Non-chalkbrood	67 ± 33.3	58±14.4	37 ± 12	44 ± 29.4	$50 \pm 28.9$	$33 \pm 23.6$
% Completed coco	oon					
Chalkbrood	34 ± 9.6	39 ± 15.3	45 ± 12.7	$50 \pm 50.0$	78 ± 22.2	$50 \pm 28.9$
Non-chalkbrood	33 ± 33.3	12 ± 12.5	25 ± 14.4	0	37 ± 23.9*	$25 \pm 25.0$
Mean days to defe	cation					
Live	$5,4 \pm 0.37$	$5.5 \pm 0.61$	$5.6 \pm 0.56$	5.6 ± 0.37	5.6 ± 0.34	$5.7 \pm 0.51$
Dead	$5.2 \pm 0.28$	5.8 ± 0.32**	$5.6 \pm 0.54$	$5.6 \pm 0.29$	$5.5 \pm 0.48$	6.1 ± 0.48
Mean days to coco	oon completi	ion				
Live	$10.0 \pm 0.65$	10.8 ± 0.32	$10.9 \pm 0.56$	10.7 ± 0.78	$10.8 \pm 0.80$	11.2 ± 0.96
Dead	10.8 ± 0.96	$10.8 \pm 1.08$	$11.8 \pm 1.04$	13.0	$10.4 \pm 1.15$	11.7 ± 0.88
Mean days to deat	th					
Chalkbrood	9.1 ± 0.28	$8.4 \pm 0.57$	$8.3 \pm 0.50$	14.0	11.5	$13.5 \pm 0.50$
Non-chalkbrood	9.7 ± 1.75	7.1 ± 0.83	$6.5 \pm 0.29$	12.1 ± 3.47	9.0 ± 2.52*	9.3 ± 1.59
Mean weight (mg)						
Live prepupae	$36 \pm 2.6$	38 ± 4.2	39 ± 2.9	$38 \pm 4.9$	$37 \pm 4.7$	$38 \pm 4.6$
Chalkbrood cada	avers					
Sporulating	13± 1.5	12± 1.7	14 ± 2.1	-	18	16
Non-sporulating	15± 1.5	13± 1.8	21 ± 0.4	11	15 ± 4.9	7 ± 3.9

Table II. Growth parameters of Megachile rotundata larvae used as controls a.

<sup>a</sup> Values are means ± SE of 4 replicates each consisting of 25 field-collected eggs reared at 30 °C each in their own cell and provisions. <sup>b</sup> Challenged with 1 x 10<sup>6</sup> ascospores of *Ascosphaera aggregata* in 2 µl buffered isotonic sall solution (BISS) per cell 24 h after field collection. <sup>c</sup> Treated with 2 µl BISS per cell 24 h after field collection. <sup>d</sup> Treated with 2 µl sterile distilled water per cell on day of collection followed by 2 µl BISS for 24 h thereafter. <sup>e</sup> Treated with 2 µl 70% ethanol per cell on day of collection followed by 2 µl BISS for 24 h thereafter. <sup>\*</sup> Difference from BISS,  $P \le 0.05$ . <sup>\*\*</sup> Difference from BISS,  $P \le 0.01$ .

The effects of Rovral 50 WP on non-challenged individuals were not significant at levels below 1000 ppm. There was a significant difference (P $\leq$ 0.01) in mortality at 1000 ppm. There was also a significant difference in development time to defecation (P $\leq$ 0.01), cocoon completion (P $\leq$ 0.01) and increased non-chalkbrood mortality prior to both of those markers (P $\leq$ 0.05). **Figure 1** below summarizes non-challenged mortality in the four different trials.



Fig 1. Effects of fungicide treatments on mortality of *Megachile rotundata* larvae reared individually their own cell and provisions at 30 °C. Means based on 4 replicates each consisting of 25 fiel collected eggs at each fungicide concentration. Statistics based on ANOVA comparing mortalities b tween treatments and controls (\*  $P \le 0.05$ ; \*\*  $P \le 0.01$ ). Challenged larvae were inoculated with 1 10<sup>6</sup> ascospores of *Ascosphaera aggregata*. a), Non-chalkbrood mortality of non-challenged larva (controls). b) Chalkbrood mortality of challenged larvae. c) Non-chalkbrood mortality of challenged larvae.

In cases of challenged individuals, Rovral reduced the effects of the fungus more so at 1000ppm than at 100ppm. The non-chalkbrood mortality in the challenged group increased only at the 1000ppm dose level. It should be noted the anti-fungal effects of Rovral were negated by its toxicity, and it exhibited no significant effect on total mortality. Rovral dosing at 100 ppm lead to an increase (P $\leq$ 0.05) in deaths after cocoon completion. Dosing at 10 ppm produced a significantly (P $\leq$ 0.05) longer time to until death in individuals succumbing to chalkbrood as well as an increase (P $\leq$ 005) in the mean weight of sporulated cadavers.

When challenged and non-challenged control groups were compared the control groups showed a significantly ( $P \le 0.01$ ) higher mortality rate in the challenged group, but no difference in non-chalkbrood mortality between the two groups. A comparison of challenged and non-challenged groups dosed at 1000 ppm revealed no significant difference in non-chalkbrood mortality.

# Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** Under the conditions tested, the exposure of developing larvae to Rovral 50 WP resulted in significantly (p<0.01) increased mortality at time of defication and at cocoon completion, prolonged development time to defication relative to untreated controls; based on mortality and developmental effects, the NOAEC is 100 ppm and the LOAEC is 1000 ppm.

**Limitations of Study:** The subjects of the study were initially obtained from a field population. Also, the endpoints of all chemicals and concentrations on each group are not made available. Additionally, the % a.i. for the chemical fungicides is not stated.

### Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 110209 Enwistle, A.R., P. A. Brocklehurst, and T. G. Jones. 1981. The effect of iprodione on seed germination and seedling emergence in onion. Association of Applied Biologists 97, 175-181.

# Purpose of Review (DP Barcode or Litigation): Litigation

# **Date of Review:** 10-02-09

**Summary of Study Findings:** This study examined the effects of iprodione on the germination of salad onion (*Allium fistulosum*) seeds. Iprodione dosing was achieved using the formulated product Rovral<sup>®</sup> (50% WP). Germination was tested in "lab" conditions (moistened filter paper) as well as "field" conditions (from compost). Under lab conditions, the study found that at 100 g a.i./kg seed, iproidone had no effect on germination time, but did reduce final germination success by 7-24%. Despite the study's claim that there was no effect on germination time, a metric called t<sub>50</sub> was prolonged by exposure at the rate above. The t<sub>50</sub> metric is defined as " time to 50% final germination" and is not explained any further. Iprodione exposure also resulted in a small, inconsistent increase in the number of abnormal seedlings. Varied storage conditions did not affect a change in seed response to iprodione exposure. When tested at 50 g a.i./kg seed, there was no effect on final germination and less of an effect on t<sub>50</sub>. Similar results were seen when testing emergence from compost. Changes in compost moisture and temperature did not cause changes in seed response to iprodione exposure. LOAEC for this experiment was 50 g a.i./kg seed, *i.e.*, the lowest treatment rate and the NOAEC was not found.

Each germination test was carried out using eight replicates of 50 seeds according to international Seed Testing Association rules (1976). Seeds were placed 1 cm apart on Whatman grade 181 filter paper in 9-cm Petri dishes or in peat-based Levington Universal compost in 21x36 cm trays. Both media were adjusted to a standard moisture content.

Seeds were stored in varied conditions, designated "good" or "bad." Good conditions were 17°C and 50% relative humidity while bad conditions were 20°C and 80% relative humidity. Germination and emergence were measured at 20°C and 60% compost moisture. Records were recorded at least three times a week until emergence ceased.

In another portion of the experiment, seed bed conditions were varied. While temperature was kept constant at 20°C, moisture content was set at 40, 55, or 70%. Moisture content was then kept at 60% while temperature was set at 5, 10, 15, 20, 25, or 30° C. Emergence was recorded daily until it ceased.

# Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study provides qualitative information on the effects of iprodione seed treatment on plants. According to the study, at 100 g/kg seed treatment level, iprodione did not affect the time at which seeds started to germinate but caused a 7 - 24% reduction in final germination and a small but inconsistent increase in the number of abnormal seedlings. Iprodione seed treatment consistent increased time to 50% generation by up to roughly 3 days.

Iprodione had no effect on the time at which seedlings started to emerge but there was a significant albeit inconsistent increase in the final percentage.

**Limitations of Study:** The study does not establish a NOAEC nor does it establish a consistent dose response for any of the variables measured. The figures presented in the study are difficult to interpret but suggest that iprodione plants in generall fair less well than untreated plants; however, these differences do not appear to be statistically significant.

### Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 110356 Yi, W. L., S.E. Law and H. Y. Wetzstein. 2003 Pollen tube growth in styles of apple and almond flowers after spraying with pesticides. Journal of Horticultural Science & Biotechnology 78 (6), 842-846.

### Purpose of Review (DP Barcode or Litigation): Litigation

### Date of Review: 10-5-09

Summary of Study Findings: This study examined the effects of various fungicides on the flower structures of fruit trees. Of interest are the observations of iprodione on the stigmas, pollen germination and tube growth of almond (*Prunis dulcis*) flowers. Iprodione was applied in the formulated product Rovral<sup>®</sup> (% a.i. not specified) at a rate equivalent to 1.12kg/ha using a laboratory apparatus (Figure 1). There were no significant effects seen on pollen tube number or on maximum tube length (Table 2), which were the only measurements taken. Statistics were calculated using GLM and Duncan's multiple range test at  $\alpha$ =0.05.

Almond studies were conducted in the Spring of 2001. Nonpareil almond budwood was shipped in chilled coolers from a Paramount Faming Company in Bakersfield, CA. Shoots were re-cut upon arrival at the lab and held at 7°C with their bases in water. Buds were emasculated before anther dehiscence. They were then evaluated for normalcy of stigma development and transferred to multi-well tissue culture plates containing tap water. Just prior to spraying, each bud was attached to an arc-shaped tray (Figure 1). The lab apparatus was calibrated to simulate a spray volume of 935 L/ ha from a tractor moving 3.2 km/hr. The buds were spaced to simulate orchard rows 7.3-meters apart. For this portion of the experiment, control buds were sprayed with tap water. Twenty-four hours after the chemical application, each stigma was pollinated by hand with the aid of a small brush and dissecting microscope. Pollination was examined using an SEM to verify uniformity. Another 24 hours after pollination, pistils were removed from flowers and fixed in a 3:1 ethanol/acetic acid solution. Tissues were softened and cleared by autoclaving at 121 °C for 20 min. in a 1% sodium sulfite solution and stained with aniline blue for at least four hours. Samples were then examined using a standard microscope. The number and growth extent of pollen tubes were observed and measured. No statistical differences were found between treatment groups and control groups.



F1G. 1

Laboratory apparatus for spray applications to simulate field-sprayer conditions. A conventional hydraulic-atomizing nozzle at operational pressure commonly used for pesticide applications provided the appropriate droplet-size spectrum, volumetric flow rate, and activeingredient concentration for each pesticide. An electronically controlled robotic arm swept the spray nozzle at controlled speeds past test flowers positioned around a circular-arc holder.

 TABLE II

 Fungicide effects on pollen germination and tube growth in almond flowers

Fungicides	No. of tubes <sup>z</sup>	Maximum tube length <sup>y</sup> (% of style length)
Azoxystrobin	14a <sup>x</sup>	33a
Myclobutanil	10a	26a
Iprodione	10a	29a
Ĉyprodinil	10a	28a
Water control	13a	33a

<sup>2</sup>Number of tubes that penetrated the stigma and grew into style. <sup>9</sup>Max. tube length expressed as the percent of the whole length of the style.

\*Values in columns followed by the same letter are not significantly different at  $\alpha = 0.05$  according to Duncan's multiple range test. Values are means of 45 observations.

All tables and figures are reproduced from the cited literature.

### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** This study provides qualitative information that at laboratory treatment rates of 1.2 g/L intended to represent a field application rate of 1.12 kg/ha to almond buds, iprodione had no significant (p>0.05) effect on pollen tube number or on maximum tube length.

**Limitations of Study:** The % a.i. of the active ingredient is never disclosed. Only one application rate is tested, and no thresholds were established. The chemical treatment background of the test flowers is not detailed.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 110872 Jeffers, S.N. 1989. The cottonball disease of cranberry in Wisconsin; potential for disease management with fungicides. Acta Horticulutrae 241, 318-323.

# Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 10-05-09

**Summary of Study Findings:** The study examines the effectiveness of several fungicides, including iprodione, at controlling cottonball disease (*Monilinia oxycocci*) in cranberry plants. It does not examine the effects of iprodione on anything but the presence and effects of *M. oxycocci*. Even in this aspect, the study finds iprodione is not significantly effective.

# **Description of Use in Document (QUAL, QUAN, INV):**

Rationale for Use: None.

**Limitations of Study:** Study examines the effects of iprodione on its intended target (a fungus) and does not consider side effects or any other characteristic pertinent to ecological risk.

### Chemical Name: Iprodione

#### CAS No: 109801

**ECOTOX Record Number and Citation: 110873** West, H.M., A. H. Fitter, and A.R. Watkinson. 1993. The influence of three biocides on the fungal associates of the roots of *Vulpia ciliata* spp. *ambigua* under natural conditions. Journal of Ecology 81, 345-350.

### Purpose of Review (DP Barcode or Litigation): Litigation

### Date of Review: 10-06-09

**Summary of Study Findings:** The study focused on the effects of various fungicides, including iprodione, on mycorrhizal colonization in the roots of winter annual grass *Vulpia ciliata* ssp. *ambigua*. Iprodione was applied to plants using the formulated product Rovral<sup>®</sup> at a rate of 0.6 g/m<sup>2</sup>. The percent a.i. of the application is not stated. The study found that iprodione did not have any effect on shoot dry matter production or plant fecundity when calculated outright. However, when the effects of infections were removed by using an analysis of covariance it was revealed that iprodione caused a reduction in shoot dry mass. Leaf and flower mass were not found to be reduced by iprodione exposure (**Figure 2**). Statistics for the experiment subjected to an analysis of variance using appropriate transformations. Percentage mycorrhizal infection was used as a covariate in some cases. Although full statistical methods and data are not given, it appears that no significance thresholds were varied.

Three experimental setups were used for this study. Two took place in the field, and one in a glasshouse. The field trials were conducted at different locations in the UK. Plots containing *V*. *ciliata* were randomly selected for both sites. Plots were treated with the appropriate biocide starting November 6/7 and in 35 day intervals thereafter until May 8/9. Plants were sampled just before flowering and at seed set. A group of plants was removed from each site in January and again in May to analyze root colonization. Sand collected from one of these field sites (not specified) was autoclaved for use in the glasshouse portion of the experiment. A portion of the collected sand was not autoclaved, and was also used for the glasshouse experiment. *V. ciliata* seeds collected from an undisclosed location "the previous Summer" were sown into 50-mm pots of sand at 6 seeds per pot. Later, they were thinned to one plant per pot. Roots were assessed for infection and the observations of them were pooled. Leaves, stems and flowers were also measured, although not at the same time as roots.



Fig 2 Effect of the three fungicides on shoot, leaf and inflorescence (flower) mass of V ciliata ssp ambigua at Santon in May 1991 Values for shoot mass are means adjusted for the effect of mycorrhizal infection density by covariance analysis For each set of data columns differently superscripted differ at P = 0.05

### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** This study primarily focuses on the efficacy of various fungicides in controlling root fungus; however, it measures the effect of iprodione indirectly through an analysis of covariance. The analysis suggests that when the effects of fungal infection are removed, iprodione appears to significantly (p<0.05) affect (reduce) shoot mass and leaf mass.

**Limitations of Study:** The percent a.i. of the formulated product used is not disclosed. It is also not made clear whether the indicated dose measured active ingredient or formulated product. The application method of iprodione is not disclosed. Data from the experiment are never presented in their entirety, and the given graphs are less than clear.

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 110874 Wicks, T. and B. Philp. 1985. Effects of iprodione and vinclozolin seed treatments on germination, emergence and plant growth in onion. Aust. J. Exp. Agric. 25, 465-469.

# Purpose of Review (DP Barcode or Litigation): Litigation

### **Date of Review:** 10-06-09

**Summary of Study Findings:** This study examines the effects of iprodione on the emergence and growth of two onion (*Allium cepa*) cultivars: White Spanish and Goldberg. Iprodione was dosed using an undisclosed commercial formulation stated to be 50% w.p. at 50, 100, 200 and 400 g product/kg seed. All treatment levels resulted in significantly (P<0.05) reduced emergence in both cultivars (**Figure 1**). Hypocotyl and radicle growth of both cultivars were also significantly reduced (**Figure 2**). Treatment significantly reduced field emergence in the Goldberg cultivar at all rates, but did not reduce emergence at any rate in the White Spanish cultivar (**Figure 3**). Plant height was only significantly reduced at the highest concentration (**Figure 4**). Delayed exposure was tested and found iprodione to be more inhibitory when exposure came after a short delay (1-2 days) than when it came after a long delay (4-6 days). The delayed exposure results can be seen in **Table 1**. Germination in organic matter had "extremely variable" results (**Table 2**). Overall, the study found that even at 25 g a.i./kg seed, iprodione exposure can affect the germination of onion seed.

Seeds were treated using multiple methods for this experiment. They default treatment is as follows: seeds were mixed in plastic bottles or bags with an amount of commercial formulation to achieve 50, 100, 200 or 400g product/kg seed. Treated seed was never stored for more than 14 days prior to use.

Eight replicates of 50 seeds were used for germination and growth tests. These tests were carried out on Whatman grade 1 filter paper moistened with 6ml of sterile distilled water and placed in 9 cm Petri dishes. Seeds were incubated at 20°C and germination was recorded after 3, 7, and 10 days. After 10 days, 100 seeds were randomly selected from each treatment and their hypocotyl and radical lengths were measured. Hypocotyl and radical measurements were combined to create an index of growth.

To test the effects of delayed exposure, four replicates of 50 seeds were allowed to incubate in the conditions described above for each day from 1 to 6 days. After their designated incubation period they were transferred to filter paper treated with iprodione at 1 or 10 mg/ml. Control seeds were used for each day and germinated on paper moistened with only water. Germination rates were recorded after 10 days of total incubation at 20°C. One treatment groups was run under water for 6 hours following treatment to test the possibility of leaching.

The effects of organic matter on emergence were tested by sewing seeds in soil of various compositions. The mixtures were as follows: 100% sand, 75% sand/25% peat, 50% sand/50% peat, 25% sand/75% peat, and 100% peat. Fifty seeds that had been dusted at 200 g product/kg seed were planted 1 cm deep in plastic cups containing 200 ml soil mixture. Emergence was

recorded 14 days after sowing. Five replicates were used for each soil mixture and the procedure was duplicated once.



Fig. 1. Effect of vinclozolin and iprodione seed treatments on the percentage germination of onion seed incubated at 20°C. Fungicide rate/kg seed:  $\Box$  untreated,  $\circ$  50 g,  $\bullet$  100 g,  $\blacktriangle$  200 g,  $\bullet$  400 g.



Fig. 2. Effect of vinclozolin and iprodione seed treatments on the 10-day growth of treated onion seed incubated at  $20^{\circ}C$ . Vertical bars show l.s.d. (P = 0.05).  $\Box$  Vinclozolin,  $\blacksquare$  Iprodione.



Fig. 3. Effect of vinclozolin and iprodione seed treatments on field emergence of onions. Vertical bars show l.s.d. (P=0.05).  $\Box$  Vinclozolin,  $\blacksquare$  Iprodione.



Fig. 4. Effect of vinclozolin and iprodione seed treatments on plant height of field onions 70 days after sowing. Vertical bars shown l.s.d. (P = 0.05).  $\Box$  Vinclozolin,  $\blacksquare$  Iprodione.

Table 1. Effect of timing of exposure to iprodione on the germination and growth of White Spanish onion

Days incubation in water before Iprodione	(mg/ml):	1	10	0		0
transferring to	Germin-	Growth	Germin-	Growth	Germin-	Growth
iprodione	ation (%)	(mm)	ation (%)	(mm)	ation (%)	(mm)
Water continuous	~	_	_	_	76	80
Iprodione continuous	49	17	6.0	3		
l day	24	8	0.0			
2 days	32	10	14	4		
4 days	74	24	60	10		
6 days	75	39	72	24		
Incubated with iprodione for						
2 days then washed for 6 h in running tap water before						
germination	36		25		91	

 Table 2. Effect of organic matter on seedling emergence from fungicide-treated onion seed 14 days after sowing

A	and	В	are	different	experiments.
---	-----	---	-----	-----------	--------------

Soil type		Number of	seedlings en	nerged from	250 seeds	
	No fu	ngicide	Iproc	lione	Vinclozolin	
	Α	В	A	В	A	В
100% sand	33	134	98	74	61	57
75% sand, 25% peat	92	63	104	59	85	77
50% sand, 50% peat	72	65	92	69	59	49
25% sand, 75% peat	74	74	49	52	50	40
100% peat	15	67	7	48	10	2.2

#### Description of Use in Document (QUAL, QUAN, INV): Qualitative

Rationale for Use: This study provides qualitative information regarding the effects of iprodione and vinclozolin seed treatments on onion seed germination and growth. At the seed treatment

levels tested, iprodione resulted in significantly (p<0.05) reduced emergence in both cultivars of onions. Hypocotyl and radicle growth of both cultivars were also significantly reduced. Iprodione treatment significantly (p<0.05) reduced field emergence in the Goldberg cultivar at all of the seed treatment rates tested, but did not reduce emergence at any of the treatment rates for the White Spanish cultivar. Plant height was only significantly reduced at the highest concentration.

**Limitations of Study:** Formulation of iprodione used in the study is not specifically stated. Neither statistical methods nor seed background are disclosed. Some of the language in the report could lead to confusion over the dose levels.

### Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 110875 Lo, H., M. A. Valentovic, P. I. Brown, and G. O. Rankin. 1994. Effect of Chemical form, route of administration and vehicle on 3,5-Dichloroaniline-induced Nephrotoxicity in the Fischer 344 Rat. Journal of Applied Toxicology 14(6), 417-422.

# Purpose of Review (DP Barcode or Litigation): Litigation

**Date of Review:** 10-06-09

**Summary of Study Findings:** This study examined the effects of changes in chemical form and dosing method of 3,5-DCA on nephrotoxicity in rats. The study focused on the hydrochloride salt and free base forms of 3,5-DCA. Different administration methods were also examined. These methods were oral (gavage) and interperitonial injection. Further, different carriers were examined. These carriers were 0.9% saline solution, sesame oil, and 25% DMSO in 0.9% saline solution.

For interperitonially-injected doses, rats were administered 0.8 mmol 3,5-DCA/ kg either as a free base or as a hydrochloride salt. Only the hydrochloride salt induced nephrotoxicity (**Tables 1 and 2**). Dosed rats expressed oliguria and proteinuria. They also showed an increase in BUN concentration and decrease in basal and lactate-stimulated PAH accumulation after 48-hours. Kidney weight in hydrochloride-treated rats was reduced after 48-hours and tubular necrosis was found in the cortex of examined kidneys. The freebase form of 3,5-DCA did not significantly alter renal function or morphology.

Orally administered doses were given at 1.5 mmol/kg. The freebase form was delivered dissolved in either sesame oil or 0.9% saline solution. The hydrochloride form was given in the same saline solution. Neither form of 3,5-DCA altered any of the renal function parameters measured by the study or kidney morphology.

Interperitonial injections containing DMSO were fatal with 24 hours to all rats dosed. Oral administration was also lethal.

Table 1.	Effect	of	intraperitoneal	administration	of	3,5-dichloroaniline	(3,5-DCA)	on urin	e volume	and
proteinu	riaª									

		Urine volum	Proteinuria*				
Compound <sup>®</sup>	Group	Day 0	Day 1	Day 2	Day 0	Day 1	Day 2
3,5-DCA-HCI	Control	12.4 ± 0.4	13.5 ± 1.5	5.6 ± 1.1°	+	+	+
	Treated	11.3 ± 2.0	$4.7 \pm 1.0^{e,d}$	3.9 ± 1.1°	+	++	++
3,5-DCA	Control	8.9 ± 1.1	9.2 ± 1.2	$5.8 \pm 0.7^{\circ}$	+	+	+
	Treated	$8.9 \pm 0.3$	9.7 ± 0.9	$5.4 \pm 0.5^{\circ}$	+	+	+

"Values are means  $\pm$  SEM for n = 4-5 rats per group. All treated animals received 0.8 mmol kg<sup>-1</sup> of the test compound, while control animals were administered vehicle only.

<sup>b</sup> Compounds tested were 3,5-dichloroaniline hydrochloride or free base (3,5-DCA ± HCI).

° Significantly different from the day 0 value within a group; p < 0.05.

<sup>d</sup> Significantly different from the appropriate control group value for that day's measurement; p < 0.05.

\* Proteinuria was determined using Multistix test strips. Semiquantitative values are 30 mg dl<sup>-1</sup> (+) and 100 mg dl<sup>-1</sup> (++).

Table 2. Effect of intraperitoneal administration of 3,5-dichloroaniline (3,5-DCA) on blood urea nitrogen (BUN) concentration and organic ion accumulation at 48 h<sup>a</sup>

		BUN concentration (mg dl-1)		Slice-to-medium (S/M) ratio		
Compound <sup>b</sup>	Group	Day 0	Day 2	PAH°	PAH + lactate	TEAª
3,5-DCA·HCI	Control	28 ± 2	20 ± 1	3.0 ± 0.2	7.9 ± 0.6	22.1 ± 0.7
	Treated	25 ± 2	71 ± 15 <sup>e,f</sup>	2.0 ± 0.2 <sup>f</sup>	4.2 ± 0.6'	22.7 ± 1.4
3,5-DCA	Control	18 ± 1	18 ± 1	4.7 ± 0.3	9.3 ± 1.1	20.2 ± 1.0
	Treated	19 ± 2	17 ± 2	4.4 ± 0.2	8.3 ± 0.7	17.8 ± 1.2

\* Values are means  $\pm$  SEM for n = 4-5 rats per group. All treated animals received 0.8 mmol kg<sup>-1</sup> of the test compound, while control animals were administered vehicle only.

<sup>b</sup> Compounds tested were 3,5-dichloroaniline hydrochloride or free base (3,5-DCA ± HCI).

<sup>c</sup> PAH = *p*-aminohippurate.

<sup>d</sup> TEA = tetraethylammonium.

\* Significantly different from the day 0 value within a group; p < 0.05.

<sup>1</sup> Significantly different from the appropriate control group value for that day's measurement; p < 0.05.

Male Fischer 344 rats between 200 and 300 g were obtained from Hilltop Lab Animals in Scottsdale, PA. Test animals were allowed at least one week to acclimate to lab conditions. Following the initial acclimatization, rats were moved to individual metabolism cages and allowed an additional day of acclimatization. Animals were kept at 21-23°C, 50-55% humidity and 6:18 L:D. Base metabolic conditions and renal function were measured on Day 0, before the treatments began. Animals were dosed with the chemicals and concentrations listed above.

Renal function was monitored at 24 and 48 h by measuring urine samples for protein, glucose, ketones and blood. Body weight, food intake and water intake were also measured at the same intervals. The rats were killed at 48 h. Left kidneys were removed and chemically analyzed using techniques described by Rankin *et al*<sup>1</sup> and Yang *et al*<sup>2</sup>. Right kidneys were removed, weighed and cut longitudinally. The cross-section was fixed in a solution and examined histologically. Blood was drawn from rats three days prior to the move to individual cages and again just before termination.

Data were analyzed using a one-way ANOVA followed by Dunnett's of Newman-Keul's test. The 0.05 level was used as the criterion for significance.

### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study shows the different effects of different forms of 3,5-DCA on renal functions in rats. It correlates the chemical with nephrotoxicity. Rats were dosed ip with 0.8 mmol 3,5-DCA/ kg (264 mg/kg) while po injections were 1.5 mmol/kg (495 mg/kg). Although some effects on the kidneys were observed, there was no acute mortality due to 3,5-DCA after 48 hours except in the group treated where DMSO was used as a co-solvent. For treatments with DMSO, there was complete mortality. These results underscore the concern regarding the selection of co-solvents in toxicity studies and how DMSO can alter uptake. These study results are consistent with the understanding that iprodione and presumably its 3,5-DCA is not acutely toxic to mammals on an acute oral exposure basis though. The relevancy of the effects of DMSO on the ip study to this risk assessment is uncertain.

**Limitations of Study:** The purity and formulation of the dosed chemicals is not given. The study also examines *in vitro* effects and does not show whole organism impacts (other than in association with DMSO).

Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation: E064288** Schwartz, A. 1991. Laboratory Evaluation of Toxicity of Registered Pesticides to Adult *Ablyseius addoensis*. S. Afr. J. Enol. Vitic. 12 (2), 87-89.

# Purpose of Review (DP Barcode or Litigation): Litigation

# **Date of Review:** 10-07-09

**Summary of Study Findings:** The study examined the effects of various pesticides, including iprodione, on the predatory mite *Ablyseius addoensis*. The percent active ingredient and formulation are not specified; instead concentrations are given as 200ml/100 l water and "dust." The application of these rates resulted in "light" mortalities of 0 and 0.1% respectively (**Table 1**). Abbott's formula was used compute the percentage mortality.

Adult *A. addoensis* were collected from mature leaves of an unsprayed Riesling vineyard. 20 mm discs were pressed from the leaves and were used as experimental substrate. A solution of water and "sticker spreader" (0.03% poly-p-menthene) was prepared at the concentration above. Leaf discs were submerged in the solution for five seconds to apply the chemical. Discs were then floated in a Petri dish of water and secured to plasticine using a pin. In "dust" treatments, leaves were dusted with an unspecified amount of chemical using a hand spreader before discs were pressed. After pressing, the same floating procedure was followed. Five to ten mites were then transferred to each disk using a sable hair brush. Mites were kept at 25°C. Mortality was assessed 24 hours after the initial transfer. Mites were considered dead if they failed to move when prodded with a brush. Each treatment was replicated between 4 and 12 times, but specifics were not given.

 TABLE 1

 The toxicity of registered pesticides to adult Amblyseius addoensis in laboratory tests.

			Mortality		
Chemical	Concentration/ 1001 water <sup>a</sup>	test mites	%	Rating	
INSECTICIDES					
dichlorvos	75 ml	44 (34) <sup>b</sup>	2.3	Light	
chlorpyrifos	200 ml	59 (66)	98.2	High	
prothiofos	50 ml	90 (86)	100	High	
dimethoate	125 ml	35 (45)	100	High	
fenthion	dust	20 (21)	100	High	
permethrin	15 ml	78 (87)	94.7	High	
carbaryl	125 g	37 (28)	100	High	
endosulfan	100 g	19 (19)	100	High	
methidation	100 g	16 (52)	100	High	
ACARICIDES	-				
propargite	100 ml	43 (40)	13,9	Medium	
bromopropylate	50 ml	75 (55)	93,3	High	
propoxur	250 ml	26 (21)	100	High	
FUNGICIDES					
sulphur	dust	19 (20)	0	Light	
triadimenol	25 ml	21 (19)	0	Light	
nuarimol	15 ml	23 (20)	0	Light	
myclobutanil	20 ml	17 (19)	0	Light	
mancozeb	200 g	72 (79)	7,0	Light	
copper oxychloride	500 g	20 (20)	0	Light	
hexaconazole	20 ml	27 (23)	0	Light	
procymidone	200ml	27 (40)	0	Light	
iprodione	200 ml	41 (40)	0	Light	
iprodione	dust	38 (40)	0,1	Light	
pirifenoks	12 ml	60 (69)	15,4	Medium	

a = registered concentrations.

b = untreated control.

### Description of Use in Document (QUAL, QUAN, INV): Qualitative

### Rationale for Use: None.

**Limitations of Study:** The percent active ingredient and formulation are never given, and the general application rate is absent in one case. No statistical analysis was performed and the sample size seems rather small. There is no background on the test subjects (not even age or condition).

### Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 109202. Beketov, M. A. and M. Liess. 2008. Potential of 11 Pesticides to Initiate Downstream Drift of Stream Macroinvertebrates. Archives of Environmental Contamination and Toxicology. 55. p247-253.

### Purpose of Review (DP Barcode or Litigation): Litigation

### Date of Review: 7-27-09

**Summary of Study Findings:** The purpose of this study was to first assess the acute toxicity of 11 toxicants (thiacloprid, imidacloprid, acetamiprid, iprodione, fenvalerate, indoxacarb, azoxystrobin, tebufenpyrad, fenoxycarb, cyprodinil, and prochloraz) and then to assess their drift initiating potential at sublethal concentrations. The species tested were *Baetis rhodani* (mayfly larvae), *Simulium latigonium* (blackfly larvae), and *Gammarus pulex* (amphipod). The test specimens were collected from streams that were described as uncontaminated, although no data were given to support this claim other than the statement that no arable land was present for several kilometers upstream from the collecting site. Blackfly larvae were obtained from existing cosm studies on-site. Following collection, the test animals were kept in a 1:1 mixture of M7 medium and water with temperature of  $15^{\circ} C \pm 2^{\circ}$  with a photoperiod of 10:14 hours (light:dark). These conditions were maintained during testing.

For acute testing, 10 active individuals were selected for each treatment. Stock solutions of the test chemicals were made using dimethylsulfoxide (DMSO); solvent controls using 3 and 0.3 mL DMSO/L were run and according to the study, no significant difference was seen on drift rate. Test animals were placed in 100-ml beakers filled with 60 ml of a solution. Conditions for the experiment were as follows; pH: 7.4, conductivity 600  $\mu$ S/cm, carbonate hardness: 180mg CaCO<sub>3</sub>/ L. The tests were run for 96 hours, during which time the solutions were not renewed and the animals were not fed. Mortality was monitored daily with death defined as the absence of any movement. **Table 2** (taken from the report) displays the 96-hour LC<sub>50</sub> data from the acute toxicity experiment. For iprodione, lethal levels were 480  $\mu$ g/L in *S. latigonium* and 3460  $\mu$ g/L in *G. pulex*.

Toxicant	LC <sub>50</sub> for 96 h (95% con	fidence interval), $\mu$ g/L	
	Baetis rhodani	Simulium latigonium	Gammarus pulex
Thiacloprid	4.60 (3.74-5.66)	NA	350 (210-570)
Imidacloprid	*8.49 (4.45-16.20)	3.73 (1.54-9.05)	270 (170-450)
Acetamiprid	NA	3.73 (1.54-9.05)	50.0 (30.0-90.0)
Iprodione	NA	480 (360-220)	3460 (2090-5720)
Fenvalerate	NA	0.12 (0.04-0.37)	0.17 (0.09-0.34)
Indoxacarb	*48.5 (NR)	NA	2520 (1330-4770)
Azoxystrobin	NA	NA	270 (170-450)
Tebufenpyrad	*2.69 (1.41-5.12)	NA	24.1 (11.1-52.5)
Fenoxycarb	NA	550 (NR)	1730 (970-3100)
Cyprodinil	NA	NA	690 (460-1040)
Prochloraz	NA	NA	2180 (1140-4180)

Table 2Median lethalconcentrations  $LC_{50}$  values andrespective 95% confidenceintervals in parentheses ( $\mu g/L$ 

\*  $LC_{50}$  for 48 h ( $\geq 10\%$ mortality in the control after 48 h)

NA – not assessed because the number of animals was limited NR – confidence intervals are not reliable Chronic toxicity testing was done using a microcosm intended to mimic a stream bed. The microcosms were made of glass and measured  $120 \times 10.5 \times 4.5$  cm. The total volume of each setup (including a reservoir) was 5 L. A flow rate of 0.007 L/ hr was used. Each unit was divided into four sections, with the top three considered upstream and the fourth considered downstream. The upstream part of each setup was raised 2-cm higher than the downstream section to achieve this flow rate. Water was re-circulated using an electric pump. New plastic components were used for each test and all glass or metal materials were washed with acetone and water after each experimental run. The test chemical was introduced to the most upstream portion of the mock-stream and concentrations were measured at 0.5, 1, 2, 4, 22, 24, 26 and 48 hours after treatment. Downstream movement of animals was also assessed at these intervals. For the chronic portion of the experiment, iprodione was only tested on *G. pulex* since insufficient numbers of the other invertebrate species were available for testing **Table 1** below shows the nominal and measured concentrations for contaminants.

Table 1 Nominal and measured (in parentheses)	Toxicant	Nominal and measured (in parentheses) pesticide concentration, $\mu$ g/L			
concentrations of the pesticides		Baetis rhodani	Simulium latigonium	Gammarus pulex	
experiments ( $\mu$ g/L)	Thiacloprid	0.3 (0.31)	0.3 NM	50 (30.3)	
	Imidacloprid	1 (0.97)	NA	30 NM	
	Acetamiprid	0.5 NM	0.5 NM	3 NM	
	Iprodione	NA	NA	500 (366)	
	Fenvalerate	0.01 NM	0.01 NM	0.01 NM	
	Indoxacarb	3 NM	20 NM	300 NM	
	Azoxystrobin	NA	NA	20 (16.50)	
	Tebufenpyrad	0.2 NM	NA	3 (2.5)	
NA – not assessed because the number of animals was limited NM – not measured due to technical measons	Fenoxycarb	NA	50 (32.6)	100 NM	
	Cyprodinil	NA	50 NM	70 NM	
	Prochloraz	NA	100	100 NM	

Iprodione treatment was observed to significantly affect (increase) the maximum observed percentage of drifted *G. pulex*. The overall results of the drift experiment can be seen in **Figure 2**. According to the study, for all the toxicants exhibiting drift-initiating activity the drift of the tested animals was already detected within 2 hrs after treatment. Maximum drift percentages were detected 4 hrs after contamination. During subsequent observation periods (22–48 hrs after contamination) the drift responses became less pronounced. Peak drift was initiated at iprodione concentrations of 366  $\mu$ g/L; this concentration is roughly 9.5 times lower than the 96-hr LC50 value for iprodione (3460  $\mu$ g/L) in *G. pulex*.


Fig. 2 Maximum observed drift responses of *Baetis rhodani, Simulium latigonium*, and *Gammarus pulex* to the sublethal concentrations (approximately 10 times below the acute  $LC_{50}$  values) of 11 investigated pesticides observed during 4 h after contamination (percentage of drifted animals as a difference from control). Asterisks indicate significant (p < 0.05) differences from the respective controls assessed as proportion of drifted individuals by contingency tables, chi-square test. The drift-initiating toxicants are grouped towards the left side of the graph

# Description of Use in Document (QUAL, QUAN, INV): Qualitative.

**Rationale for Use:** The study provides qualitative evidence that under the conditions tested, the 96-hr LC<sub>50</sub> values for *S. latigonium* and *G. pulex* are 480 and 3460  $\mu$ g/L, respectively. The study also demonstrated that iprodione can initiate drift of amphiopd larvae at concentrations around 9.5 times lower (366  $\mu$ g/L) than the 96-hour LC<sub>50</sub> value (3460  $\mu$ g/L).

**Limitations of Study:** The study uses wild-caught test animals, but does not provide data to show that they have not been exposed to the toxicants being tested, or any other chemical. There is no breakdown of chemical concentration by time after the initial contamination event and no time-specific data provided to show downstream drift. Also, DMSO was used as a co-solvent and it is unclear how the solvent may have affected chemical uptake.

# Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 64250 Reigher, Z. J. and C. S. Throssell. 1997. Effect of Repeated Fungicide Applications on Creeping Bentgrass Turf. Crop Science. 37. p 910-915.

# Purpose of Review (DP Barcode or Litigation): Litigation

# Date of Review: 7-24-09

**Summary of Study Findings:** This report examines the effects of various fungicides on growth (root and shoot) and carbohydrate production on creeping bentgrass (*Agrostis palustris*).

A three-year field study was conducted in soil with an organic concentration of 4.7% and pH of 7.1. Plot sizes were 1.5x3.0 m. Seed was applied at a rate 37 kg/ ha. Starter N, P and K were applied at 27, 33, and 45 kg/ ha respectively. The fields were then fertilized with 145 kg/ha N, 39 kg/ha P and 73 kg/ha K annually. The plots were mowed six times weekly. In 1990 they were cut to 0.76 cm. In 1991 and 1992 they were cut to 0.6 cm. "Minimal" pesticides were applied to control weeds and insects, but they are not listed.

Fungicide treatments were applied using  $C0_2$  powered backpack sprayers at 207 kPa in 1632 l water/ha. Most treatments were applied every 7-10 days (16 times per year), but the exact time period is not specified. Treatment two was applied only after the appearance of "dollar spot" on certain plots. Rates are as shown in the following table:

	8 18 8	
Treatment	Application schedule	kg ha <sup>-1</sup>
1. Untreated check	_	0.00
<ol> <li>Chlorothalonil</li> </ol>	curative†	9.50
3. Chlorothalonil	weekly‡	6.36
4. Benomyl	weekly‡	3.05
<ol> <li>Fropiconazole</li> </ol>	weekly‡	0.84
6. Iprodione	weekly‡	3.05
7. Chlorothalonil	⟨ / alternated weekly§	6.36
benomyl	ANY	3.05
8. Chlorothalonil	alternated weekly§	6.36
propiconazole	11.7	0.84
9. Chlorothalonil	alternated weekly§	6.36
iprodione		3.05

 Table 1. Treatments for observation of the effect of repetitive applications of fungicides on creeping bentgrass.

<sup>†</sup> Applied when disease activity was present in these plots on 22 Aug. 1990, 14 Aug. and 13 Sep. 1991, and 15 July 1992.

<sup>‡</sup> Applied every 7 to 10 days from May through Sep. of 1990, 1991, and 1992; 16 applications per year.

§ Fungicides alternately applied every 7 to 10 days from May through Sep. of 1990, 1991, and 1992; 8 applications of each fungicide per year.

Plots were rated visually immediately before each fungicide application. Visual inspections were graded on a scale from 1-9 for both color and quality. For quality, 1 represented dead turf, 5 acceptable turf and 9 excellent turf. Color was on much the same scale with 1 corresponding to chlorotic 5 being acceptable and 9 dark green.

Clippings from each plot were harvested every two weeks from May to September. Plots were not mowed for 3 days prior to harvest. Harvests were cut using a 50-cm wide greens mower. One pass was made over each plot. Clippings were dried at 57°C for "at least" hours. They were then ground to pass through a 1-mm mesh screen. Carbohydrates were then extracted in water for one hour and quantified using anthrone.

Root measurements were made in June, July and August of 1992 (third year). Vegetation and thatch were separated into 0 to 5 cm and 5 to 10 cm groups. They were then combined and washed using a hydropneumatic eluration system. With 925  $\mu$ m primary and 437  $\mu$ m secondary filters. Ashed weights were used for statistical analysis.

Soil samples were taken using a 2.5-cm diameter soil probe. They were divided into the groups 0-1.3 cm and 1.4 to 6.3 cm. Cores from each depth were combined and soil pH was determined from a slurry of soil and distilled water.

Thatch depth was measured on September 9, 1992. It was measured fat three locations around each of three 4.8 cm diameter cores taken from each plot. Earthworm casts were counted impromptu on the same date as thatch sampling. Counts of yellow tuft and pink snow mold were also performed. ANOVA was performed on all final data using the least significant difference to separate means. The results of this analysis of are displayed in the **Tables 2** and **3**. Neither mean clipping weight nor mean carbohydrate concentration of clippings were significantly (p>0.05) affected by iprodione treatments under the conditions tested. Means of rooting (**Table 5**) were not statistically (p>0.05) affected by iprodione treatment. Means of disease incidence, earthworm casts, thatch of plots of creeping bentgrass (**Table 6**) were also not significantly (p>0.05) by iprodione treatment.

			19	90				1991					1992		
Source	df	June	July	Aug.	Sep.	May	June	July	Aug.	Sep.	Мау	June	July	Aug.	Sep.
Treatment	8	**	**	NS	NS	**	**	**	**	**	NS	NS	**	**	**
Schedule (S)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**
Fungicide (F)	2	**	**	*	*	**	**	**	**	**	NS	NS	**	**	NS
S × F	2	NS	NS	NS	NS	NS	NS	*	NS	**	NS	NS	NS	NS	NS
Treatment means			_					—— g n	n - 2						
Untreated check		11.4	15.5	16.5	7.5	3.8	2.3	2.0	3.6	2.3	4.2	7.7	3.8	4.0 c	4.7
Curative chlorothalonil		10.3	17.5	17.7	9.2^	3.9	2.4	2.0	3.6	2.3	4.5	8.7	2.9	3.2	4.6
Weekly chlorothalonil		10.7	14.5	18.0	10.1	4.31	3.4 1	3.21	3.7*	2.71	5.0*	10.3 1	3.5 N	4.31	5.91
Weekly benomyl		10.3	15.5	15.5	8.8	3.4	2.3	2.5	3.7	1.2	5.1	10.5	4.2	4.3	5.0
Weekly propiconazole		14.0	19.6	19.4	10.6	6.9	5.2	6.8	5.2	4.1	4.7	11.7	5.8	6.2	6.2
Weekly iprodione		11.2	16.8	17.0	9.5	4.1	2.9	2.1	3.8	2.5	5.2	10.4	3.9	4.5	4.8
Chlorothalonil + benomyl		10.7	15.1	14.4	9.0	3.7	2.9	3.2	4.0	2.1	5.0	10.7	4.5	4.6	7.3
Chlorothalonil + propiconazole		12.5	18.8	19.2	10.7	5.8	4.6	5.8	5.2	3.4	4.6	11.1	5.1	6.5	6.7
Chlorothalonil + iprodione		10.2	15.7	16.6	9.3	4.1	3.5	2.8	3.9	2.6	5.0	10.5	3.8	4.6	6.2
LSD .		1.8	2.6	NS	NS	1.4	0.8	1.0	0.6	0.6	NS	NS	1.0	0.9	1.3
Fungicide means															
Propiconazole		13.2	19.1	19.3	10.7	6.4	4.9	6.2	5.1	3.7	4.7	11.4	5.5	6.4	6.4
Iprodione		10.7	16.3	16.8	9.4	4.1	3.2	2.5	3.8	2.6	5.1	10.4	3.8	4.6	5.5
Benomyl		10.5	15.3	14.9	8.9	3.5	2.6	2.9	3.8	1.7	5.1	10.6	4.4	4.4	6.1
LSD		1.3	1.8	2.7	1.4	1.1	0.6	0.7	0.5	0.4	NS	NS	0.7	0.7	NS

 Table 2. Analysis of variance summary and means of clipping weights harvested from plots treated with repeated applications of fungicides.

\*\*,\*,NS Significant at 0.01, 0.05, and nonsignificant, respectively.

			19	990				1991					1992		
Source	df	June	July	Aug.	Sep.	Мау	June	July	Aug.	Sep.	May	June	July	Aug.	Sep.
Treatment	8	NS	NS	NS	•	NS	•	**	*	•	NS	NS	NS	•	**
Schedule (S)	1	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	**
Fungicide (F)	2	NS	NS	NS		NS	NS	*	**	**	NS	NS	NS	NS	**
S × F	2	NS	NS	NS	NS	NS	NS	<b>NS</b>	NS	NS	NS	NS	NS	NS	NS
Treatment means								g n	n-1						
Untreated check		64	37	49	84	86	83	38	77	18	34	56	12	60	46 /
Curative chlorothalonil		64	42	41	96	80	84	33	73	19	37	53	14	61	44
Weekly chlorothalonil		59	37	39	81 ~	80	61	21 5	59 5	15 N	36	46	13	45	33 5
Weekly benomyl		63	41	49	93	86	86	25	65	11	39	66	11	60	44
Weekly propiconazole		58	41	48	85	88	74	17	60	11	30	51	14	50	35
Weekly inrodione		62	38	47	95	81	92	32	79	17	34	56	12	55	53
Chlorothalonil + benomyl		58	38	44	93	89	78	19	63	12	37	52	12	52	33
Chlorothalonil + propiconazole		59	45	45	77	79	71	19	61	13	37	45	12	43	32
Chlorothalonil + iprodione		60	41	46	85	84	78	27	76	17	36	58	13	47	40
LSD (0.05)		NS	NS	NS	13	NS	16	ĩi	14	5	NS	NS	NS	12	8
Fungicide means															
Propiconazole		59	43	46	81	84	72	18	61	12	34	48	13	47	33
Iprodione		61	40	46	90	82	85	30	- 78	17	35	57	13	51	46
Benomyl		61	40	46	93	88	82	22	64	11	38	59	12	56	39
LSD (0.05)		NS	NS	NS	9	NS	NS	7	10	4	NS	NS	NS	NS	6

Table 3. Analysis of variance summary and means of water-soluble carbohydrate concentrations of clippings harvested from plots treated with repeated applications of fungicides.

\*\*,\*,NS Significant at 0.01, 0.05, and nonsignificant, respectively.

Table 5.	Analysis of	variance summa	ry and means	of rooting me	easured in 1992	2 of creeping	bentgrass treated	with repeated applicati	ons
of fun	gicides.			0			0		

			June			July			Aug.	
Source	df	0-5 cm	5-10 cm	total	0-5 cm	5-10 cm	total	0-5 cm	5-10 cm	total
Treatment	8	•	NS	**	**	**	•	NS		
Schedule (S)	1	**	NS	**	**	NS	**	NS	NS	NS
Fungicide (F)	2	NS	•	•	**	**	•	NS	NS	NS
S × F	2	NS	NS	NS	•	NS	NS	NS	NS	NS
Treatment means						— mg m <sup>-3</sup> —				
Untreated check		1574	519	2093	1518	453	1971	1059	290	1355
Curative chlorothalonil		1737-	535	2271	2012	637	2648	1681	489	2175
Weekly chlorothalonil		2175	6721	28474	2709 1	652 4	3366 f	1599	626	2226
Weekly benomyl		1630	530	2159	1849	672	2521	1126	351	1482
Weekly propiconazole		1859	703	2567	1818	774	2592	1054	591	1650
Weekly iprodione		1726	504	2236	2108	621	2730	1538	382	1920
Chlorothalonil + benomyl		1956	601	2562	2658	591	3254	1380	423	1803
Chlorothalonil + propiconazole		2480	784	3265	1864	794	2664	1625	469	2093
Chlorothalonil + iprodione		2434	708	3142	3723	560	4283	1365	362	1732
LSD (0.05)		575	NS	611	840	168	891	NS	199	530
Schedule means										
Alternate		2292	698	2990	2750	652	3402	1457	418	1874
Weekly		1742	581	2322	1925	688	2618	1238	443	1686
Fungicide means										
Propiconazole		2170	744	2018	1830	784	2628	1220	520	1960
Inrodione		2078	606	2689	2013	501	3500	1335	272	1007
Benomyl		1793	565	2363	2251	632	2888	1253	387	1640
LSD (0.05)		NS	143	433	591	117	626	NS	NS	NS

\*\*,\*,NS Significant at 0.01, 0.05, and nonsignificant, respectively.

Source	df	Thatch†	Yellow tuft†	Snow mold‡	Worm casts†
Treatment	8	**	NS	**	**
Schedule (S)	1	NS	NS	NS	**
Fungicide (F)	2	*	NS	**	**
S × F	2	**	NS	NS	**
			no.		28
			infected	% plot	no.
Treatment means		mm	plants m <sup>-1</sup>	damaged	<b>m</b> <sup>-1</sup>
Untreated check		8	19 (1	2	23( '
Curative chlorothalonil		9	10 1	2 4 .5	14 7
Weekly chlorothalonil		10	811	5 g 11'	1 3
Weekly benomyl		9	42	3	9
Weekly propiconazole		7	21	35	40
Weekly iprodione		8	27	4	30
Chlorothalonil + benomyl		8	29	7	4
Chlorothalonil +					
propiconazole		8	21	39	5
Chlorothalonil + iprodione		9	55	8	4
LSD (0.05)		1	NS	12	13
Fungicide means					
Propiconazole		7	21	37	22
Iprodione		8	41	6	17
Benomyl		8	35	5	6
LSD (0.05)		1	NS	8	9

Table 6. Analysis of variance sommary and means of disease incidence, earthworm casts, and thatch of plots of creeping bentgrass treated with repeated applications of fungicides.

\*\*,\*,NS Significant at 0.01, 0.05, and nonsignificant, respectively. † Rated 9 Sep. 1992.

‡ Rated 9 Sep. 1992. ‡ Rated 27 Mar. 1993.

## Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The three-year study provides qualitative evidence that under the conditions tested and the endpoints measured, iprodione treatment at 3.05 kg/ha did not significantly (p>0.05) affect any of the variables measured for bentgrass.

**Limitations of Study:** The formulation of iprodione used in the study is not discussed and as such, there is uncertainty regarding actual exposure concentrations. Also, meterological information is not provided and it is uncertain as to the extent that rain may have been a factor.

## Chemical Name: Iprodione

CAS No: 109801

**ECOTOX Record Number and Citation:** 70300 Benson, D.M. 1991. Control of Rhizoctonia Stem Rot of Poinsettia During Propagation with Fungicides that Prevent Colonization of Rooting Cubes by *Rhizoctonia solani*. Plant Disease. 75 (4), 394-398.

## Purpose of Review (DP Barcode or Litigation): Litigation

## **Date of Review:** 10-07-09

**Summary of Study Findings:** This study primarily measures the effectiveness of iprodione and other fungicides at controlling *Rhizoctonia solani* in Poinsettia plants. It uses the same procedures and measurements as seen in study by Benson (1992) entitled "Fungicides as Foliar Sprays of Rooting Cube Soaks in Propagation of Poinsettia<sup>1</sup>." Additionally, the studies may use the same chemical concentrations. This cannot be verified as the previous paper did not specify the percent active ingredient applied. Plants dosed at 0.3 g a.i./l via root-cube soaking showed reduced rooting, but no difference in plant height. Foliar spraying at the same concentration also produced a reduction in rooting, and no change in plant height. Specific data are not presented.

The methods and procedures used in this experiment were identical to those in the review for Benson 1992. For the rooting cube soaking portion of the experiment, strips of five cubes (one strip) were soaked in 200 ml of a solution containing the appropriate concentration of fungicide. Control strips were soaked in untreated water. Each strip was allowed to absorb its liquid completely before cuttings from a stock poinsettia population were stuck into each cube. For spray-application testing it is assumed, but not specified, that cuttings were placed in rooting cubes soaked only in water. It is stated that each cutting was sprayed to runoff and that runoff occurred at approximately 60 ml (72 mg) per strip. The sprayed cuttings were allowed to dry before they were placed under the misting apparatus.

The cuttings were moved to a mist bench where they were misted two minutes of every hour from 7am to 7 pm for the first day and then two minutes of every three hours after 7 pm on the first day. Temperatures averaged 21.4°C during the experiment. It is never stated how many replicates were run during the experiment, but at days 14, 21 and 28 after planting two replicates of five cuttings (one strip) for each treatment along with one control strip were selected for root ratings. Rooting was scored using a numerical rating system on a 1-7 scale with one being the lowest level of development and seven being the highest. Roots were also counted and measured. On Day 28 root counts were significantly lower in both the soaked and sprayed groups when compared to the control. There was also a significant difference in the root counts of sprayed cuttings versus those exposed in soaked cubes. Rooted cuttings were then transplanted and allowed to grow for 58 days in undisclosed conditions. The plant heights were measured at after 30 and 58 days.

<sup>&</sup>lt;sup>1</sup> Benson, D.M. 1992. Fungicides as Foliar Sprays or Rooting Cube Soaks in Propagation of Poinsettia. HortScience 27 (9), 1006-1008.

Height of iprodione-treated poinsettias was not statistically different than untreated plants These study results are in contrast to the Benson 1992 paper demonstrated a (p>0.05). significant effect of iprodione on reducing plant growth relative to untreated control plants.



Table 1. Plant height and Rhizoctonia stem rot caused by Rhizoctonia solani for poinsettias transplanted as apparently healthy rooted cuttings treated in ropting cubes with foliar fungicide sprays 89 days earlier Correstant (1)

Fungicide	Rate (g a.i./L)	Rooted cuttings (no.)*	Plant ht (× cm)*	Stem ro (%)
Chlorothalonil 40.4F	0.73*	11	9.3 od	55
Quintozene 75W	0.45	6	8.5 d	50
Ethazol + thiophanate				
methyl 40W	0.24	7	10.9 bcd	43
Benomyl 50W	0.30	10	10.9 bcd	40
Iprodione 50W	0.60	11	16.4 ab	17
Flutolanil 50W	0.30	12	14.8 abc	8
Metalaxyl +				
benomyl 42W	0.50	12	17.9 a	0
Control		12	18.3 a	0

"Rooted cuttings transplanter 29 days after fungicide treatment." "Plant height means followed by the same letters are not significantly different according to the Waller-Duncan k ratio; k = 100,  $\frac{p}{2} = 0.05$ . Percentage of transplanted rooted cuttings with stem rot symptoms 60 days after transplanting

Rate expressed as milliliter a.i./ L.

### Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** Although the study methodology is near identical to that described in Benson 1992, this paper reports no significant effect of iprodione on plant growth whereas the later study shows a significant effect on growth.

**Limitations of Study:** This paper focuses on the effectiveness of iprodione as a fungicide and not on its effects plant growth. It is the opinion of the reviewer that the experiments referred to in both papers were run simultaneously and in the same location.

#### **Chemical Name:** Iprodione

CAS No: 109801

ECOTOX Record Number and Citation: E089884 Helver, N. 1991. Laboratory Pesticide Screening Method for the Aphid Predatory Midge Aphidoletes aphidimyza (Rondani) (Diptera: Cecidomyiidae).Biocontrol Sciences and Technology. 1, 53-58.

#### Purpose of Review (DP Barcode or Litigation): Litigation

#### Date of Review: 10-07-09

Summary of Study Findings: This study is focused on the describing a research procedure for testing aphid predatory midges. While it does report a mortality rates (corrected by Abbott's formula) for eggs, larvae and adult midges, which are listed below, only one concentration was tested. Further, the study found this concentration to be "safe" and established no thresholds. No statistical analysis or comparison to a control was reported. Iprodione used in this study was the formulated product Rovral<sup>®</sup> WP (% active ingredient of the formulated product is not reported). According to the study, iprodione at 500 mg a.i./L, was tested. It is not clear from the study how much of the actual test material was applied to the test apparatus.

					Adults	5.865			Larvac	
		Constantial	% 100	otality			9	mortali	ty	
Common name	Trade name	(mg a.L.A)	24 N	48 h	mort	Classification	24 h	48 h	72 h	Classification*
Acuricides					· · · ·					
dicafel + tetradifon	Childion	125-6+339-45	89-7	89-3	٠	н	0	3	3	s
dienochlor	Peninc	312	5	19-6	0-6	S	Ť	ĩ	0	š
fenbutatin oxide	Torque	250	37-7	42.8	2.0	SH	ō	Ó	8	5
quinomethionate	Morestan	250	59-1	71-7	7-7	SH	50	67.5	87-5	SH
Funcicides										
benomvi	Benjate	500		0	7.9	e	1.5		5.1	
bugirinate	Nimerod	500	- ñ		1.5	ŝ	<u> </u>	á	2.5	5
chlorothalonil	- Recalse	1100	∩	5.0.	-0.5	<b>-</b>	ക്ഷം	3.3	10.1-	
iprodione	Royal WP	\$00	11	14-1	4-1	s	S.	6	0	e
hexacanazole	Anvil	15 10		- 71	4.5	e ri	369.6	4.8	ŏ.o	5
manch	Manch 80	-1200 /	3-100	HOD -	10	\ H		0	6 6	
propiconazole	Tilt	100	719	88	1.0	C2145 MH	ñ	ŏ	1.1	11/2 S
pyrazombos	Afugan	150	100	100	•	54	mő	100	100	800
triforinc	Saprol	237-5	600	17.8	<b>ه</b> ا	SH .		0		S
Handhield.			92		- 0	7680	*		*©	HC:714P
inclusion and inclusion	Gum		NR-2000							
OBBOXYOUN	Grasp	200	Q	0	1.0	5	1.0	4-9	20-6	s
Insecticides										
Bacilha	Dipel	4-8 × 10' UI/mg'	0	0	1-4	S	0	0	0	S
Duringiensis		(3 g product/l)								
buprofezia	Applaud	75	33-5	52-6	3-6	SH	15	47-5	53-8	SH
cypermethrin	Ambush C	62	190	100	•	н	100	100	100	н
diazinon	Dinzinon	160	100	100	•	н	100	100	100	Ĥ
diffubenzuron	Dimilia	125	11-5	13-7	0-3	S	0	0	0	S
fenpropathrin	Rody	50	HO	100		н	62-1	67-9	71 8	SH
heptenophos	Hostaquick	412-5	ECO	КŬ	100	н	100	100	100	HO
nicotine	XL All Nicoline	950	100	KOD.		н	22.5	67-5	97-4	MBIO
oxemy]	Vydate L	360	100	100	+	H	100	100	100	н
al distant on the	Beiman	360	93.0	67.0						

The results are corrected to 0% control mortality using Abbots correction formula.

The figures in square brackets are the persistence in days.
'4-8 × 10' International Units of Potency per sig (as determined against Trichophesic w).
S = Safe: < 30% mortality. SH = Slightly harmful: 30%-79% mortality. MH = Moderately harmful: 80%-79% mortality. H = Harmful: >99% mortality. \* = No eggs laid.

Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study reports mortality rate at a defined dose. At 500 mg, iprodione exposure for 48 hours resulted in less than 15% mortality in adults, less than 5% mortality in eggs and no mortality in larvae  $(1^{st} \text{ instar})$ .

**Limitations of Study:** The study is focused on describing a procedure rather than finding chemical effects. The exact exposure value used in the study is unclear given the information provided in the report. The presumption is that each leaf contained 500 mg of iprodione.

## 3,5 DCA Degradate Open Literature

### **Open Literature Review Summary**

Chemical Name: Iprodione (3,5-DCA Degradate)

CAS No: 113201

**ECOTOX Record Number and Citation:** van Leeuwen, C.J., D. M. M. Adema, and J. Hermens. 1990. Quantitative structure-activity relationships for fish early life stage toxicity. Aquatic Toxicology. 16, 321-334.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-24-09

**Summary of Study Findings:** The study examines the effects of 3,5-dichloroaniline (3,5-DCA) and other chemicals on the early life stages (ELS) of fish for the purpose of quantitative structure-activity relationship (QSAR) improvement and revision. Parameters studied included survival, embryo-hatchability and growth.

The 3,5-DCA used for this study was >97% pure.

Reconstituted water was used in all of the tests. It was prepared from groundwater by adding several salts. The resulting water quality has been found suitable for breeding aquatic test species in the past. The water had the following characteristics: CaCO<sub>3</sub> 210 mg/l, mean dissolved oxygen 7.7 mg/L, mean pH 8.0-8.2, Na<sup>+</sup> 1.19mmol/L, K<sup>+</sup> 0.20 mmol/OL, Ca<sup>++</sup> 1.46 mmol/L, Mg<sup>++</sup> 0.73 mmol/L, Cl<sup>-</sup> 2.72 mmol/L, SO4<sup>--</sup> 0.73 mmol/L, HCO<sub>3</sub><sup>--</sup> 1.39 mmol/L..

Authors report that in "several instances", dimethylsulfoxide (DMSO) was used as a cosolvent for the test compounds and that DMSO concentrations were kept below 100  $\mu$ L/L.

Fertilized zebra fish (*Brachydanio rerio*) in the blastula (<6 hours after fertilization) from a stock culture at TNO laboratory. 50-100 eggs were placed in 1-liter glass containers filled with one liter of test solution. Non-viable eggs were removed after one day and the number of viable eggs in each test container was reduced to 40. If the number of viable eggs fell below 25 after 48 hours, the container was discarded. Each group was exposed in a semi-static manner for 28 days. Chemical solutions were renewed three times a week and tanks were gently aerated. Approximately 4-5 days after hatching the fry from each treatment were transferred to two, larger containers. The fry were fed *Brachionus rubens* from a laboratory culture. After one week, this food was supplemented with baby brine shrimp (*Artemia salina*) enriched with the commercial nutritional concentrate Selco<sup>®</sup>. The tanks were maintained at  $24 \pm 2^{\circ}$ C with a 12:12 photoperiod. Dead eggs and fish were removed and counted daily. At the end of the test period, fish were anaesthetized and examined.

While specific chemical concentrations were not given, but the study does state that a ratio of 1.8 was used to separate the different levels for each chemical. Concentrations were verified before and after water changes and were found to deviate less that 10% from the (unspecified) nominal concentrations. **Table IV** below shows the results obtained from the experiment.

The study authors report that "retardation of growth appeared to be the most sensitive parameter in all tests, although a number of embryotoxic effects such as skeletal deformities, enlarged yolk sac and edemas were frequently observed in the higher test concentrations; however, the data were not treated statistically as most of the larvae involved had died before the end of the test period".

#### TABLE IV

Results of 28-day early life stage tests with B. rerio and chloroanilines (mg/l).

Compound	$\log P_{oct}^{a}$	LC 50 and 95% C.L.	NOLC	NOEC <sup>b</sup>
Aniline	0.94	39 (30 -51)	5.6	1.8
3-CA	1.91	6.8 (5.8 - 8.0)	5.6	1.0
3 5-diCA	2.88	1.3 (1.0 - 1.8)	1.0	0.32
2.4.5-triCA	3.69	0.12 (0.06 - 0.18)	0.056	0.056
2.3.4.5-tetraCA	4.57	0.10 (0.06 - 0.18)	0.056	0.032
pentaCA	5.08	0.056 ( 0.032- 0.10)	0.010	0.010

<sup>a</sup>All data are experimental values taken from De Bruijn et al. (1989), while the value for 3,5-dichloroaniline was calculated using  $\pi$  constants derived by the same authors.

<sup>b</sup>No observed effect concentration for survival, hatching and growth (length).

The data obtained were used to create QSARs for  $LC_{50}$ , NOLC, and NOEC. These formulas are listed in **Table V** below.

# TABLE V

QSARs for the aniline derivatives from Table IV. Ecotoxicological data are expressed in µmol/l.

	r	S	Eq.
$\log 1/LC_{50} = 0.82(\pm 0.08) \log P_{oct} - 3.26$	0.983	0.27	(8)
$\log 1/NOLC = 0.81(\pm 0.10) \log P_{oct} - 2.85$	0.970	0.36	(9)
$\log 1/NOEC = 0.66(\pm 0.05) \log P_{oct} - 2.05$	0.991	0.16	(10)

r = correlation coefficient and s = the standard error of estimate.

The researchers conducted a literature search for other zebra fish toxicity data, but could find no results. They advocate using the fathead minnow (*Pimphales promelas*) as a surrogate species. Then, the paper uses the title "Summary of 28-32 day early life stage toxicity tests with zebra fish. *B. rerio* and fathead minnowm *P. promelas*." for one of its charts. By its own admission, this chart cannot possibly be accurate because of the lack of existent zebra fish data. The study then sites similarities in sensitivity between *P. promelas* and *Poecilia reticulata* as a means of readjusting the QSAR formula stated earlier to:

$$\log 1/\text{NOEC} (\mu \text{mol/l}) = (0.90 \pm 0.05) \log P_{\text{oct}} - 3.80 n = 30 r = 0.956 s = 0.33$$

The study then presents the results for a few scenarios run through their QSARs and presents the results.

# Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study develops a "working" QSAR for early life stages in fish. The study provides an estimated no observed lethal concentration and a no observed effect concentration of 5.6 and 1.0 mg/L, respectively. Although the NOEC is for survival, hatching and growth (length), the study does not report which of these is affected and to what extent.

**Limitations of Study:** A number of the study's connections and justifications seem tenuous. There is also no data given as to the dosing levels used in determining various toxicity levels. The study also does not specify which treatments used dimethylsulfoxide (DSMO) as a solvent and which did not. Aeration was used, but study reports that measured concentrations were close to nominal. However, no data are provided to support this claim. Although the NOEC is for survival, hatching and growth (length), the study does not report which of these is affected and to what extent.

**Chemical Name:** Iprodione (3,5-DCA Degradate)

CAS No: 113201

**ECOTOX Record Number and Citation:** 5375 Maas-Diepeveen, J.L. and C. J. van Leeuwen. 1986. Aquatic toxicity of aromatic nitro compounds and anilines to several freshwater species. Laboratory for Ecotoxicology, Institure of Inland Water Management and Waste Water Treatement, Ministry of Transport and Public Works. Leystad, Netherlands.

# Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-25-09

**Summary of Study Findings:** The study examines the short-term effects of 3,5-DCA on water fleas (*Daphnia magna*), guppies (*Poecilia reticulata*), zebra fish (*Brachydanio rerio*), algae (*Chlorella pyrenoidosa*) and bacteria (*Photobacterium phosphoreum*). It then takes the results of these effects experiments and develops a quantitative structure-activity relationship (QSAR) equation based on the octanol/water coefficient ( $K_{ow}$ ) of the chemical.

The 3,5-DCA used for this experiment was >96% pure.

The procedural techniques for this experiment are not detailed in the paper, but are referenced as being the same as those "described by van Leeuwen *et al* (1985)"<sup>1</sup>, although this may be for only a portion of the procedure and not the whole experiment.

Chronic toxicity testing with daphnids, 10 daphnids were used, with one daphnid per jar containing 50 mL medium for 21 days; daphnids were fed  $3x10^8$  *C. pyrenoidosa* cells/L/day. The mean size of daphnids was determined from treatments and controls.

Studies with zebrafish (*Brachydanio rerio*) were carried out at  $25\pm1^{\circ}$ C in 10 mL glass [jars] containing 50 mL of test solution. Test solution had a hardness of 250 mg/L (as CaCO<sub>3</sub>) and pH of 8.2±0.2. Tests were conducted in singular with 25 eggs per jar with eggs obtained from laboratory culture. Test solutions were renewed weekly; fish were not fed during test and the study was terminated after 7 days. Each day the dishes were inspected for mortality.

No background is given on the test organims, although it may be explained in the referenced paper.

It is stated that isopropanol (99 % pure) was used as a solvent; however, the amount of the solvent is not specified.

**Table IV** below shows the results of the aniline exposure experiments. The results of these experiments were combined to create QSAR equations for each species. The formulas are based on the entire class of chemicals (anilines), not any one chemical in particular. The equations can be found in **Tables VIII** and **IX** below.

<sup>1</sup>Van Leeuwen, C.J. Luttmer, W.J. Griffioen, P.S. 1985a. The use of cohorts and populations in chronic toxicity studies with *Daphnia magna*. I. A Cadmium exmple. Ecotoxicol. And Environm. Saf. 9, 26-39

#### TABLE IV

Results of short-term and long-term toxicity studies with anilines (mg/l).

NO	Compound	14-ð <sup>b</sup>	48-h	21-đ	21-	-4	96-h	15-min
		1050	LC50 (95%C.L.)	LC50 (95% C.L.)	LR	т	EC50 (95%C.L.)	EC50 (95%C.L.)
					r <sub>m</sub>	L		
		P.reticulata	a D <sub>a</sub> magna	D.magna	D.mar	gna	C.pyrenoidosa	P.phosphoreum
1	Aniline	125.6	0.08(0.06-0.10)	0.04(0.03-0.06)	0.01	0.32	94 (77-114)	60 (5665)
2 <sup>a</sup>	2-01	6.3	0.13(0.09-0.19)	-	-	-	32(26-38)	13(10-19)
3 <sup>8</sup>	3-01	13.4	0.10(0.09-0.12)	0.26(0.10-0.32)	0.032	0.32	21 (17-25)	16 (14-18)
4 <sup>a</sup>	4-01	26.0	0.05(0.04-0.06)	-	-	-	4.1 (3.8-4.3)	5.9(5.3-6.5)
5 <sup>a</sup>	2,4-dic1	6.3	0.50(0.40-0.62)	3.2(1.0-10.0)	0.1	3.2	10 (9.2-11)	3.4(3.3-3.6)
6 <sup>8</sup>	2,5-diCl	1.7	2.92(2.15-3.97)	-	-	-	10(8,1-11)	3.6(3.2-3.9)
7 <sup>a</sup>	3,4-dicl	6.3	0.10(0.08-0.11)	-	-	-	4.2(4.0-4.4)	1.74(1.66-1.82)
8 <sup>a</sup>	3,5-diCl	3.9	1.12(0.97-1.29)	-	-	-	7.5(6.6-8.4)	10(8.2-13)
9	2,3,4-triCL	1.4	0.73(0.62-0.86)	1.2(0.3-3.2)	0.1	1.0	1.7(1.4-1.9)	1.34(1.27-1.42)
10	2,4,5-triCL	2.0	3.40(2.46-4.69)	-	-	-	2.2(1.8-2.7)	1.84(1.76-1.92)
11	2,3,4,5-tetraCl	0.4	0.64(0.56-0.73)	0.18(0.10-0.32)	0.1	0.32	0.7(0.5-1.0)	1.49(1.45-1.53)
12	2-CH3	81.3	0.52(0.31-0.86)	2.2(0.32-3.2)	0.1	1.0	55 (40-259)	33(31-36)
13	3-013	36.3	0.15 —	-	-	-	44 (4048)	26 (22-30)
14	4-CH3	10.7	0.20(0.14-0.29)	-	-	-	138(130148)	8.0(7.6-8.5)
15	2-C2H5	74.7	8.05(6.24-10.4)	7.0(3.2-10.0)	1.0	3.2	38(12-124)	10.3(9.9-10.7
16	3-C2H5	27.1	0.42(0.35-0.51)	-	-	-	22(20-24)	6.2(5.8-6.5)
17	4-C2H5	29.1	0.09(0.08-0.11)	-	-	-	6.2(6.0-6.4)	0.21 (0.20-0.22)

a Selected by the BC and the IRC. <sup>,b</sup>Data from Hermens et al., (1984).

#### TABLE VIII:

```
QSAR-equations for the aniline compounds.
Lineair relationships<sup>a</sup>.
```

QSAR-equation	log 1,	/C= <u>a</u> lo	g Poct	calc)+ <u>b</u>	
C (µmol/l)	<u>a</u>	b	no.	5	r
LC50 14-d P.reticulata	0.92	-3.72	1-11	0.27	0.946
LC50 48-h D.magna	-0.39	0.55	1-11	0.50	0.553
EC50 96-h C.pyrenoidosa	0.82	-3.61	1-11	0.27	0.932
EC50 15-min P.phosphoreum	0.70	-3.18	1-11	0.29	0.900

<sup>a</sup>No.: compounds 1-11 of Table II; <u>s</u>: standard error of the estimate and <u>r</u>: correlation coefficient.

#### TABLE IX

```
QSAR-equations for the aniline compounds. Introduction of \Sigma \sigma^{>a}
```

QSAR-equation		$\log \frac{1}{C} = \underline{a} \log P_{OCt}(calc) + \underline{b} \Sigma \sigma + \underline{c}$						
С (µл	nol/1)	<u>a</u>	Þ	<u>c</u>	no.	5	<u>r</u>	
LC 50	14-d P.reticulata	0.24	1.17	-3.04	1-11	0.22	0.970	
1C50	48-h D.magna	0.06	-0.77	0.10	1-11	0.52	0.587	
EC 50	96-h C.pyrenoidosa	1.56	-1.29	-4.37	1-11	0.20	0.969	
EC50	15-min.P.phosphoreum	0.58	0.19	-3.07	1-11	0.30	0.902	

<sup>a</sup>See Table VIII.

Most of the short term QSARs showed a good correlation to actual results. The exception is the short term QSAR for *D. magna*. There was no correlation found between the effects of anilines and the Kow of those chemicals for *D. magna* which prevented an accurate QSAR from being created.

#### Description of Use in Document (QUAL, QUAN, INV): Quantitative

**Rationale for Use:** The study creates aniline QSARs for multiple aquatic invertebrate species. It also measures the effects of 3,5-DCA exposure on those species. The 48-hr  $LC_{50}$  for *Daphnia* 

*magna* is 1.12 (0.97 – 1.29) mg/L, and the 96-hr  $EC_{50}$  for green algae (*Chlorella pyrenoidosa*) is 7.5 (6.6 – 8.4) mg/L. No 21-day LC<sub>50</sub> value is reported for *D. magna*.

**Limitations of Study:** There is no justification for the statement that the QSARs created in this study are accurate. While the methods and setup used may be good, they are not specified, which makes the paper difficult to assess on its own. The experimental doses used are not listed, nor is the background of the test animals.

**Chemical Name:** Iprodione (3,5-DCA Degradate)

CAS No: 113201

**ECOTOX Record Number and Citation:** E005810 McLeese, D.W., V. Zitko, and M. R. Peterson. 1979. Structure-Lethality Relationships for Phenols, Anilines and Other Aromatic Compounds in Shrimp and Clams. Chemosphere 2, 53-57.

# Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-25-09

**Summary of Study Findings:** This study measures the 96-hourLT<sub>50</sub> (Time to Lethality for 50%) for shrimp (*Crangon septemspinosa*) and soft shelled clam (*Mya arenaria*) and then developed QSAR equations to predict  $LT_{50}$  values for chemicals of similar structure.

Test animals were collected locally. Shrimp were held in running sea water at 10°C for at least a week before testing began. Clams were held in running sea water at 4°C for an unspecified length of time prior to testing. During the 96-hour lethality testing, three of each species were kept in 4-L tanks and exposed simultaneously. The tanks contained aerated sea water and were kept at 10°C for the duration of the experiment. Test chemicals were dissolved in either ethanol of dimethyl sulfoxide (DMSO) in such a way that 1ml of solvent achieved the desired concentration in the treatment tanks. Control groups were dosed with 1 ml of ethanol or dimethyl sulfoxide. The test concentrations are not specified, but the paper does state that 5 different test treatments were used and that treatment concentrations differed by a factor of two. Chemical concentrations in each tank were measured at the beginning of the experiment and after solution was changed/refreshed (48 hours).

The time to 50% mortality for each was calculated using a logarithmic graph of the time until 50% death vs. the concentration of chemical at which they occurred. The geometric of the  $LC_{50}$  times of the highest step with no deaths and the next highest concentration at which all three animals died. The methods do not specify if the tests were species specific, and it is difficult to tell what exactly is meant by the designation of the two concentration levels used.

The octanol-water partition coefficient  $(K_{ow})$  was obtained from established literature or were calculated from partition coefficients of related compounds. Dissociation constants were taken mostly from literature. Values were corrected for test conditions.

According to the report, measured concentrations remained "practically constant throughout the 48 h."

For shrimp, the 96-hr  $LC_{50}$  of 3,5-DCA 2.5 mg/L. A lethal concentration was not found for clams. This could be because when initially exposed to a contaminant, the clams closed their shells, which may have prevented or altered exposure. The tables below summarize the QSAR equations created and their accuracy.

Equation Num	ber of compounds	log(1/LT)=	Correlation coefficient
All c	ompounds, lethal thr 45	reshold to <u>Crangon</u> 1.03*logP + 2.41	0.778
Pheno 2	ls and anilines 33	1.02*logP + 2.48	0.780
3	33	0.74*logP + 1.17*(0H)+ 0.81*(NH2) + 0.16*(CH3)+ 0.42*(Cl) + 0.97*(NO2)+1.09	0.909
Δ	33	0.57*PI + F + 0.86*R+4.65	0.798
Pheno 5	ols 23	0.48*logP + 0.54*(DpH)+2.93	0.960
Pheno	ols, lethal threshol 8	d to <u>Mya</u> 0.62*logP + 0.79*(DpH)+1.43	0.972

Table 3. Relationships between lethal thresholds and structural parameters of phenols and anilines.

Remarks:

P = octanol/water partition coefficient (OH), (NH2), (CH3), (C1), (NO2) = number of hydroxy, amino, methyl, chloro, and nitro groups in the molecule, respectively
Non-linearthic substituent constant (4)

PI = lipophilic substituent constant (4) F,R = "aromatic" substituent constants of Swain and Lupton (4)

(DpH) = pKA phenol - pKA compound (5)

Table 4. Distribution of differences between observed and calculated log(1/LT).

Equation	>0.9	Difference betu 0.7-0.9	Number of ween observe 0.5-0.7	compounds ed and calcula 0.3-0.5	ated log(1/LT) 0.1-0.3	<0.1
1 2 3 4 5 6	8 3 3 3 0	7 1 2 2 1 0	10 7 4 3 1 1	4 6 11 9 3 0	7 5 8 6 7 3	9 6 5 10 8 4

#### Description of Use in Document (QUAL, QUAN, INV): Qualitatative

**Rationale for Use:** The study develops QSAR equations and finds toxicity data for aquatic invertebrate species. Although there are uncertainties regarding exposure conditions, the study does provide a 96-hr  $LC_{50}$  of 2.5 mg/L for the marine shrimp, *Crangon septemspinosa*. It is not possible to estimate the toxicity of 3,5-DCA to clams (*Mya arenaria*) since the maximum exposure concentration tested of 3,5-DCA with this species is not provided.

**Limitations of Study:** The study uses wild caught test organisms and their prior exposure history is uncertain although the organisms were acclimated to laboratory conditions. Exposure concentrations are not reported although the study asserts that exposure concentrations were close to nominal and did not fluctuate even though the exposure tanks were aerated.

**Chemical Name:** Iprodione (3,5-DCA Degradate)

CAS No: 113201

**ECOTOX Record Number and Citation:** 16533. Hulzebos, E.M, D.M. M. Adema, D. Breemen, W. A. van Dis, H. A. Herbold, J. A. Hoekstra, R. Baerselman, and C.A.M van Gestel. 1993. Environmental Toxicology and Chemistry. 12, 1079-1094.

# Purpose of Review (DP Barcode or Litigation): Litigation

# Date of Review: 8-24-09

**Summary of Study Findings:** The study examines the toxicity of a 3,5-dichloroaniline (3,5-DCA), among other chemicals, to lettuce (*Lactuca sativa*) in soil and in nutrient solution with the intent of establishing a quantitative structure activity relationship (QSAR) related to the octanol-water partition coefficient of the chemical.

The chemical used in the test was >95% pure. Plants used were cultured at  $21 \pm 4^{\circ}C$  at 40-80% humidity. The photoperiod was 16:8 L:D. Tests were carried out by two different labs.

Table 1 displays the octanol-water partition ( $K_{ow}$ ) coefficient and  $LC_{50}$  used/found for the QSAR formula.

Soil for the experiment was gathered from an orchard in two different collections. It is not specified whether the soil was analyzed for chemical residues before the experiment was started. 3,5-DCA was mixed with acetone and a small amount of quartz sand to serve as a carrier. Specific concentrations are not listed, but a factor of 3.2 was used to separate the different trials. Duplicates were run for the control group and three unspecified dose levels.

For the soil test, 0.25-L plastic trays with 400g of soil were sown with 10 seeds. The trays were covered with glass plates until germination. After germination and plate removal, demineralized water was added daily to counteract evaporation. Only the first five germinating seeds were used, any germinating after the first five were removed and discarded. Harvests took place after seven and 14 days. When harvesting, shoots were cut at the soil level. The fresh weight of each plant was determined immediately after harvesting. By the end of the soil trials, DCA presence had dropped to a value  $\leq$  30% of its original concentration

For the nutrient solution test, an unspecified number of seeds were sown in 0.25-L plastic trays filled with perlite saturated with a nutrient solution containing a concentration of the test chemical. The trays were covered with glass plates until the seeds germinated. After one week, five seedlings with roots longer than 3 cm were selected and transferred to1-L pots filled with "nutrient solution and the test compound." The solution in the pots was changed three times weekly at which times oxygen concentration and pH were measured. Chemical concentrations were generally close to their nominal values. Shoots were harvested and weighed after 16 and 21 days.

A literature search was done to verify that the values found in this experiment were in line with those previously obtained. It was concluded that the results were similar.

The data gathered were used to create a QSAR equation for both the soil and nutrient solution tests. EC50 values were based on harvested shoots; dead plants or ungerminated seeds were not taken into account.

The nutrient solution formula uses  $K_{ow}$  and is as follows:

$$y = -0.54x + 2.83$$
  $r = -0.86$   $n = 12$ 

Due to the assumed effects of degradation, volatilization, and water adsorption,  $K_{ow}$  was not a sufficient metric for calculating a QSAR formula in soil. The soil sorption coefficient ( $K_p$ ) was used instead and is justified through several inexact connections. The soil QSAR formula (comparing  $K_p$  and EC<sub>50</sub>) was:

$$y = 1.16x + 0.15$$
  $r = 0.90$  and  $n = 12$ .

		EC50		
Compound	$(\mu g/g \text{ soil}, t = 7 \text{ d})$	$(\mu g/g \text{ soil}, t = 14 \text{ d})$	(mg/L  solution, t = 16-21  d)	Log K
(Chloro)(methyl)phe	nols			
Phenol <sup>a</sup>	SI 96 (72-129)	79 (58-107)	1 20 (8-46)	1.46
Phenol <sup>o</sup>	SF 146 (119-180)	168 (145-195)	7 14 (7 8-24)	1.40
2-MCP <sup>a</sup>	§ 4 52 (-)	43 (14-130)	2 16 (4-64)	1.40
3-MCP <sup>a</sup>	ap 21 (12-37)	7 (3-15)	4 56 (0 5-59)	2.15
2,4-DCP <sup>a</sup>	a / 27 (16-39)	53 (12-299)	5 2 4 (0 1-9 5)	2.30
3,5-DCP <sup>6</sup>	92 60 (18-195)	32 (-)	$(-1)^{-1}$	3.00
2,4,6-TCP <sup>a</sup>	an 19 (12-31)	16 (5-51)		3.62
2,3,5-TCP <sup>a</sup>	al 17 (8-35)	9 (8-11)	$\frac{1}{2}$ 20(10-40)	3.09
2,3,5-TCP <sup>6</sup>	as 8.5 (6.3-11)	8.9 (7.5-11)	g 0.79 (0.6-1)	3.83
PCP <sup>a</sup>	<i>G</i> ( 7 (4−15) ✓	8 (4-15)		5.85
PCP <sup>b</sup>	a 2 2.7 (2.2-3.5)	3.2 (2.7-3.7) 🗸		5.24
4C2MP <sup>a</sup>	32. < 100	>32 < 100	10.03(0.02-0.04)	5.24
4C3MP <sup>a</sup>	32. < 100	>32, <100	(2, 4.0, (2.4-0.5))	2.78
Catechol <sup>a</sup>	>1.000	>1,000	3 2.3 (0.7-7)	3.10
$\beta$ -Naphthol <sup>b</sup>	(291 (218-388)	88 (72-107)	$10^{-5.0}(1.3-21)$	0.88
o-Cresol <sup>a</sup>	67 (52-86)	>100	(5 4.9 (3.9-6)	2.70
m-Cresol <sup>b</sup>	<b>69</b> (51-94)	96 (62 147)	16 23 (10-31)	1.95
Nonvlphenol <sup>a</sup>	559 (331-946)	50 (03-147)	(7 50 (42-61)	1.96
Chloroanilines	/00 555 (551-540)	025 (502->1,000)	$ \chi  > 0.1, < 0.32$	
Anilinab	10 (10 - 50)			
Amine	25 49 (43-56)	56 (49-64)	19 7.9 (6.9-8.9)	0.90
	10 0 32 (0.4-294)	33 (24-45)	20 17 (5.2-55)	0.90
2-MCA <sup>b</sup>	$32 (\pm 50)$	$>32^{\circ}(\pm 50)$	21 31 (27-37)	1.90
3-MCA°	108 17 (13-20)	15 (12-19)	22 5.9 (5.0-6.9)	1.88
2,4-DCA <sup>2</sup>	104 32 (24-43)	29 (24-36)	23 7.0 (5.7-8.7)	2.78
2,4-DCA"	<i>a</i> 24 (17–33)	>10, <32	24 6.9 (3.4-14)	2 78
3,4-DCA°	111 > 10 (almost 10)	>10 (almost 10)	25 1.7 (1.5-1.9)	2.69
3,5-DCA	16 (13-20)	13 (10-16)	2( 5.0 (4.2-5.9)	2.00
2,4,5-TCA <sup>0</sup>	25 (18-35)	17 (15-20)	2713(12-15)	3.45
2,4,6-TCA <sup>6</sup>	27 (20-36)	23 (20-28)	28 3.5 (3.1-3.9)	3.52
2,3,4,5-TeCA <sup>o</sup>	47 (40-56)	24 (21-28)	25 0.39 (0.33-0.45)	3.52
2,3,5,6-TeCA°	64 (42-99)	16 (13-19)	30 0 62 (0 53-0 73)	3.54
PCA <sup>o</sup>	647 (333-1,255)	471 (296-751)		4.10
PCA*	>1,000	>1.000		
Chloro(nitro)benzenes	5	- 1,000	39 - <sup>3</sup> w	
1 4 DCPb	1,000	>1,000	<b>3</b> <sup>2</sup> 9.3 (7.5-12)	2.84
1,4-DCD	213 (156-290)	248 (212-298)	<sup>3*</sup> 5.1 (4.2-6.2)	3.52
1,2,3-1CD	5.8 (4.5-7.4)	3.8 (3.4-4.2)	35 0.028 (0.022-0.036)	4.14 <sup>c</sup>
1,2,3-1CD	3 <i>i</i> >1, <3.2	1 (0.2-5)	ND	4.14
135700	2 30 (43-74)	48 (41-56)	36 0.6 (0.53-0.69)	4.02
1.3.5-TCB <sup>a</sup>	770 115 (93-142)	123 (105–144)	37 2.0 (1.6-2.6)	4.15
1,3,3-1CD	ND	ND	38 >0.32, <1	4.15
1,2,3,4-1CB	67 (45-98)	32 (27-38)	3 0.63 (0.53-0.76)	4.64
1,2,4,5-TECB	4.2 (2.5-7.3)	1.3 (1.2-1.5)	₩ > 0.07 (0.06-0.09)	4.82
1,2,4,3-1ECB"	2 (1-6)	2 (1-4)	4! > 0.1, <1	4.82
PCB	128 228 (93-554)	56 (39-81)	42 ±1.0	5 17
PCB	862 (76->1,000)	±320	$(\overline{\mathbf{x}}) > \mathbf{S}_{\mathbf{x}}$	5 17 -
CONID <sup>b</sup>	130 >1,000	>1,000	$\bigcirc >S_{m}$	
C2NB <sup>o</sup>	5.0 (4.0-6.0)	5.4 (4.7-6.2)	1.8 (1.6-2.0)	2.24
C2NB <sup>-</sup>	132 >3.2, <10	>3.2, <10	41, 2.0(0.2-22)	2 24
C3NB	1 33 12 (10-14)	12 (11-13)	(6, 4, 6, (4, 2-5, 1))	2.11
2,3-DCNB <sup>a</sup>	(44 20 (17-25)	12 (9-17)	40 >0.32. <1	3.05
N compounds		. ,	1	5.05
Ethylenediamine <sup>a</sup>	>1.000	692 (570-840)	49 208 ( )	2.01
Dipropylamine <sup>a</sup>	134 383 (262-559)	370 (297-461)	· 200 (-)	-2.04
<b>Dibutylamine</b> <sup>a</sup>	139 510 (383-680)	361 (294-444)	27700, < 320	1.67
Acrylamide <sup>a</sup>	(32 101 (60-170)	152 (124-186)	51 6(05 60)	2.83
	, , , , , , , , , , , , , , , , , , , ,	(124-100)	(0.3-08)	-0.67
			5	continued

Table 1. Effect of 76 priority pollutants on the growth of *Lactuca sativa* in soil and in nutrient solution, expressed as EC50 values with 95% C.I.s (all values are based on nominal concentrations)

The authors compare the results from this study of chloroanilines to open literature and note that the  $EC_{50}$  for *Lemna minor* (duckweek) of 10 mg/L is roughly similar to the value obtained in this study for lettuce in nutrient solution (6.9 – 7 mg/L). They also report that the  $EC_{50}$  values of about 10 mg/L for 2,4-DCA in three different green algae. As such, the authors imply that the terrestrial plant data obtained in their data are in relatively close agreement with vascular and nonvascular aquatic plant toxicity data for the same compound.

# Description of Use in Document (QUAL, QUAN, INV): Qualitative

**Rationale for Use:** The study provides the basis and formulation of QSARs for 3,5-DCA in two different mediums, *i.e.*, soil and nutrient media. The 7- and 14-day EC<sub>50</sub> for 3,5-DCA are 16 and 13  $\mu$ g/g (mg/kg) in soil, respectively, and the 16 - 21-day EC<sub>50</sub> in nutrient medium is 5 mg/L. The endpoints are presumably based on fresh weight of emergent shoots.

**Limitations of Study:** Exposure concentrations are not provided although the report states that values were close to nominal for many of the compounds. For the chlorobenzenes at time 0, concentrations varied between 15 - 131% of nominal and were attributed to the high volatility of the compounds. By the end of the study, concentrations had dropped to  $\leq$ 30% of the initial concentration. However, exposure concentrations declined to <30% of nominal toward the end of the study. The exact nature of the EC<sub>50</sub> is not well described, but is presumed to be based on fresh weight of emergent shoots. As this measurement is typically associated with terrestrial plants, it is not considered applicable to aquatic plants.