Appendix H. Summary of available ecotoxicity information for all vinclozolin degradates and formulated products

Vinclozolin Open Literature Reviews

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 108963 Bayley, M., P. F Larsen, H. Bækgaard, and E. Baatrup. 2003. The Effects of Vinclozolin, an Anti-Androgenic Fungicide, on Male Guppy Secondary Sex Characters and Reproductive Success. Biology of Reproduction 69: 1951-1956.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-19-09

Summary of Study Findings: This study examines the effects of vinclozolin exposure on guppy (*Poecilia reticulata*) reproduction and condition.

The experimental animals in this experiment came from a stock group maintained in the author's lab since 1997. In both stock and experimental tanks the fish were kept at 25 ± 2°C at with a pH of 7.3 and conductivity of 600 μS/cm. They were fed daily with newly hatched artemia and commercial fish food.

Three experiments were carried out during this study. One observed the effects on male sexual phenotype, one the effects on females before insemination, and one the effects on pregnant females.

For each of these experiments, exposure was the same. Guppies were dosed through their food which was treated with a solution vinclozolin dissolved in acetone. The control group's food was treated with acetone alone. The concentrations of vinclozolin used were 0.1, 1.0 and 10.0 μg vinclozolin/mg dry food. Fish were given an average of 18 mg dry food per gram of fish per day, which resulted in a nominal concentration of 1.8, 18 and 180 mg vinclozolin/ kg body weight.

There was no mortality observed in any of the treatments.

For the first experiment, 75 virgin females were randomly chosen from a tank of virgin females and placed in five groups of 15 fish per group in smaller aquaria. Two groups were used as controls and each of the other three received one of the food treatments described above for 30 days. Each group of 15 was then divided into groups of three and placed in tanks that were smaller still. After an acclimatization period, adult males from the lab stock were added for seven days to ensure insemination. The aquaria were then monitored three times daily for 45 days for fry, which were removed immediately to avoid cannibalism. A slight reduction in the size of the first clutch was seen amongst the treated groups, but no reduction was of a statistically significant level.

The second experiment involves 23 groups of three grown (and assumed pregnant) females that were removed from the stock aquarium. Eight groups served as controls with the rest divided evenly amongst the three dose levels. Tanks were monitored for 45 days in the same manner as

described above. Just as in the virgin female trials, there was no significant effect seen in clutch size.

The third experiment focused on 75 virgin males split into five groups. Two of the groups were controls, and each of the remaining groups was treated with one of the doses described above. Animals were dosed for 30 days and kept in their aquaria for on additional day. Eight control males and five males from each dosing group were randomly selected and transferred to clean tanks containing three virgin females each. They remained in the tanks for seven days to ensure insemination. The females were monitored for 45 days and their egg laying habits were recorded. After their 7-day mating period, males were placed individually with adult females to measure mating behaviors. After observation of mating behavior, a sperm count was performed on each male. Exposed males showed significantly reduced clutch sizes compared to control group males. A standard dose response pattern emerged and can be seen below (**Figure 1a**.); the number of first clutch juveniles per aquarium was statistically different than controls in the 1 ($p<0.05$) and 10 µg/mg ($p<0.01$) dry food treatments. The authors conclude from this that exposure of male guppies from 10 to 14 wks had a deleterious effect on first-clutch size after copulation with virgin females. Exposure of virgin females from 10 – 14 wks had no significant effect on first clutch size (**Figure 1b**) and exposure of pregnant adult females had no significant effect on first clutch size after cessation of exposure (**Figure 1c**) According to the authors, oral [dietary] exposure to vinclozolin from 10 to 14 wks caused a significant reduction in sperm count that was dose dependent (Figure 2). Sperm counts however were only statistically different ($p<0.05$) from controls in the 1 and 10 µg/mg diet treatment (**Figure 2**). A loose positive correlation was made between the sperm counts of individuals and the number of offspring they produced (**Figure 3**); while this correlation was significant ($p<0.05$), the adjusted R^2 value ($R^2=0.162$) suggests that it is not very predictive. A dose-response relationship was observed in mating display behaviors, although only the highest dose level was significantly different from the control. No correlation was found between mating displays and either offspring (clutch size) produced or sperm count.

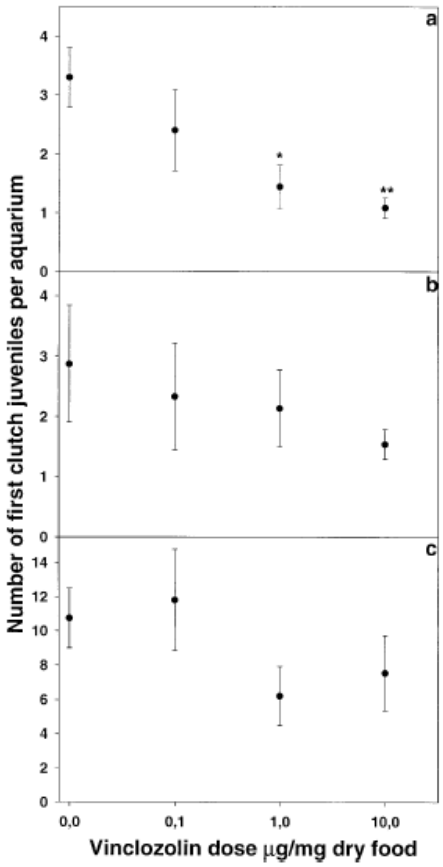

FIG. 1. Effects of dietary vinclozolin exposure on guppy reproductio presented as the average size of the female first clutch. **a**) Exposure c male guppies from 10 to 14 wk has a deleterious effect on first-clutc size after copulation with virgin females. **b**) Exposure of virgin female from 10 to 14 wk has no significant effect on first-clutch size. **c**) Exposur of pregnant adult females has no significant effect on first clutch afte cessation of exposure. Error bars show the SEM. For all three experiments n = 8 for controls and 5 for treatments. Error bars show the SEM. * <0.05, ** $P < 0.01$.

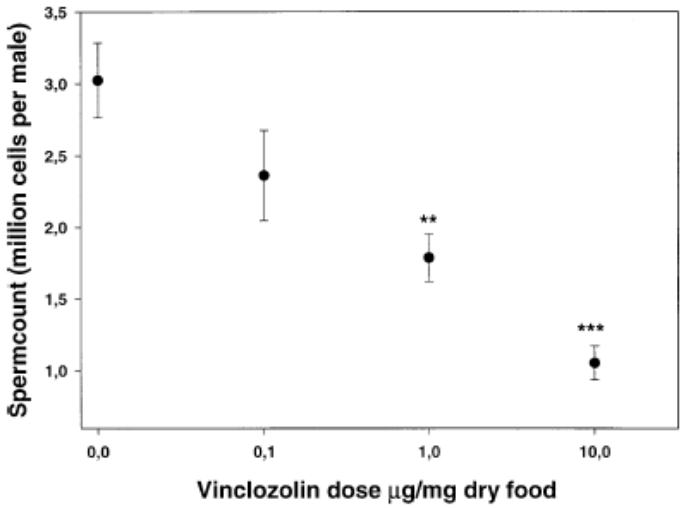

FIG. 2. Oral exposure to vinclozolin from 10 to 14 wk causes a sig icant reduction in male sperm count. The sperm count was performed individual males after they had been kept with their designated vir female for fertilization for 7 days and subsequent measurement of th sexual behavior. Error bars show the SEM. $^{**}P < 0.01$, $^{***}P < 0.001$

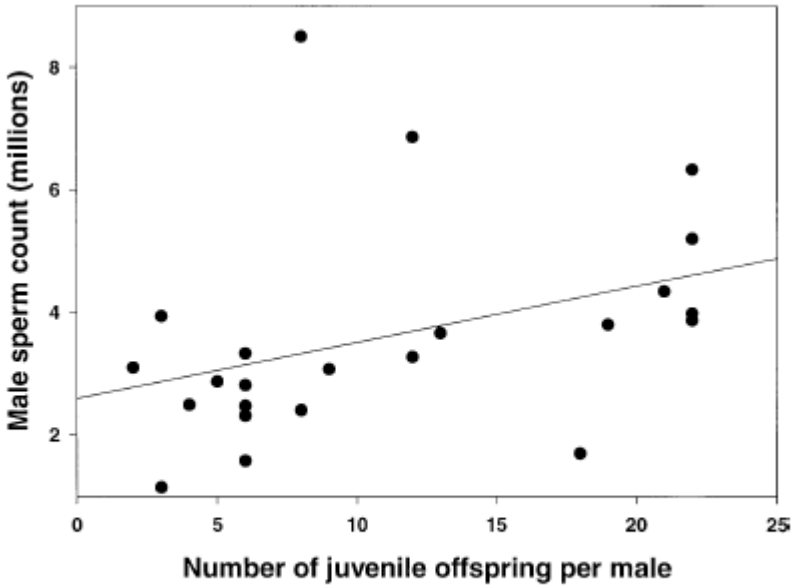

FIG. 3. Male sperm count is significantly correlated to the size of the first clutch. Pearson correlation coefficient $R = 0.45$, adjusted $R^2 = 0.162$, $P = 0.033$.

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

Rationale for Use: Although actual exposure levels are a major uncertainty in this study, the results indicate that exposure of male guppies from 10 to 14 weeks significantly reduced clutch size and significantly reduced sperm count at 1 and 10 µg/mg diet.

Limitations of Study: The source and purity of the vinclozolin used in the study are not reported and exposure concentrations were not verified through any measurements. Fish were exposed to vinclozolin in their diets and it is unclear whether all of the vinclozolin-treated feed was

consumed and/or whether there was any aversion to the food in any of the treatment groups. As such, actual exposure levels are highly uncertain in this study.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 104562 Haeba, M., H., K. Hilscherová, E. Mazurová and L. Bláha. 2008. Selected Endocrine Disrupting Compounds (Vinclozolin, Flutamide, Ketoconazole and Dicofol): Effects on Survival, Occurrence of Males, Growth, Molting and Reproduction of *Daphnia magna*. Env. Sci. Pollut. Res. 15 (3), 222-227

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-04-09

Summary of Study Findings: This study examines the acute, sub-chronic and chronic effects of vinclozolin on *Daphnia magna*.

Study animals came from a long term laboratory culture that had been maintained for more than three years under controlled conditions (20 ± 2°C, 16/8 L/D). These conditions were used during each of the trials. Dimethylsulfoxide (DMSO) was used as a solvent in each of the trials.

The acute toxicity test was carried out using animals less than 24 hours old. Animals were exposed for 48 hours, during which time they were not fed. Water was not renewed or changed. Immobilization was assessed as mortality and was recorded after 24 and 48 hours. Vinclozolin treatments did not result in mortality at any level including its solubility limit (> 3 mg/L).

Sub-chronic toxicity testing was conducted with gravid 10-14 day old females. Animals were exposed to a sub-lethal dose based on previous acute testing. The exposure medium was renewed every 48 hours and each dose was replicated in 10 individuals. The first batch of eggs was discarded due to incomplete exposure. The second set of eggs laid was removed and had its sex ratio analyzed. Vinclozolin significantly ($p<0.05$) altered the sex ratio, reducing the number of males by a factor of 2 (**Table 2**) in the highest concentration of vinclozolin tested, i.e., 1 mg/L. Based on these study results, the NOAEC for sex ratio from this study would be 0.1 mg/L or 100 µg/L.

Table 2: Overview of the effects of tested compounds (at indicated concentrations) on the sublethal parameters in sub-chronic (4–6 day) and chronic (21 day) bioassays with *D. magna*

	Assay type	Dicofol (0.1 mg/L)	Ketoconazole (1 mg/L)	Flutamide (1 mg/L)	Vinclozolin (1 mg/L)
Sex ratio (males/total)	*sub-chronic*	increase (3-fold, p<0.05)	no	no	decrease (2-fold, p<0.05)
	chronic	no	no	no	No
Neonate numbers	*sub-chronic*	no	no	no	No
	chronic	no	no	decrease (2-fold, p<0.05)	no
Size of maternal organisms	*chronic*	no	no	suppression (64%, p<0.05)	no
Maturation	*chronic*	no	no	delayed (50%, p<0.05)	no
Molting	*chronic*	no	no	no	no

A chronic (21 day) reproduction assay was started with females younger than 24 hours. Ten replicates were used per treatment and the exposure media were renewed every 48 hours. Dose level is not stated. Subjects were kept individually in 50 ml beakers of medium. They were evaluated on the following parameters: number of offspring and their sex ratios, molting frequency, maturation time, and reproductive organ length. Results from this test are not reported.

Animals from the sub-chronic and chronic trials were fed every other day at the time of medium exchange. No differences in feeding behavior between treated groups and the controls were noted.

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

Rationale for Use: The study provides information on vinclozolin's sublethal effects on *D. magna;* however, the study does not report measured concentrations and the use of DMSO as a solvent may have affected the uptake of the test compound. Actual response in terms of sex ratio has to be roughly extrapolated off a box graph. Acute toxicity testing failed to establish an EC_{50}.

Limitations of Study: Results are not well reported. Doses are often not well disclosed (if at all) and endpoints provided little useful information.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 71989 Kiparissis, Y., T. L. Metcalfe, G. C.Balch and C. D. Metcalfe. 2003. Effects of the antiandrogens, vinclozolin and cyproterone acetate on gonadal development in the Japanese medaka (*Oryzias latipes*). Aquatic Toxicology. 63, 391-403.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-17-09

Summary of Study Findings: The study examines the effects of vincolzolin as an endocrine disrupting substance on Japanese medaka (*Oryzias latipes)*. The chemical forms used for this study were: the formulated compound Ronilan® EG (50 % a.i. by weight) and "pure compound" (vinclozolin).

The methods used in this study are the same as those described by Gray and Metcalf (1997)[1]. They are briefly outlined. Eggs were obtained from Carolina Biological Supply Company, Burlington, NC and hatched. Exposures at the nominal concentrations listed in **Table 1** started one day post-hatch and ended 100 days post-hatch. Fish were kept in a semi-static (48hr) renewal system. As the fish grew, they were moved to progressively larger containers (range: 1-10 L). Photoperiod was 16:8 L:D and temperature was kept between 22 and 24°C. The fish were fed on a diet of newly hatched brine shrimp twice daily. Each treatment started with 40-75 fish.

Table 1
Range of nominal concentrations (µg/l) of cyproterone acetate and vinclozolin tested in *in vivo* tests with Japanese medaka (*O. latipes*)

Chemical	Nominal concentrations (µg/l)
Cyproterone acetate	0, 1, 10
Vinclozolin (Ronilan®)	0, 1000, 5000
Vinclozolin (pure compound)	0, 2500

Chemicals were dissolved in acetone and administered to each system in 10µl aliquots. Control treatments were given an equal volume of acetone as the experimental treatments.

At the end of the dosing period, fish were sacrificed. They were weighed, measured and examined histologically.

Ronilan® EG Treatment

The sex ratio was not significantly different between the Ronilan-treated and control groups. The highest concentration of Ronilan (5000 ppb; 5 ppm) resulted in two fish (7%) with intersex organs (**Table 2 below**).

Gray, M.A. Metcalfe, C.D. 1997. Induction of testis-ova in Japanese medaka (*Oryzias latipes*) exposed to p-nonylphenol. Environ. Toxicol. Chem. 16, 1082-1086

Table 2
Numbers (percentages) of phenotypic male and female Japanese medaka (*O. latipes*) identified histologically after exposure to the antiandrogens, vinclozolin and cyproterone acetate. (The number (percentage) of the intersex condition, testis–ova observed in male medaka is also presented).

Chemical	Concentration	*N*	Female	Male	Testis–ova
Vinclozolin (Ronilan®)	0	53	22 (42)	31 (58)	0
	1000	45	20 (44)	25 (56)	0
	5000	53	23 (43)	30 (57)	2 (7)
Vinclozolin	0	36	15 (42)	21 (58)	0
	2500	46	25 (54)	21 (46)	0
Cyproterone acetate	0	36	15 (42)	21 (58)	0
	1	75	32 (43)	43 (57)	1 (2)
	10	56	24 (43)	32 (57)	2 (6)

Significant effects (P<0.01) in testicular development were observed in the two groups treated with Ronilan. **Table 4** reports study results.

Table 4
Percent of phenotypic male medaka (*O. latipes*) at different stages of spermatogenesis after 3 months of exposure to antiandrogens. Percent of medaka showing testicular effects is also presented.

Treatment (µg/l)	Stages of spermatogenesis[a] (%)				Testicular effects[b] (%)			
	N	Immature	Intermediate	Advanced	*N*	Normal	↑Fibrosis	↓Spermatozoa
Vinclozolin (Ronilan)								
0	30	0	13	87	30	100	0	0
1000	24	0	38	62	24	48**	28	40**
5000	29	0	78	22***	27	43**	33	20**
Vinclozolin								
0	20	0	30	70	20	100	0	0
2500	18	17	78	5**	15	86	7	7
Cyproterone acetate								
0	20	0	30	70	20	100	0	0
1	36	23	57	20**	27	92	4	4
10	25	15	47	38*	22	68**	14	18**

[a] Stages of spermatogenesis were determined in medaka with total body length > 1.6 cm.
[b] Increases in testicular fibrosis and decrease in the density of mature spermatozoa were determined in medaka which were at the ntermediate or advanced stages of spermatogenesis.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

Ovary development in female fish was also affected (Table 5). Most notably, the highest dose level increased atresia (P<.001) and caused differences in vitellogenic development.

Table 5
Percent of phenotypic female medaka (*O. latipes*) at different stages of oogenesis after 3 months of exposure to antiandrogens. Percent of medaka showing ovarian effects is also presented.

Treatment (µg/l)	Stages of oogenesis[a] (%)					Ovarian effects (%)			
	N	Pre-VtG[b]	Early VtG	Late VtG	Post-VtG	*N*	Normal	Mild atresia	Moderate atresia
Vinclozolin (Ronilan)									
0	20	19	33	24	24	22	100	0	0
1000	15	20	47	33	0	20	90	5	5
5000	23	17	48	35	0	23	53**	30	17**
Vinclozolin									
0	15	20	20	47	13	15	93	7	0
2500	21	29	38	33	0	25	96	4	0
Cyproterone acetate									
0	15	20	20	47	13	15	93	7	0
1	28	63	19	18	0*	32	97	0	3
10	24	42	29	29	0*	24	88	4	8

[a] Stages of oogenesis were determined in medaka with total body length >1.6 cm.
[b] VtG = vitellogenic oocytes.
* $P < 0.05$.
** $P < 0.01$.

Vinclozolin Group

The vinclozolin treated groups saw no difference in sex ratios and had no intersex individuals (**Table 2**). Testicular development in males was not inhibited, but spermatogenesis was affected (P<.01). While there appear to be differences in ovary development and vitellogenesis in female fish, there was no statistical difference between the control and treatment groups (**Table 5**). All morphometric female measurements in this group were significantly different from those in the control group (**Table 3**) as seen below.

Table 3
Number of phenotypic male and female Japanese medaka (*O. latipes*) in the three experiments with vinclozolin and cyproterone acetate. Mean ±S.D. of the morphometric parameters, total length (TL), weight (Wt) and condition factor (CF) are presented for all treatments (values with the same letters are not significantly different from each other).

	Vinclozolin (Ronilan®) (µg/l)			Vinclozolin (µg/l)		Cyproterone acetate (µg/l)		
	0	1000	5000	0	2500	0	1	10
Males (*N*)	31	25	30	21	21	21	43	32
TL (cm)	2.0±0.2 a	2.0±0.3 a	2.2±0.3 b	2.1±0.2 a	1.8±0.2 b	2.1±0.2 a	1.8±0.2 b	1.9±0.2 c
Wt (mg)	66.6±15.1 a	64.3±22.3 a	80.5±25.5 b	82.9±24.2 a	47.6±17.5 b	82.9±24.2 a	50.7±17.7 b	59.1±21.0 c
CF	0.78±0.08 a	0.81±0.14 a	0.78±0.12 a	0.82±0.09 a	0.80±0.08 a	0.82±0.09 a	0.78±0.08 b	0.78±0.09 b
Females (*N*)	22	20	23	15	25	15	32	24
TL (mm)	2.1±0.3 a	1.9±0.3 b	2.2±0.3 a	2.1±0.2 a	1.8±0.2 b	2.1±0.2 a	1.9±0.2 b	2.1±0.2 a
Wt (mg)	70.2±24.2 a	57.6±22.7 a	85.1±26.0 b	85.1±26.3 a	48.9±14.9 b	85.1±26.3 a	54.9±17.8 b	70.2±20.8 c
CF	0.79±0.07 a	0.86±0.16 a	0.82±0.12 a	0.86±0.06 a	0.80±0.06 b	0.86±0.06 a	0.80±0.06 ab	0.75±0.15 b

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

Rationale for Use: The study uses sound experimental and statistical methods to examine the histological effects of vinclozolin as an endocrine disruptor. It uses both the pure chemical, and a formulated compound, illustrating the differences between the two.

Limitations of Study: The background on the fish used for the study is not completely elucidated. It is not clear from the study report whether the concentrations reported for the formulated product (Ronilan EG) are expressed in terms of active ingredient or in terms of formulated product. While the study reports the technical grade compound as "pure", the purity is not reported in terms of percent active ingredient.

Primary Reviewer: TJ Graven, Biologist

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

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 90333 Lorenzini, G., L. Guidi, A. Panattoni, A. 1987. Evaluation of Fungicide Effects on Ozone Injury to Tobacco Plants. Annual Applied Biology. 110 (supplement)

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-10-09

Summary of Study Findings: This study focuses on the ability of certain pesticides to counteract the effects of ozone damage in tobacco plants (*Nicotiana tabacus*). Eight young to mature potted plants per treatment were grown in filtered air and then exposed to ambient air. The study is characterized as a field study conducted in the summer of 1985 and 1986. One experiment consisting of two ozone treatments were conducted in 1985 and 5 experiments consisting of 2 to 4 ozone treatments were conducted in 1986. Plants were treated with Ronilan (50%) at a rate of 1500 g a.i./ha. According to the methods, treatments were foliarly applied to run-off [drench]. Four days after the end of treatments, the percentage of necrotic area was determined from the leaves. The study gives rough details of the experimental setup and statistical methods before concluding that vinclozolin (Rovral®, 50% a.i.applied at 1500 g a./i./ha) did not prevent or combat ozone damage.

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

Rationale for Use: Although the study might be interpreted as evidence that vinclozolin treatment did not result in damage to the tobacco plants, the plants did incur roughly the same amount of damage as plants treated with tap water alone.

Limitations of Study: The results of the study are difficult to interpret from the information provided. A number of data points are missing without explanation. At face value, vinclozolin treatment did not appear to be substantially different from tap water alone. It is not possible to determine whether the application is in terms of active ingredient or formulated product and it is not clear how many applications of vinclozolin were made to the plants.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 51826 Makynen, E.A. M. D. Kahl, K. M. Jensen, J. E. Tietge, K. L.. Wells, G. Van Der Kraak, and G. T. Ankley. 2000. Aquatic Toxicology. 48, 461-475.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-07-09

Summary of Study Findings: The study examines the effects of vinclozolin on fathead minnows (*Pimphales promelas*). It examined early life stage dosing beginning at >6-hr old and exposed for 34 days, then placed in clean water for 4 – 6 months. The study also examined adult dosing for 21 days. Animals were measured for reproductive success, growth rate and in some cases androgenic receptor binding. In the early life trials, the only difference noted was a reduction in body weight of the highest dose groups. The adult trials saw very few effects, but did note an increase in vinclozolin metabolites with increased vinclozolin concentrations. Based on reductions in female gonadosomatic index, the NOAEC would be 200 μg/L and the LOAEC would be 700 μg/L based on the adult study. For the ELS study, the NOAE would be 600 μg/L based on reduced 34-day body weight and the LOAEC would be 1200 μg/L; this effect though appeared to be transitory as 90-day fish weights were not statistically different between vinclozolin treated and control fish.

All experiments were conducted in filtered water from Lake Superior. The water had the following parameters: pH 7.1-8.0, dissolved oxygen, 5.5-7.8 mg/l, hardness 44.5-46.0 mg/l CaCO3. The desired concentration of vinclozolin was achieved by passing the water through a column of glass wool coated with the chemical.

Vinclozolin used in this experiment was 99% pure. Metabolites M1 (2-{[(3,5-dichlorophenyl)-carbamoyl]oxy}-2-methyl-3-butenoic acid) and M2 (3',5'-dichloro-2-hydroxy-2-methylbut-3-enanilide) were obtained from the US EPA and M3 (3,5-dichloroaniline) was purchased from Aldrich Chemical Co.

For the early life assays, newly fertilized eggs were obtained from the EPA lab in Duluth, MN. They were exposed until approximately 30 days post-hatching. Following this period, they were held in clean water until they reached maturity. Some of the animals were allowed to breed to determine the reproductive effects of the early dosing. The dosing levels were as follows: 75, 150, 300, 600 and 1200 μg/l with a flow rate of 6 ml/min. The tanks used were partitioned into three equal sections, each with its own egg basket. Approximately 70 eggs from spawns at the source facility were placed into each egg basket immediately (<4 hrs) after fertilization. This created three replicates of each dose level. After 24 hours, the eggs were examined for viability and culled to 50 eggs per basket. The test systems were then maintained at 25 ± 1°C with a 16:8 L:D photoperiod until the eggs hatched (4-5 days). On the sixth day, the fry were released from

the egg cups. They were fed brine shrimp three times daily. Animals were counted on Day 14 as a basis for future survival determination. On Day 34, 25 fish were removed from each tank partition and transferred clean 5 l sections of a 120-L tank to be raised to maturity. After 90 days the animals were again culled, this time to 15 individuals. At seven months of age, one pair of fish was taken from each original group of eggs (3 pairs from each different treatment group). Each three pair set was placed in a 20-L aquarium containing spawning substrate for 30 days. They were observed daily for spawning activity and any eggs laid were assessed for viability.

In the early life stage study, mean (±std deviation) exposure levels in the 75, 150, 300, 600 and 1200 treatments were 89±25.2, 162±576, 267±99.8, 543±177 and 1170±363 µg/L, respectively.

Table 2 summarizes the results for this experiment. The only significant difference observed between controls and dosed fish was weight of 34-day old fish in the 1200 µg/L treatment; weights of these fish were 33% lower. None of the other endpoints, i.e., survival, sex ratio or fecundity were statistically different from controls. By 90 days, none of the fish weights from any of the vincolozolin treatments were significantly different from controls.

Table 2
Survival, growth, sex ratio and fecundity of fathead minnows exposed as early embryos and larvae to vinclozolin for 34 days[a]

Target Concentration (µg l^{-1})[b]	10-Day[c] survival (%)	10–34-Day survival (%)	34-Day weight (mg/fish)	34–90-Day survival (%)	90-Day weight (mg/fish)	90-Day to 7-month survival (%)	Sex ratio[d] (%)	Fecundity[e]
Control (0)	84 ± 14	79 ± 9	100 ± 27	95 ± 7	515 ± 69	98 ± 4	51 ± 10	121 ± 30 (11)
75	84 ± 9	78 ± 14	102 ± 24	95 ± 3	553 ± 64	100 ± 0	69 ± 20	98 ± 13 (26)
150	87 ± 7	77 ± 12	96 ± 22	98 ± 3	493 ± 30	91 ± 9	52 ± 16	96 ± 40 (8)
300	82 ± 5	85 ± 8	103 ± 16	95 ± 4	542 ± 30	98 ± 4	54 ± 13	94 ± 47 (13)
600	70 ± 31	75 ± 16	101 ± 22	95 ± 5	567 ± 79	92 ± 10	64 ± 13	49 ± 43 (9)
1200	70 ± 13	74 ± 6	67 ± 27*	96 ± 3	527 ± 77	92 ± 6	52 ± 16	92 ± 52 (9)

[a] Values indicated by an asterisk differ significantly from controls. Values are expressed as mean ± S.D., ($n = 6$, unless otherwise indicated).
[b] See Table 1 for specific concentrations.
[c] Days post-hatch.
[d] Percent male.
[e] Eggs/spawn (total number of spawns), from three pairs of fish, except for the 150 µg l^{-1} treatment, where there were two spawning pairs.

For the adult exposure portion of the study mature animals were obtained from the EPA lab in Duluth, Minnesota. The animals were paired and placed in 4.5-L tanks with flow rates of 16.7 mL/ min. The dose levels for this experiment were 200 and 700 µg/l. The dose concentrations were achieved in the same manner as in the early life experiment. Conditions were also the same as those used earlier. Reproductive activity (behavior and egg deposition) was observed during the 21-day dosing period. After cessation of dosing, the fish were anaesthetized and their blood was sampled for analysis. Fish were then weighed and their gonads were removed. The remaining carcass was used to determine vinclozolin and metabolite concentrations. **Table 3** shows those concentrations. In the adult trial, reproductive success could not be determined because of a near complete lack of spawning in all groups including controls; the study authors state that it was not possible to determine the effects, if any, of vinclozolin on fecundity and viability. There was no discernable difference in the expression of sexual characteristics and most measured androgens were similar (**Table 3**). Gonadosomatic index (GSI) in females (**Table 4**) was significantly different than controls ($p<0.05$) in fish treated with 700 µg/L; the GSI was roughly 63% lower in the vinclozolin-treated fish relative to controls. Additionally, plasma β-estradiol concentrations were significantly different in male fish treated with 700 µg/L; estradiol levels in males treated with 700 µg/L were 1.8X higher than controls. There was no qualitative difference in histological sections of testes between control and treated fish; the testes

were well developed with mature spermatozoa. Histological sections of the ovaries did not reveal any qualitative differences except that oocytes in females from the high treatment were substantially smaller than those from eith the lower vinclozolin treatment or controls. The authors state the the reduction in oocyte was seemingly due to retarded maturation of the oocytes, which resulted in a higher prevalence of primary oocytes and that the results were consistent with the reduced GSI observed in females from the 700 μg/L treatment.

There was a dose-dependent increase in tissue residues corresponding to the two water concentrations (**Table 3**). At the higher concentration, females accumulated significantly more ($p<0.05$) vinclozolin than males from the same tank. For a given sex, vinclozolin concentrations in individual fish varied by a factor of 4 to 5. M1 was detected in all fish with greater concentrations in fish from the higher exposure concentration. M2 and M3 were also detected primarily in fish from the higher exposure concentration but there did not appear to be any sex-related difference in tissue concentration.

Table 4
Effects of a 21-day vinclozolin exposure on gonadal status and plasma steroid concentrations (μg l^{-1}) in mature male and female fathead minnows[a]

Target concentration (μg l^{-1})[b]	GSI (%)[c]		β-Estradiol		Testosterone		11-Keto-testosterone	
	Male	Female	Male	Female	Male	Female	Male	Female
Control	1.38 ± 0.43(4)	11.61 ± 3.60(4)	0.80 ± 0.16(3)	3.52 ± 1.22(4)	5.32 ± 4.82(4)	3.79 ± 3.63(2)	7.09 ± 9.11(2)	NM[d]
200	1.27 ± 0.45(4)	14.48 ± 5.27(4)	0.57 ± 0.10(4)	5.53 ± 3.75(4)	14.73 ± 12.9(4)	3.39 ± 0.29(3)	NM	NM
700	1.27 ± 0.16(4)	4.24 ± 1.58(4)*	1.46 ± 0.22(2)*	6.68 ± 4.76(4)	6.40 ± 3.05(4)	4.75 ± 2.90(3)	16.08 ± 9.18(4)	NM

[a] Values are expressed as mean ± S.D. (*n*). Values indicated by an asterisk differed significantly from controls.
[b] See Table 3 for specific water and tissue concentration data.
[c] Gonadal-somatic index calculated as (gonad wet weight/body wet weight) × 100.
[d] NM, not measured due to inadequate plasma volumes.

A third portion of this experiment was carried out to assess the binding affinity of vinclozolin to androgenic receptors. Vinclozolin and M1, at concentrations up to 50 μM, were ineffective in competing with testosterone for binding sites in brain extracts. At very high levels, M2 should "limited ability" to compete for testosterone binding sites; however, solubility limited testing at higher concentrations. Similar results were obtained in the competition studies using ovary cytosolic extracts.

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

Rationale for Use: The study observes both early and late life dosing. Experimental conditions are well described and exposure is quantified through measured concentrations

Limitations of Study: Failure of the fish to spawn in the adult study.

Primary Reviewer: TJ Graven, Biologist

Secondary Reviewer: Thomas Steeger, Ph.D., Senior Advisor

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 106557 Martinovic, D., L. S. Blake, E. J. Durhan, K. J. Greene, M. D. Kahl, K. M. Jensen, E. A. Makynen, D. L.Villeneuve and G. T Ankley. 2008. Reproductive Toxicity of Vinclozolon in the Fathead Minnow: Confirming an Anti-Androgenic Mode of Action. Environmental Toxicology and Chemistry 27.2, 478-488.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-04-09

Summary of Study Findings: The 21-day reproduction study found that exposure to vinclozolin reduced the number of eggs produced by fathead minnows (*Pimphales promelas*). The magnitude of the reduction of egg production corresponded to the dose level. The study reports complete reproductive failure at a treatment concentration of 700 µg/L The hatching success of the eggs was not impacted by vinclozolin. The study also found that vinclozolin decreased expression of secondary sexual characteristics in males. Vinclozolin also blocked the expression of 17β-trenbolone-induced tubercles in females was considered by the authors as evidence of the anti-androgenic activity of vinclozolin. Vinclozolin exposure resulted in increased gonadal weight, androgen receptor and 11β-hydroxysteroid dehydrogenase mRNA transcription and *ex vivo* gonadal production of testosterone and 11-ketotestosterone.

Adult *P. promelas* used in the study were obtained from the culture facility at the U.S. EPA lab in Duluth Minnesota. The vinclozolin used for the study was 99% pure and not in any formulated mixture.

In experiment one, 20L tanks were divided into two sections. One male and one female (both randomly selected from their respective groups) were placed in each half of each tank. Each tested concentration level had 10 pairs of fish, including the control pair. The fish were kept at 25°C and fed twice daily with a photoperiod of 16: L:D. The fish were given three weeks to acclimate to their new habitat. Survival, general appearance, reproductive behavior and reproductive success were monitored during this period. Eggs laid were removed from the substrate daily, counted, and examined to determine fertility. Pairs that did not reproduce within the initial three week period were excluded from the study. Water from Lake Superior was passed through a column containing glass wool treated with vinclozolin. The resulting flow-through was then diluted with UV treated Lake Superior to achieve the desired concentration of vinclozolin. The solutions were delivered to exposure ranks at a rate of 45 ml/min which achieved a renewal period of 3.5 hours. Each pair was allowed to acclimate for Vinclozolin concentrations in the tanks were measured at least twice weekly using high performance liquid chromatography. Toxcity testes were performed using the general experimental design and techniques described by Ankley *et al*[1]. The exposures lasted 21 days and were conducted with the following target (measured) vinclozolin concentrations: 100µg (60µg), 400µg (255µg) and 700µg (450µg). During the experimental run, tanks were maintained in the same manner as during acclimatization with the exception that eggs were only removed from a randomly selected

subset of pairs. Once the eggs were collected, they were maintained in clean water for five days to determine hatching success.

Following 21 days of exposure, anaesthetized with tricaine methane sulfonate. They were weighed and measured. Urine was collected and stored at -80°C for later analysis. Nuptial turbercles were counted and ranked by their size. Blood was collected from the caudal vein and centrifuged to separate hemaglobin and plasma. Plasma vitellogenin concentrations were later measured using ELISA. Plasma 17β-estradiol and testosterone were measured using a radioimmunassay. Dorsal pads (from the head) gonads, liver tissues were removed, weighed and analyzed. Gonads were examined histologically as well.

A similar initial setup used in experiment one was used in experiment two. For experiment two, flow rate was increased to 0.4 ml/min and water sampling technique was changed slightly. Also, the tanks were not divided into two and 15 fish (12 female, 4 male) were placed in each. Fish were acclimated for 7 days. Chemicals were dosed in the following combinations: 500 ng 17β-trenbolone (TB)/L, 200 µg vinclozolin/L, 700µg vinclozolin/ L, 500 ng TB/L + 200 µg vinclozolin/ L, 500 ng TB/L + 700 µg vinclozolin/L. The exposure period was shortened to 13 days. Tissue and plasma samples were taken from four females in each exposure tank after 48h, 96h, and 13 days. Analytical procedures were similar to those done in experiment one.

No mortality was observed in 21-day study nor was any abnormal behavior observed. There was concentration dependent decline in the cumulative number of eggs produced (**Figure 1**); fish exposed to 450 µg/L failed to reproduce. Fish from all of the vinclozolin treatments produced fewer eggs than control fish. Fertility and hatching success of deposited effects were not affected.

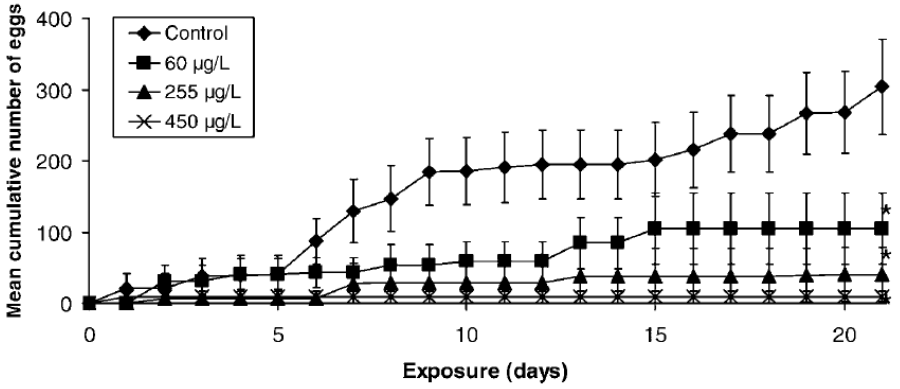

Fig. 1. Effects of vinclozolin on fecundity of fathead minnows in a 21-d test. Data are expressed as mean ± standard error of the mean (n = 10) cumulative number of eggs per female, per day and per treatment. Values with an asterisk differed significantly from control.

Females exposed to vinclozolin had significantly higher body weight at each of the vinclozolin concentrations tested (**Table 1**). The gonadosomatic index of males exposed to 255 (2.03 ± 0.21) and 450 µg/L (2.07 ± 0.16) was significantly higher than controls (1.38 ± 0.10); while the GSI is significantly higher at the two higher treatment concentrations, the extent of the increase did not appear to be dose-dependent as the GSI was roughly similar although the concentrations differed by a factor of 1.7X.

Table 1. Body weights androgen receptor expression of female fathead minnows exposed in control and vinclozolin treatments.

Treatment µg/L	Female Body Weight in grams (mean ± std error)	Androgen Receptor mRNA transcription (copies/ng RNA)
0	**1.40 ±0.07 g**	**2,128.3 ± 456.9**
60	**1.71 ± 0.09 g***	**1,302.9 ± 126.6**
255	**1.75 ± 0.11 g***	**2,056.7 ± 144.52**
450	**1.67 ± 0.07 g***	**1,996.3 ± 167.2**

Exposure to vinclozolin at 255 and 450 µg/L caused a significant reduction in the tubercle score (**Figure 2**) relative to controls; dorsal pad index was significantly reduced in males treated with 450 µg/L.

The incidence of oocyte atresia in controls was 20% whereas in the 60, 255 and 450 µg/L treatments, the incidence was 40, 40 and 90%, respectively. The severity of the atresia was significantly increased in females treated with 450 µg/L.

Plasma vitellogenin concentrations were significantly elevated in females treated with 255 and 450 µg/L (**Figure 3**) while vitellogenin levels were low in males regardless of treatment level (**Figure 4**).

Ex vivo testosterone production by gonad preparations from females had significantly increased testosterone production in fish exposed to vinclozolin at 60, 255 and 450 µg/L (**Figure 3**). In males, exposure to vinclozolin at 255 and 450 µg/L significantly increased 11-ketotestosterone relative to controls. Exposure to 450 µg/L increased testicular androgen receptor and 11β-hydroxysteroid dehydrogenase mRNA transcription (**Figure 5**). According to the study, androgen receptor expression was down-regulated in the ovary of females exposed to vinclozolin at 60 µg/L, but in none of the other vinclozolin treatments, relative to controls.

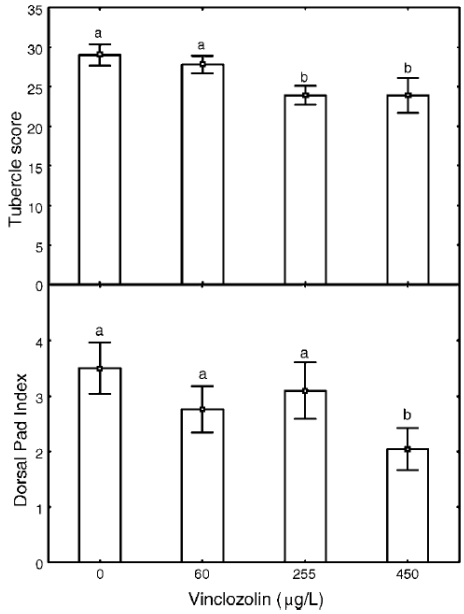

Fig. 2. Effects of vinclozolin on expression of secondary sexual characteristics in male fathead minnows (mean ± standard error of the mean; $n = 10$) following a 21-d exposure. Treatments labeled with common letter(s) do not differ significantly from each other ($p \leq 0.05$).

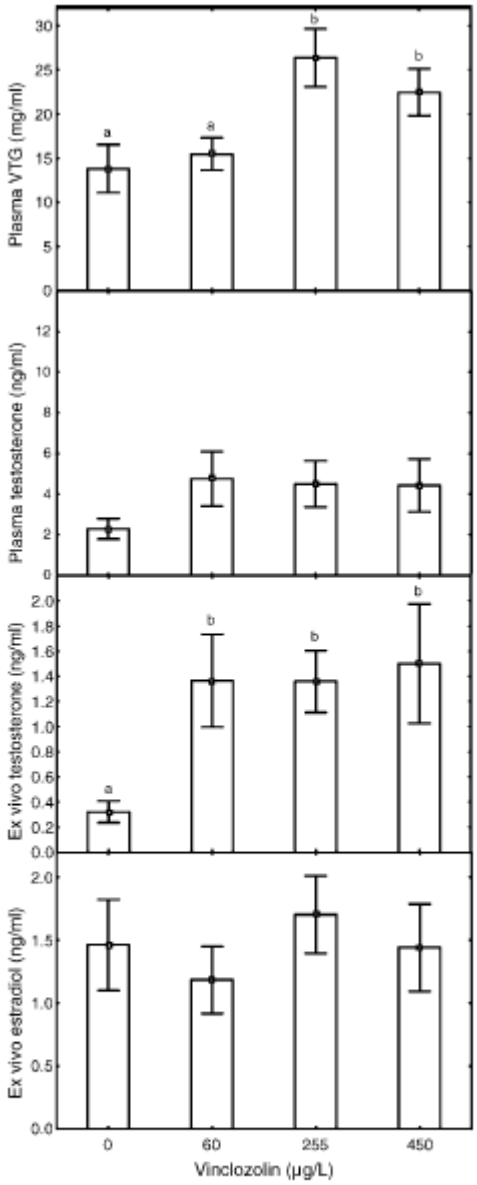

Fig. 3. Effects of vinclozolin on concentrations of plasma vitellogenin (VTG), testosterone, and ex vivo gonadal synthesis of testosterone and 17β-estradiol in female fathead minnows following a 21-d exposure (mean ± standard error of the mean; $n = 8–10$). Treatments labeled with common letter(s) do not differ significantly from each other ($p \leq 0.05$).

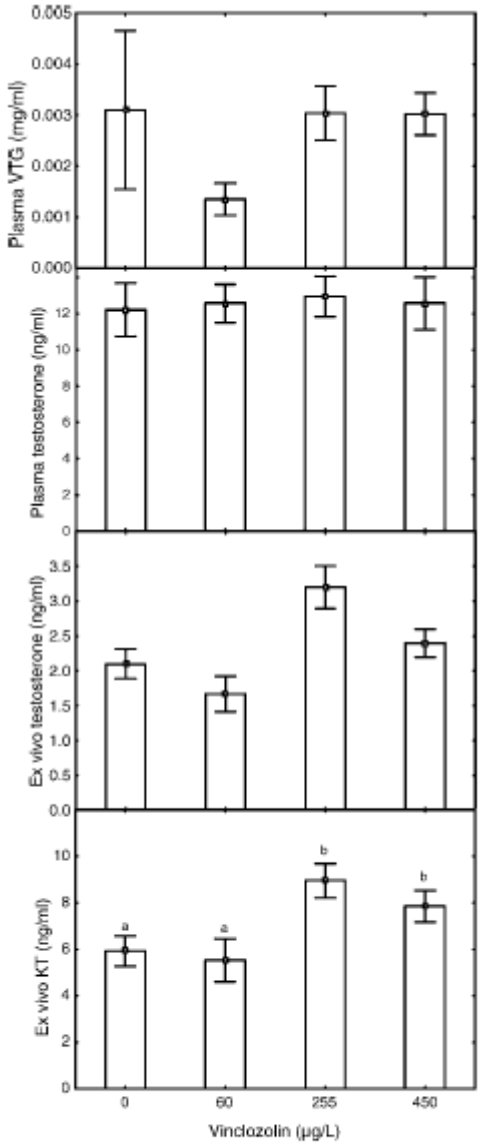

Fig. 4. Effects of vinclozolin on concentrations of plasma vitellogenin (VTG), testosterone, and ex vivo gonadal synthesis of testosterone and 11-ketotestosterone (11-KT) in male fathead minnows following a 21-d exposure (mean ± standard error of the mean; n = 9–10). Treatments labeled with common letter(s) do not differ significantly from each other ($p \leq 0.05$).

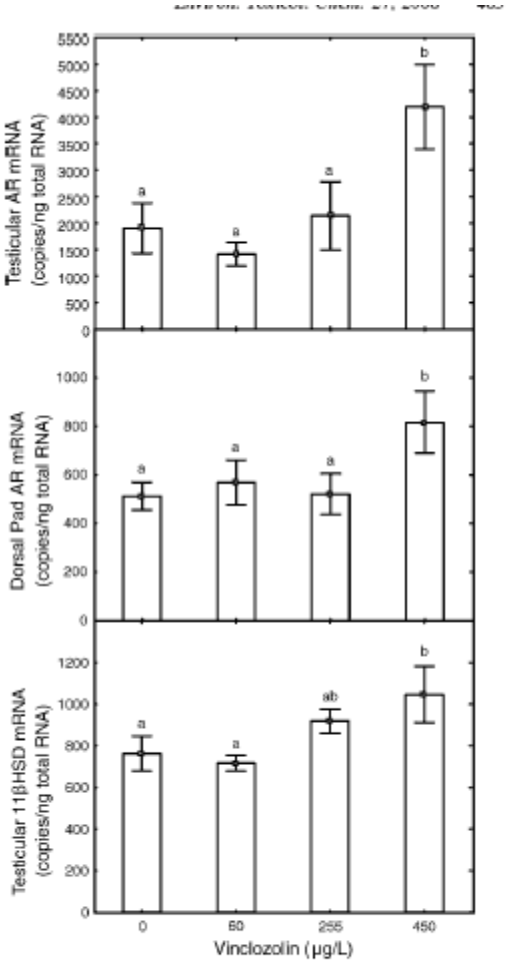

Fig. 5. Effects of vinclozolin on expression of testicular and dorsal pad androgen receptor (AR), and testicular 11β-hydroxydehydrogenase (11βHSD) mRNAs of male fathead minnows following a 21-d exposure (n = 6–10). Treatments labeled with common letter(s) do not differ significantly from each other ($p \leq 0.05$).

In the second study, no mortality was observed and neither body mass nor GSI was affected. Cotreatment of fish with vinclozolin and 17β-trenbolone blocked masculinization (as evidenced by tubercle formation) caused by trenbolone treatment alone. None of the fish treated with vinclozolin at 200 or 700 µg/L plus trebolone exhibited tubercles. Similar to the first study, treatement with 700 µg/L resulted in up-regulation of androgen receptor mRNA in the testes; however, cotreatment with trenbelone blocked this response.

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

Rationale for Use: This study quantifies the effects of vinclozolin on an aquatic vertebrate. It uses sound laboratory techniques, which are explained in detail, and presents its results in depth.

Limitations of Study: Some of the responses reported for vinclozolin-treated animals show statistically significant differences from controls, the responses do not necessarily reflect dose-responsive differences as the

Primary Reviewer: TJ Graven, Biologist

Secondary Reviewer: Thomas Steeger, Ph.D., Senior Advisor

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 66731 McGary, S., P. F. P Henry and M. A. Ottinger. 2001. Impact of Vinclozolin on Reproductive Behavior and Endocrinology in Japanese Quail (*Cotrunix coturnix japonica*). Environmental Toxicology and Chemistry. 20, 2487-2493

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-12-09

Summary of Study Findings: Three replicates were used for this study, but statistical analysis indicated that variance between the groups was not significant so the results from all groups were pooled.

The study was replicated in triplicate with Eggs were randomly collected from a bred Japanese quail colony at the University of Maryland's Department of Animal and Avian Sciences in College Park, Maryland. Collected eggs were stored at 7°C until a sufficient number had been collected (5 days). The eggs were then incubated at 37°C with 65% humidity. On day four of the incubation fertile eggs (determined by candling) were dosed by injection into the air space of each egg. The treatment groups were as follows; control (no injection), sham (injected with 5 µl corn oil), 25, 50, 100 ppm vinclozolin. The vinclozolin treatments were dissolved in ethanol and the ethanol was allowed to evaporate; vinclozolin-treated eggs were all injected with 3 µl corn oil. Their concentration was calculated based on an average egg weight of 10 g. Injection sites were drilled prior to injection and immediately sealed with surgical tape following injection. The study states that hatchlings were maintained in mixed sex groups at **95 ° C** until adult plumage and thermoregulatory capabilities were developed. At four weeks of age, males were transferred to individual cages while females remained in group cages. The study gives contradicting accounts of how hatchlings were selected for measurement, but a number (20 per treatment) were sacrificed and measured.

Adult birds were weighed and examined bi-weekly. Males were examined for cloacal gland development and cloacal foam production. Sexual behavior was observed and analyzed once maturity was attained. At seven weeks of age all birds were sacrificed for measurement.

There was no observed effect on mounting latency or mounting attempts in any of the treatment groups. Mounting frequency and cloacal contacts were significantly reduced in the 25 and 50 ppm treatments.

Frozen brains of hatchlings and adults were prepared and analyzed for gonadotropin releasing hormone (GnRH-I) activity. GnRH-I was unaltered in the females of the study. GnRH-I levels in hatchling males was significantly altered by all treatment groups, although it should be noted that the sham treatment was also significantly different from the control group. Adult males showed significantly higher GnRH-I levels only in the 25 ppm treatment. The graphs below display these results.

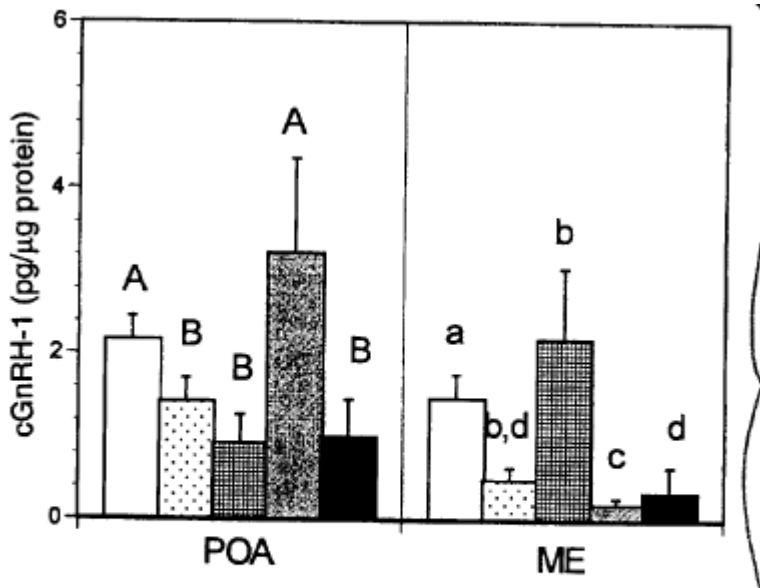

Fig. 1. Hypothalamic gonadotropin-releasing hormone I (GnRH-I) in hatchling male Japanese quail treated with vinclozolin. Mean ± standard error. Capital letters denote medial preoptic area (POA) and lowercase letters denote median eminence (ME) mean comparisons across treatments. □, Control, n = 29; ⊡, oil, n = 20; ▦, 25 ppm, n = 17; ▨, 50 ppm, n = 12; ■, 100 ppm, n = 9.

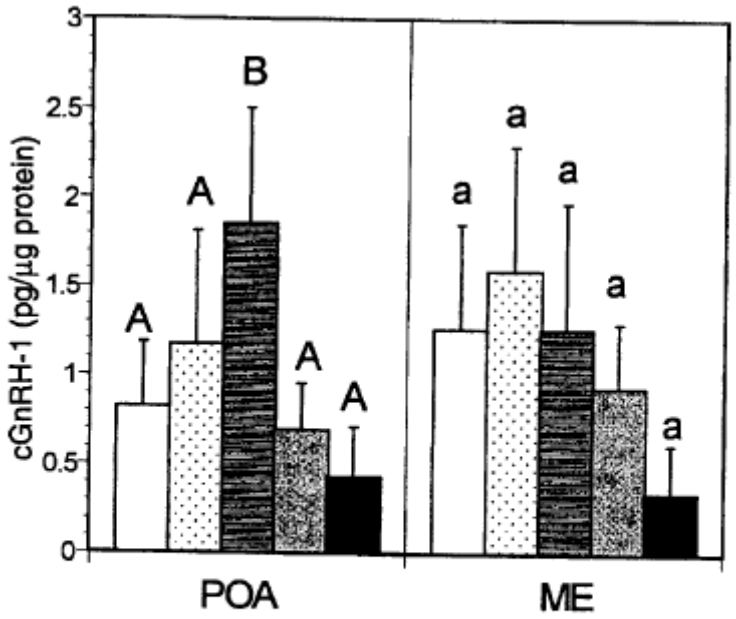

Fig. 2. Hypothalamic gonadotropin-releasing hormone I (GnRH-I) in adult male Japanese quail treated with vinclozolin. Mean ± standard error. Capital letters denote medial preoptic area (POA) and lowercase letters denote median eminence (ME) mean comparisons across treatments. □, Control, n = 9; ⊡, oil, n = 13; ▤, 25 ppm, n = 6; ▨, 50 ppm, n = 12; ■, 100 ppm, n = 4.

Adult and hatchling plasma was analyzed for estradiol and androgen level. No statistical difference was found in the level of any steroid in any group.

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

Rationale for Use: Not applicable

Limitations of Study: Purity and source of the vinclozolin used in the study are not specified. The method of exposure, i.e., injection through the egg shell into the egg air space has limited relevancy to what may occur in the field. Dosages were not selected based on measured residues in eggs but rather were selected to result in effects on endocrine-mediated processes. The study also states that hatchlings were maintained at 95° C, this is presumed to be a typographical error; however, even at 95^{o}F, the brood chamber would be relatively warm. According to the results, the oil (vehicle control) was significantly different ($p<0.0051$) from controls for median eminence GnRH-I and for preoptic area; as such, there is a significant solvent effects and the study is classified as scientifically unsound.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 74217 Niemann, L., B. Selzsam, W. Haider, C. Gericke and I. Chahoud. 2004. Effects of Vinclozolin on Spermatogenesis and Reproductive Success in the Japanese Quail (*Coturnix coturnix japonica*). Archives of Environmental Contamination and Toxicology. 46, 528-533

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-18-09

Summary of Study Findings: The study examines the effects of vinclozolin on the reproduction and development of Japanese quail (*Coturnix coturnix japonica*).

The vinclozolin used for dosing in the study was 99.1% pure.

Thirteen to 19 week old Japanese quail received dietary exposures for six weeks at either 125 or 500 ppm.

The test animals were obtained from a breeder at around six weeks of age. They were then allowed 5 weeks to acclimate to lab conditions. During this time the animals were kept separately in cages. Conditions for the entire lab period were: temperature 22 ±2° C, relative humidity 50 ±10%, and photo period 16:8 L:D. Animals were fed a "standard" diet and given water *ad libitum*. From age 8 weeks onward, animals were mated once daily, five times a week to identify reproductively viable individuals. Following the adaptation period, animals were divided into pairs (only proven breeders were used) and split into three groups (Control, Exp. 1 and Exp. 2). The animals were maintained in their new pairs untreated for another two weeks to acclimate and ensure reproductive viability. Test subjects were then dosed orally (through diet) for six weeks. The treated food was sampled weekly to assess actual chemical concentrations. These samples found concentrations to be sufficiently close (averages: 124.5, 482.65 ppm). Eggs were analyzed for residues and adults as well as the chicks they produced were monitored daily for behavioral changes, signs of toxicity and mortality. The results of egg residue analysis can be seen in **Table 1**. Further analysis of egg characteristics did not reveal any meaningful results. The only significant difference found between treatment and control groups with regard to actual reproduction was a shift in sex ratios. In the control and pre-treatment assessment, males were more commonly born than were females. In the treatment groups, the sex ratio shifted significantly and the number of males was reduced.

Table 1. Residues of vinclozolin in eggs (mean values)

Week of treatment	0 ppm	125 ppm	500 ppm
1st	<0.05[a]	1.99	6.21
5th	<0.05[a]	2.31	4.69

[a] Limit of detection.

There were no adverse clinical signs observed in adult birds as a result of vinclozolin exposure. The few deaths that occurred during the study were due to unrelated maladies and occurred in the low-dose and control groups.

Adult birds were weighed upon arrival, immediately before treatments started and just before termination. Body weights did differ significantly between groups in either sex and food consumption was not affected by dietary concentration.

During the last week of treatment, blood was taken from each animal and analyzed for the concentrations of the hormones testosterone, estradiol, progesterone (females only), T3 and T4. Plasma levels of these chemicals were not found to be significantly different among any of the treatment groups.

At the end of the 6-wk exposure period study, adult birds were sacrificed and examined histologically. Further chemical examination was performed on selected organs. No treatment-related gross pathological legions were found in any of the groups. Examination of livers found what appeared to be differences in condition in treated animals, but the frequency of these occurrences was similar in the control group, which prevented any conclusion from being made. Differences were found when examining the quail testis; the incidence of testicular atrophy was higher in the vinclozolin-treated animals and the number of spermatids per testes was statistically different ($p<0.05$) in birds treated with 500 ppm diet. On average, spermatids were 26% lower in the testes of birds treated with 500 ppm diet. While the number of spermatids were lower in the 125 ppm treatment(8.8% lower), the difference was not statistically significant.

Table 3. Atrophy of germinating epithelium in quail testis

	0 ppm	125 ppm	500 ppm
Atrophy incidence	0/6	2/6	4/6

Table 4. Spermatid count

	0 ppm		125 ppm		500 ppm	
	n	Mean ± SD	*n*	Mean ± SD	*n*	Mean ± SD
Spermatids × 10^6 per testis	16	339 ± 55	16	309 ± 33	18	250 ± 58*

Note. *n*, number of drakes examined.
* $p < 0.05$, ANOVA followed by Dunnett's test.

Sex ratio of chicks was significantly different ($p<0.05$) among offspring of quail feed at 500 ppm (**Table 7**); treated animals had a ratio of 39:43 (male:female) while controls had a ratio of 45:24. While the number of males in the 125 ppm treatment was lower than controls, the difference was not statistically significant.

Table 7. Distribution between male and female quail chicks during the pretreatment and the administration period

	0 ppm		125 ppm		500 ppm	
	Male	Female	Male	Female	Male	Female
P, 2nd week	16	11	18	7	20	10
T, 2nd week	11	8	11	14	14	15
T, 4th week	17	10	14	11	13	16
T, 6th week	17	6	17	12	12	12
Total	45	24	42	37	39*	43*

Note. P, pretreatment phase; T, treatment phase.
* $p < 0.05$, chi-square test.

The eggshell thickness (mm) of cracked eggs was significantly different ($p<0.05$) from vinclozolin-treated quail compared to controls. In both the 125 and 500 ppm treatments, eggshell thickness averaged 0.146 mm compared to controls at 0.135 mm (**Table 6**). Egg weight was also significantly different in vinclozalin-treated quail compared to controls; however, the higher weight of the vinclozolin treatments was apparent and statistically significant before treatments were initiated.

Table 6. Egg parameters (mean ± SD) during the pretreatment (2nd week; 13 weeks old) and treatment (14th to 19th weeks) phases

	Total number of eggs laid	Egg weight (g)	Number and percentage of cracked eggs	Eggshell thickness (mm)	
				Intact eggs	Cracked eggs
Pretreatment					
0 ppm	104	11.8 ± 0.9	7 = 7%	0.150 ± 0.014	0.131 ± 0.010
125 ppm	108	12.4 ± 0.9*	7 = 6%	0.154 ± 0.013	0.144 ± 0.010
500 ppm	104	12.2 ± 1.2*	13 = 12%	0.158 ± 0.015*	0.130 ± 0.01*
Treatment					
0 ppm	621	11.9 ± 1.0	44 = 7%	0.151 ± 0.017	0.135 ± 0.015
125 ppm	616	12.4 ± 0.9*	67 = 11%†	0.151 ± 0.017	0.146 ± 0.015*
500 ppm	615	12.2 ± 1.2*	55 = 9%	0.152 ± 0.016	0.146 ± 0.021*

Note. * $p < 0.05$, ANOVA followed by Dunnett's test. † $p < 0.05$, chi-square test.

The study authors concluded that fertility and reproductive performance were not affected up to the highest concentration; however, spermatid counts and histology provided evidence of an inhibition of spermatogenesis at both dietary concentrations. There was no evidence of systemic toxicity in the adult birds and plasma hormone levels, although highly variable, did not demonstrate treatment-related effects. The authors further state that no clear-cut indications of antiandrogenic effects in quail; however, there was transfer of the test substance into the eggs.

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

Rationale for Use: The study provides documentation of the link between parental dosing and egg residue. It also establishes a shift in expressed sex ratios brought on by vinclozolin exposure; however, the shift was not reported as statistically significant. Spermatids were also significantly reduced in the testes of treated birds and this difference was statistically significant in males treated with 500 ppm.

Limitations of Study: It is unclear from the study why the sex ratio of chicks is skewed in favor of males (45:24) in control animals. Statistical differences in egg weight and eggshell thickness existed in vinclozolin-treated birds prior to treatment. As such, there is uncertainty regarding the process for randomly assigning birds to treatments. The statistical differences in egg weight are therefore likely an artifact of pretreatment conditions and cannot be attributed to vinclozolin treatment.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

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 ±25 cm^2 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® 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® 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.

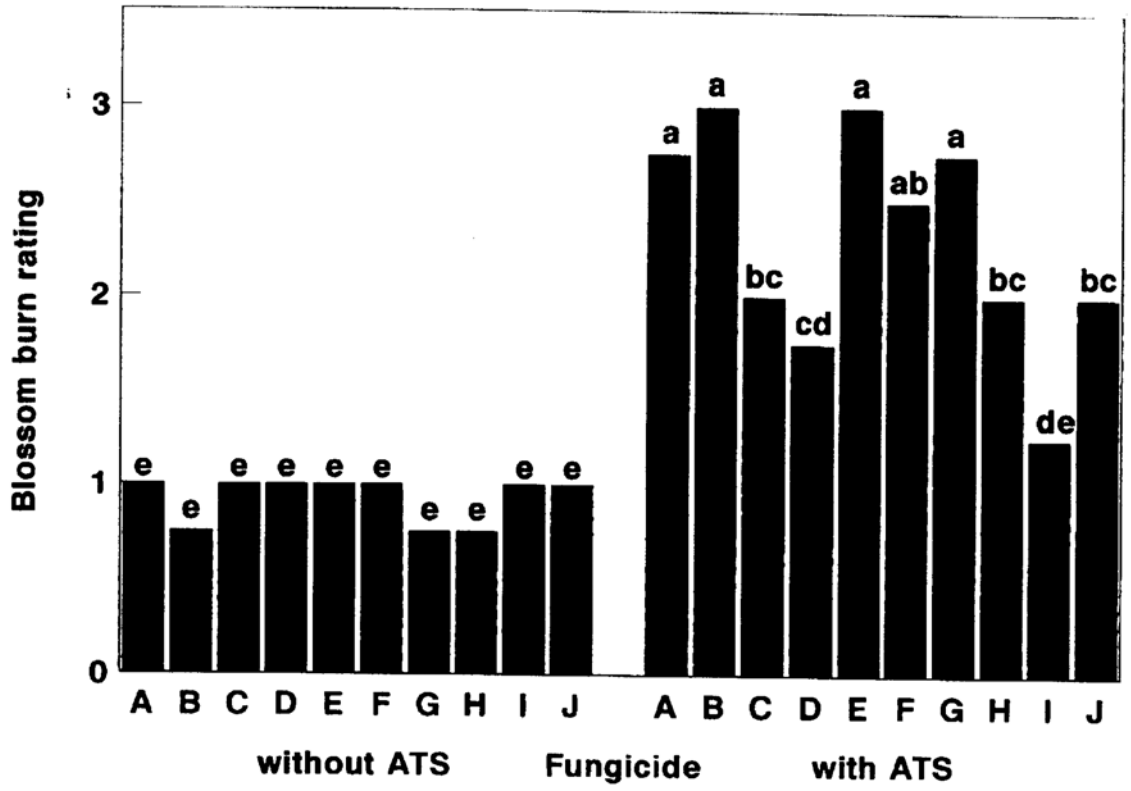

FIG. 2
Phytotoxicity of nine fungicides to peach blossoms when applied alone or simultaneously with ATS (scale: 0 = no damage, 3 = severe). Means with lower case letters in common are not significantly different at $P = 0.05$. Treatments: A—water control; B—benomyl; C—chlorothalonil; D—captan; E—triforine; 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

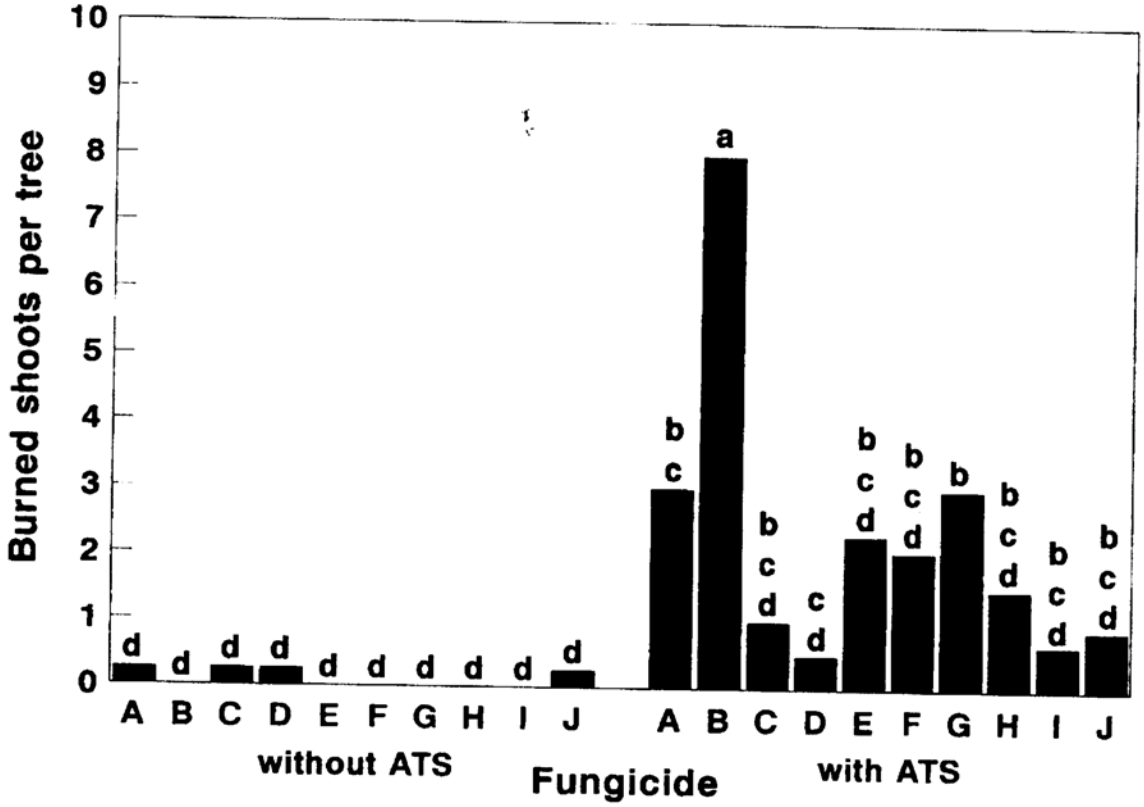

FIG. 3
Phytotoxicity of nine fungicides to shoots of peach trees when applied alone or simultaneously with ATS. Means with lower case letters in common are not significantly different at $P = 0.05$. Treatments: A—water control; B—benomyl; C—chlorothalonil; D—captan; E—triforine; F—propiconazol; G—vinclozolin; H—iprodione; I—sulphur; J—thiophanate-methyl (full details of the fungicides and rates of application are given in Table 1).

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.

Primary Reviewer: TJ Graven, Biologist

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 ($p<0.01$), aniline hyroxylase ($p<0.01$), 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.

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

	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*	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***	0.71 ± 0.20***	0.23 ± 0.06
NADPH-cytochrome c reductase (nmol/mg x min)	107 ± 19	158 ± 23***	154 ± 30***	106 ± 18
Aniline hydroxylase (nmol/mg x min)	0.71 ± 0.17	1.41 ± 0.33***	1.10 ± 0.28*	0.68 ± 0.19
Aldrin epoxidase (nmol/mg x min)	0.14 ± 0.03	0.23 ± 0.07*	0.46 ± 0.06***	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***	1.20 ± 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

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.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 84997 Ronis, Martin J. J. Badger, Thomas M. 1995. Toxic Interactions between Fungicides That Inhibit Erosterol Biosynthesis and Phosphorothioate Insecticdes in the Male Rat and Bobwhite Quail (*Colinus virginianus*). Toxicology and Applied Pharmacology. 130, 221-228.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-12-09

Summary of Study Findings: This study examines the effects of ergosterol biosynthesis inhibiting fungicides (EBIFs), including vinclozolin, and phosphorothioate insecticide interaction on bobwhite quail (*Colinus virginianus*) and Sprague-Dawley (SD) rats (*Rattus norvegicus*) by measuring inhibition of plasma butyryl cholinesterase (BChE). It found that vinclozolin in combination with either parathion or malathion did not increase BChE induction in rats, but did induce BChE in quail. The study also examined the metabolic implications of chemical combinations by measuring cytochrome P450 (CytP450) enzyme levels to evaluate their activity. An *in vitro* experiment was carried out to determine the metabolic rates and metabolites of parathion, malathion and diazanon.

Experimental setup was the same as in report 53711 (Ronis, 1998). Male bobwhite quail (200 g) and male SD rats (300g) were dosed with vinclozolin at 400 mg/kg bw for three consecutive days based on previous study results indicating that 3 consecutive doses would induce hepatic microsomal enzyme actiivity. Rats received a single bolus of parathion (0.4mg/kg) or malathion (150 mg/kg) in corn oil. The rats were killed 12 hours after parathion ingestion and 4 hours after malathion ingestion. Quail were given a 12 mg/kg dose of malathion 48 hours after their last vinclozolin ingestion. They were terminated 12 hours after malathion treatment. For vinclozoline treatment along, quail were sacrificed 48 hours after their final dose.

Acute toxicity was assessed by measuring BChE. Significant ($P<0.05$) BChE inhibition was found in the quail dosed with vinclozolin and malathion. No other pertinent inhibitory effects were seen.

A significant ($p<0.05$) difference (31% increase relative to controls) in liver weight (hepatosomatic index) was seen in quail treated with vinclozolin, but not in any other experimental group of concern. Vinclozolin treatment also resulted in a significant ($P<0.005$) difference in hepatic CytP450 activity (roughly 10X increase relative to controls). **Table 2** below shows the results for the *in vivo* experiments.

TABLE 2

Effect of EBIF Treatment on Liver/Body Weight Ratio and Hepatic Cytochrome P450 Content in Male Rate and Bobwhite Quail

Treatment[a]	Liver/body weight (%)	Cytochrome P450 (nmol/mg)
Rat		
NC	4.5 ± 0.35	0.89 ± 0.15
P	5.6 ± 0.29*	2.95 ± 0.27**
V	4.7 ± 0.24	2.62 ± 0.22**
C	7.4 ± 0.53**	3.10 ± 0.44**
K	5.7 ± 0.8	1.30 ± 0.19
Quail		
NC	1.3 ± 0.1	0.034 ± 0.009
P	1.7 ± 0.1*	0.220 ± 0.050**
V	1.7 ± 0.1*	0.340 ± 0.009**
C	1.4 ± 0.1	0.080 ± 0.002*
K	2.6 + 0.3**	0.200 + 0.003**

Note. Data as mean ± SE for $N = 5$ or 6/group.

[a] NC, control; P, propiconazole; V, vinclozolin; C, clotrimazole; K, ketoconazole.

* $p < 0.05$.

** $p < 0.005$.

Reproduced from Ronis and Badger 1995.

In vitro tests were carried out to test the metabolism and metabolite formation. This was accomplished using microsomal protein from test animals. Parathion was found to produce an increase ($p<0.05$) in both paraoxon and p-nitrophenol formation in vinclozolin-treated rats (**Table 3**).

TABLE 3

Parathion Metabolism by Hepatic Microsomes from Control and EBIF-Treated Male Rat

	Product formed (nmol/mg/min)		
Treatment[a]	Paraoxon	*p*-Nitrophenol	Activation/ detoxication ratio
NC	1.8 ± 0.1	0.3 ± 0.03	6.3
P	6.4 ± 0.9*	2.6 ± 0.60*	2.4
V	4.3 ± 0.7*	2.1 ± 0.30**	2.0
C	3.3 ± 0.7*	1.2 ± 0.38*	2.8
K	2.3 + 0.2	0.2 + 0.13	11.0

Note. Data as mean ± SE for $N = 5$/group.

[a] NC, control; P, propiconazole; V, vinclozolin; C, clotrimazole; K, ketoconazole.

* $p < 0.05$.

** $p < 0.005$.

Reproduced from Ronis and Badger 1995.

Similar effects were observed with malathion and diazinon. The both of which can be seen below in **Tables 4 and 5**.

TABLE 4
Effect of EBIF and Parathion Treatment on Rat Hepatic Microsomal Metabolism of [^{14}C]Diazinon

	Metabolites[b]					
Treatment[a]	Total	Oxon	OH-diaz	Pyr	OH-pyr	A/D Ratio[c]
NC	3.5 ± 0.5	1.4 ± 0.1	0.2 ± 0.02	1.9 ± 0.3	0.4 ± 0.02	0.64
PT	4.1 ± 0.5	1.4 ± 0.1	0.2 ± 0.04	2.0 ± 0.2	0.4 ± 0.09	0.51
P	8.1 ± 1.5*	4.3 ± 0.6**	0.3 ± 0.06*	3.2 ± 0.8	0.3 ± 0.03	1.14
P/PT	7.9 ± 0.9**	3.9 ± 0.4**	0.3 ± 0.04	3.4 ± 0.4*	0.4 ± 0.10	1.00
V	8.0 ± 0.1**	4.3 ± 0.4**	0.3 ± 0.06	3.0 ± 0.4	0.3 ± 0.06	1.10
V/PT	9.6 ± 0.9**	4.7 ± 0.5**	0.4 ± 0.10	3.8 ± 0.4**	0.5 ± 0.1	0.96
C	5.3 ± 0.9	3.2 ± 0.5*	0.3 ± 0.07	1.4 ± 0.4	0.4 ± 0.05	1.50
C/PT	7.3 ± 0.7*	4.0 ± 0.4**	0.4 ± 0.13	2.4 ± 0.2	0.4 ± 0.13	1.20
K	4.8 ± 0.6	1.8 ± 0.2	0.3 ± 0.05	1.9 ± 0.5	0.4 ± 0.05	0.70
K/PT	4.5 + 0.8	2.1 + 0.2*	0.2 + 0.07	2.0 + 0.6	0.3 + 0.09	0.87

Note. Data as rate of product formation nmol/mg/min. Mean ± SE for $N = 5$/group.
[a] NC, control; PT, parathion; P, propiconazole; V, vinclozolin; C, clotrimazole; K, ketoconazole.
[b] Oxon, diazonon (active metabolite); OH-diaz, hydroxydiazinon; pyr, 2-isopropyl-4-methyl-6-hydroxypyrimidine; OH-pyr, 2-(2-hydroxy-2-propyl)4-methyl-6-hydroxypyrimidine.
[c] Activation/detoxication ratio: oxon/other metabolites.
* $p < 0.05$.
** $p < 0.005$.

Reproduced from Ronis and Badger 1995.

TABLE 5
Effect of EBIF Treatment on Hepatic Microsomal Metabolism of Malathion in Male Rat and Bobwhite Quail

	Product formation (nmol/mg/min)		
Treatment[a]	Maloxon	Malathion acids	Activation/ detoxication ratio
Rat			
NC	ND	1112 ± 49	—
P	ND	1082 ± 105	—
V	ND	1122 ± 41	—
C	13 ± 4.4**	1047 ± 61	0.012
K	ND	1194 ± 85	—
Quail			
NC	81 ± 12.5	29 ± 2	2.8
P	242 ± 29.0**	102 ± 28*	2.4
V	267 ± 30.0**	115 ± 30*	2.1
C	103 ± 8.0	53 ± 7*	1.9
K	82 + 11.0	114 + 23*	0.7

Note. Data as mean ± SE for groups of $N = 5$ or 6 animals.
[a] NC, control; P, propiconazole; V, vinclozolin; C, clotrimazole; K, ketoconazole.
* $p < 0.05$.
** $p < 0.005$.

Reproduced from Ronis and Badger 1995.

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

Rationale for Use: Although the dosing regime used in this study has questionable relevancy to exposures that might be expected to occur under typical use conditions for the chemical, the study provides information on the ability of vinclozolin to induce microsomal enzyme activity. Following 3 consecutive treatments with vinclozolin at 400 mg/kg bw, liver cytochrome P450 enzyme activity was increased by an order of magnitude relative to controls and increased liver weights likely reflected the induction of microsomal enzyme activity. The study did not report any other sublethal effects on the birds.

Although hepatic cytochrome P450 activity was statistically ($p<0.005$) different in rats (roughly 2.9X higher than controls), the rat hepatic-somatic index was not statistically different than controls.

Limitations of Study: The paper acknowledges the idea that *in vitro* results are not necessarily indicative of real world *in vivo* occurrences. Study conditions are not well described and the dosing regime for the animals is difficult to relate to what could actually occur in the field.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 76452 Ronis, M.J.J., M. Ingelman-Sundberg, and T. M. Badger. 1994. Induction, Suppression and Inhibition of Multiple Hepatic Cytochrome P450 Isozymes in the Male Rat and Bobwhite Quail (*Colinus virginianus*) by Ergosterol Biosynthesis Inhibiting Fungicides (EBIFs)

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-18-09

Summary of Study Findings: This study examines the effects of vinclozolin on hepatic microsomal monooxygenase systems of vertebrates (rats and quail).

The study's experimental setup was identical to that seen in reports 53711 and 84997 (Ronis 1995, 1998). Male Sprague-Dawley rats (300 g; n=5) and male Bobwhite quail (200 g; n=6) were each administered three consecutive doses of vinclozolin (95% ai) at 400 mg/kg bw/day and were killed 48 hours after the final dose. Experimental conditions are not reported.

The results of monooxygenase activity analysis are shown in **Table 2** below. Vinclozolin significantly ($P<0.005$) raised cytochrome P450 (Cyt-P450) levels by a factor of 3 - 4 fold in both rats and quail. It also significantly ($P<.0.05$) elevated levels of cytochrome b_5 and Cyt-P450 reductase by factors of 2 - 4 fold in both animals (**Figure 2**). In quail, vinclozolin induced P450 content 10-fold ($p<0.005$).

Alkoxyresorufin metabolism was increased by vinclozolin in both taxa *in vivo* (**Figure 3**). However, when these processes were examined *in vitro* vinclozolin inhibited alkoxyresorufin metabolism. It should be noted that there was no correlation found between the *in vivo* and *in vitro* effects of the chemical in this study.

EROD and CYP 2B1/2-dependent BROD were increased 12- and 300-fold by vinclozolin treatment, respectively. Vinclozolin increased CYP 1A2-dependent MROD and BYP2B1/2-dependent PROD 30- to 40-fold, respectively. The degree of induction was lower in quail than in rats though.

Androstendione, 2β- and 6β-hydroxytestosterone formation was significantly ($P<0.05$) increased in rats exposed to vinclozolin. The formation of unknown compound 2 and 16α-hydroxytestosterone was also significantly increased ($P<0.005$). Little effect was observed on testosterone metabolism in quail exposed to vinclozolin. Results can be seen in **Figure 4**.

Vinclozolin increased lauric acid hydroxylation in both rats and quail (**Table 2**)

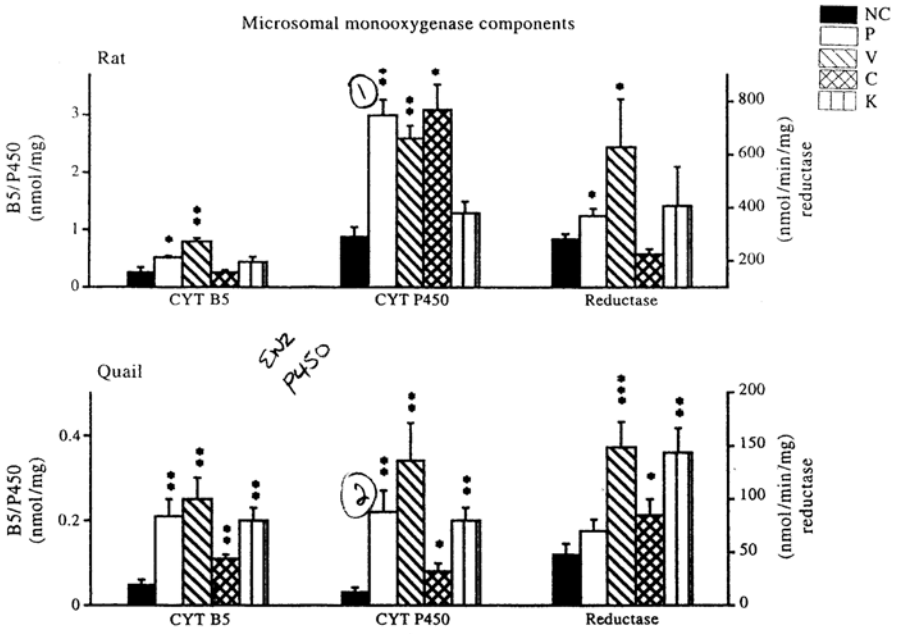

Fig. 2. Concentrations of microsomal monooxygenase components in untreated male rat and quail microsomes and following EBIF treatment. Cytochrome P450 and cytochrome b_5 concentrations were determined spectrally [20]; cytochrome P450 reductase was measured as nmol of cytochrome *c* reduced/min/mg by microsomes in the presence of NADPH [21] at 37° (rat) and 42° (quail). Abbreviations: NC, control; P, propiconazole-treated; V, vinclozolin-treated; C, clotrimazole-treated; and K, ketoconazole-treated. Data are expressed as means ± SEM for rat (N = 5) and quail (N = 6). Key: (*) significant at $P < 0.05$, (**) significant at $P < 0.005$, and (***) significant at $P < 0.0005$.

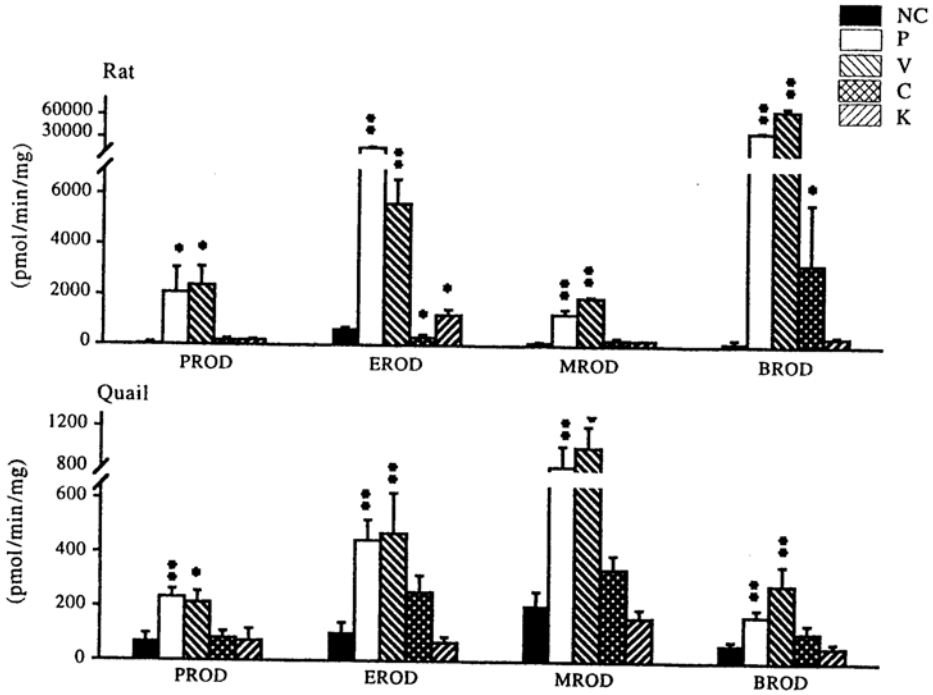

Fig. 3. Metabolism of a series of alkoxyresorufins by untreated male rat and quail microsomes or following EBIF induction. Abbreviations: PROD, pentoxyresorufin *O*-dependentylase; EROD, ethoxyresorufin *O*-deethylase; MROD, methoxyresorufin *O*-demethylase; BROD, benzyloxyresorufin *O*-debenzylase; NC, control; P, propiconazole-treated; V, vinclozolin-treated; C, clotrimazole-treated; and K, ketoconazole-treated. Data are expressed as means ± SEM for rat (N = 5) and quail (N = 6). Key: (*)significant at $P < 0.05$, and (**) significant at $P < 0.005$.

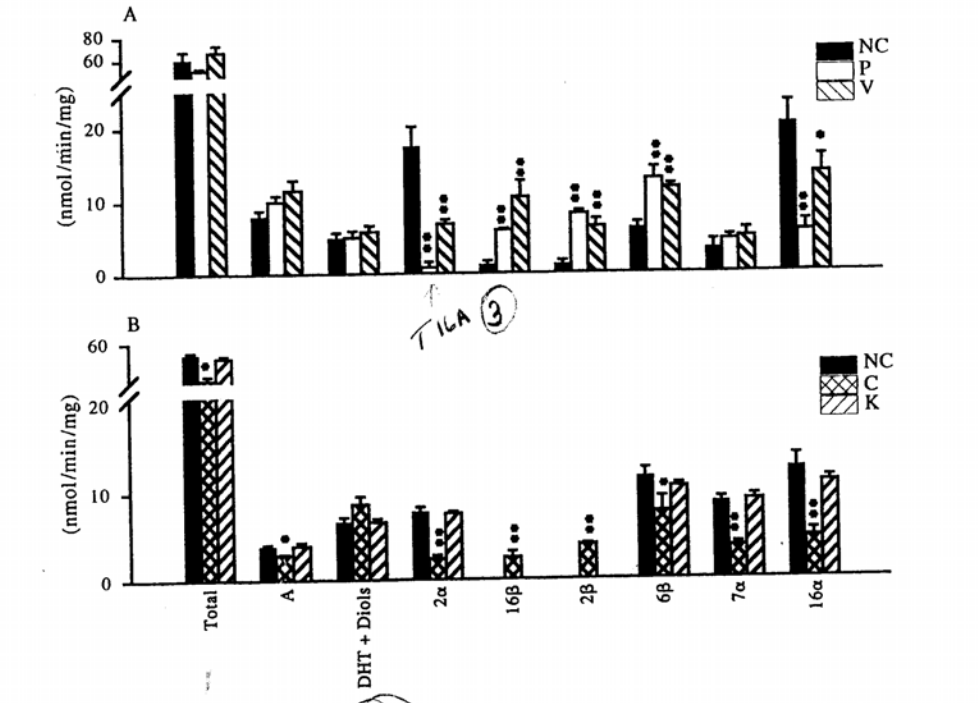

Fig. 4. Metabolism of testosterone by male rat microsomes following EBIF induction. (4A) NC, control; P, propiconazole-treated; and V, vinclozolin-treated. (4B) NC, control; C, clotrimazole-treated; and K, ketoconazole-treated. Abbreviations: A, androstenedione; DHT, dihydrotestosterone; Diols ($3\alpha(\beta)$ 5α-androstanediols); 2α, 2α-hydroxytestosterone; 16β, 16β-hydroxytestosterone; 2β, 2β-hydroxytestosterone; 6β, 6β-hydroxytestosterone; 7α, 7α-hydroxytestosterone; and 16α, 16α-hydroxytestosterone. Data are expressed as means ± SEMS of duplicate assays for N = 5 animals/group. Key: (*) significant at $P < 0.05$, (**) significant at $P < 0.005$.

Table 2. Induction of monooxygenase activities by EBIF treatment in male rat and bobwhite quail

Treatment	CCl_4-Dependent lipid peroxidation	Erythromycin *N*-demethylase	Lauric acid		
			ω-OH	(ω-1)-OH	*X*
Rat					
Control	0.16 ± 0.05	1.5 ± 0.16	2.6 ± 0.4	1.2 ± 0.2	0.2 ± 0.02
Propiconazole	0.18 ± 0.03	5.7 ± 0.35*	3.7 ± 0.4	1.8 ± 0.3	1.5 ± 0.4†
Vinclozolin	0.13 ± 0.05	2.1 ± 0.52	3.8 ± 0.5	2.3 ± 0.2†	0.8 ± 0.2†
Clotrimazole	0.09 ± 0.05	5.1 ± 1.40	2.2 ± 0.3	1.0 ± 0.3	0.8 ± 0.2†
Ketoconazole	0.03 ± 0.02*	1.6 ± 0.20	2.0 ± 0.3	1.2 ± 0.3	0.5 ± 0.1†
Quail					
Control	0.015 ± 0.005	1.3 ± 0.10	1.4 ± 0.1	<0.1	<0.1
Propiconazole	0.035 ± 0.005*	1.8 ± 0.07*	3.9 ± 0.7†	1.0 ± 0.2†	0.4 ± 0.1†
Vinclozolin	0.048 ± 0.014	1.9 ± 0.08*	4.1 ± 0.6*	1.4 ± 0.2†	0.6 ± 0.1†
Clotrimazole	0.014 ± 0.005	1.2 ± 0.1	2.1 ± 0.3	0.3 ± 0.1†	0.2 ± 0.1†
Ketoconazole	0.010 + 0.004	1.7 + 0.1†	1.3 + 0.2	<0.1	<0.1

Data are presented as means ± SEM (nmol/mg/min) for N = 5 (rat), N = 6 (quail).
*,† Significantly at: different from control *$P < 0.005$, and †$P < 0.05$.

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

Rationale for Use: The paper's methods and procedures seem sound and demonstrate the sublethal effects of vinclozolin. Under the conditions studied, vinclozolin significantly and simultaneously affected different subfamilies of hepatic P450 enzyme activity in both rats and birds. The authors speculate that the induction and inhibition of broad microsomal enzyme activity may affect the ability of animals to metabolize other chemicals that may be dependent on the same enzyme systems for activation and/or deactivation, e.g. activation of malathion.

Limitations of Study: The parameters examined in this experiment do not show a relationship to effects on a whole organism level. Even within the study there was a lack of consistency from one situation to another (*in vivo* vs. *in vitro* results.) While the dosing regimen used in the study, i.e., 3 consecutive doses at 400 mg/kg bw/day, is known to be effective for inducing hepatic monoxygenase activity, its relevancy it risk assessment is unclear.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 53711 Ronis, M.J.J., M. Celander, and T. M.. Badger. 1998. Cytochrome P450 enzymes in the kidney of the bobwhite quail (*Colinus virginianus)*: induction and inhibition by ergosterol biosynthesis inhibiting fungicides. Comparative Biochemistry and Physiology. 121, 221-229

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-11-09

Summary of Study Findings: Male Bobwhite quail (200 g) and Sprague-Dawley rats (300 g) (*Ratus norvegicus*) were both exposed to vinclozolin (along with other chemicals in different trials). Their hepatic and renal tissues were then analyzed to explore enzymatic activity, testosterone metabolism, and alkoxyresorufin metabolism. The most useful results of the study are those found in quail analysis because they are the first examination of avian enzymatic in response to vinclozolin. The study found that the production of testosterone metabolizing enzymes and the alkoxyresorufins may be used as future markers for vinclozolin exposure.

Rats and quail were both housed in cages with a 12:12 L:D period. They were given *ad libitum* access to food and water. Control groups contained 12 quail, treated groups had 6. The group sizes for rats were not given. Control group quail were gavaged once a day for three consecutive days with 1 ml corn oil. Treatment group quail were treated in the same way with 400 mg/kg vinclozolin (95% pure) in corn oil. Treatment methods for rats were not specified. All birds were sacrificed 48 hours after their last gavage. Rat sacrifice details are not given. Liver and kidney preparations were made for each group. The preparations were then analyzed. For statistical analysis, significance was analyzed using a one-way ANOVA with $P<0.05$ significance level.

Based on the results presented in **Table 3** of the study, kidney microsomes of vinclozolin-treated quail had significantly different ($p<0.05$) levels of 2β-OH testosterone and 15β-OH testosterone 2. The 2β-OH testosterone was roughly 38% higher than controls and the 15β-OH testosterone was roughly 67% higher than controls. None of the other variables used to measure testosterone metabolism were significantly different than controls. None of the variables used to measure alkyoxyresrufin metabolism in quail kidney microsomes were statistically different between vinclozoline and control quail (**Table 4**).

Table 3
Testosterone metabolism in rat and quail kidney microsomes

Product	Male rat kidney[a]	Male quail kidney[a]				
		Control	Propiconazole	Vinclozolin	Clotrimazole	Ketoconazole
Androstenedione	116 ± 11	198 ± 29	261 ± 26	197 ± 6	259 ± 16	249 ± 23
DHT	91 ± 11	58 ± 14	71 ± 16	50 ± 3	56 ± 4	51 ± 9
5-Androstene-3α(β)17β-diols	n.d.	175 ± 14	164 ± 16	184 ± 11	194 ± 22	188 ± 33
5α-Androstane-3α(β)17β-diols	79 ± 8	173 ± 14	158 ± 11	145 ± 13	151 ± 31	128 ± 41
2α-OH-T	121 ± 11	184 ± 14	141 ± 8	132 ± 14	140 ± 14	165 ± 18
2β-OH-T	48 ± 8	210 ± 19	249 ± 32	289 ± 11*	169 ± 34	161 ± 30
6β-OH-T	35 ± 5	n.d.	n.d.	n.d.	n.d.	n.d.
6α-OH-T	20 ± 6	61 ± 10	70 ± 18	95 ± 3	42 ± 2	31 ± 4*
15β-OH-T2	n.d.	24 ± 3	33 ± 11	40 ± 6*	14 ± 6	7 ± 6*
7α-OH-T	8 ± 4	5 ± 2	5 ± 4	8 ± 2	2 ± 1	2 ± 1
16α-OH-T	70 ± 9	48 ± 9	68 ± 9	74 ± 17	48 ± 29	24 ± 15
15α-OH-T2	5 ± 4	12 ± 1	17 ± 8	21 ± 4	8 ± 3	6 ± 3
Total	588 ± 47	1181 ± 18	1252 ± 38	1239 ± 20	1116 ± 30	1028 ± 51*

[a] Data presented as pmol mg^{-1} min^{-1}, mean ± S.E.M. for four sets of kidney microsomes from male rats, six pools of kidney microsomes prepared from two control quail and three pools of kidney microsomes prepared from two fungicide-treated quail.
[b] Product identification based on R_f value relative to testosterone according to the published values of Waxman [29] for pure standards in the same solvent system; n.d. not detectable.
*Statistically significant at $P<0.05$ vs control quail.

Table 4
Alkoxyresorufin metabolism in rat and quail kidney microsomes

Activity	Male rat kidney[a]	Male quail kidney[a]				
		Control	Propiconazole	Vinclozolin	Clotrimazole	Ketoconazole
EROD	0.9 ± 0.1	0.69 ± 0.08	0.45 ± 0.16	0.59 ± 0.10	0.48 ± 0.25	0.03 ± 0.01**
MROD	1.0 ± 0.2	0.96 ± 0.13	1.54 ± 0.6	0.54 ± 0.45	0.83 ± 0.71	0.12 ± 0.10**
PROD	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
BROD	2.1 ± 0.18	0.43 ± 0.20	1.07 ± 0.23	0.71 ± 0.35	0.11 ± 0.07	n.d.**

[a] Data presented as pmol mg^{-1} min^{-1}, mean ± S.E.M. for six sets of kidney microsomes from male rats, six pools of kidney microsomes prepared from two control quail and three pools of kidney microsomes prepared from two fungicide-treated quail.
n.d., not detectable.
** Statistically significant at $P<0.005$ vs control quail.

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

Rationale for Use: Under the conditions tested, exposure to three consecutive doses of vinclozolin at 400 mg/kg bw, male bobwhite quail exhibited a statistically significant induction in two markers for testosterone metabolism, *i.e.*, testosterone 2β- and 15β-hydroxylase, activity and did not show any significant difference in the measures of alkoxyresorufin metabolism. The authors note that kidneys from vinclozolin-treated quail had increases in expression of P450 enzymes cross-reactive with antibodies raised against CYP3A-like proteins in fish and significantly induced a CYP 1A1 cross-reactive P450 enzyme.

Limitations of Study: The study is lacks background data on the test animals particularly the rats. The dosing regime, *i.e.*, 3 consecutive oral doses of 400 mg/kg bw/day is unusual for an oral study and it is difficult to understand how this exposure could be related to what may actually occur in the field.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

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® (vinclozolin 50 % a./i. treated at 10 g Ronilan®/acre) or Rovral® (iprodione 50 g % treated at 50 g Rovral®/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.

Table I. Unitary Mean Fresh Weights of the Lettuces

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

[a-c] Significantly different from the control at the 1% (*a*) and 5% (*b*) level or nonsignificant (*c*), respectively. [d] Means ± SD of the weights of 20 lettuces. [e] Month and day, year 1983.

Table II. Carotenoid Content of the Fresh Lettuce

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

[a-c] As in Table I. [d] Means ± 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

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 69835 M. Tillmann, U. Schulte-Oehlmann, M. Duft, B.Markert, and J.Oehlmann. 2001. Effects of Endocrine Disruptors on Prosobranch Snails (Mollusca: Gastropoda) in the Laboratory. Part III: Cyproterone Acetate and Vinclozolin as Antiandrogens. Ecotoxicology. 10, 373-388

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-17-09

Summary of Study Findings: The study examines the effects of vinclozolin on marine invertebrates (snails).

The species exposed were the ramshead snail (*Marisa cornuarietis)* and the marine dogwhelk (*Nucella lapillus*) and the netted whelk (*Nassarius reticulates*). Test animals came from breeding stock in the examiners' laboratory. All exposures were conducted as semi-static renewal assays. *Marisa* were kept in tap water, while *Nucella* were kept in artificial seawater. Test conditions were as follows; temperature 22°C ±1° for *Marisa* , 14°C ±1° for *Nucella*, 12:12 L:D photoperiod.

Juvenile *Marisa* were exposed to vinclozolin at the nominal concentration of 0.03, 0.1, 0.3 and 1.0 µg/L for 5 months in 60-L glass aquaria. Ethanol (12.5 µg/L) was used as a solvent control. 30 specimens were collected for analysis at the beginning of the experiment and at monthly intervals until completion.

By the end of the 5 month period, the first males had attained sexual maturity in the control group. No males attained maturity in any of the treated groups. Females did not show any effects of vinclozolin exposure, although no spawning occurred, which makes fecundity measurements impossible. Males showed a significant decrease in the extension of male accessory sex organs in the two lowest (0.03 and 0.1) treatment groups. This response was only detected in the first two to three months of the experiment. No differences in penis or penis sheath length were found during the last two months of the test.

Adult *Nucella* were exposed to the same range of treatments as described above for three months. The mean penis and prostate gland length were significantly reduced when compared with the control group. The relative number of males with ripe sperm stored in the seminal vesicle was also reduced. Responses did not seem to be dose dependent.

The results of the study with *M. cornuarietis* can be seen in **Figure 5**. For the first two months of the study the length of the penis was significantly different than controls in the 0.03 and the 1.0 µg/L treatments; by the third month of the study, only the males in the lowest treatment (0.03) had statistically different penis length and by the fourth month of the study there was no difference in penis length between vinclozolin-treated and control snails.

For penis sheath, males treated with 0.03 and 1.0 µg/L were statistically different than controls for the first two months of the study; however, for months three and four of the study there was no difference in penis sheath between vinclozolin-treated and control animals.

Based on the information in **Figure 6**, there were significant differences ($p<0.05$ in penis length, prostate length and presence of sperm in the vesicular seminalis of *N. lapillus* across the first three months of the study. In all cases, the measurement parameters in vinclozolin-treated snails were lower than in controls. It is noteworthy that across all three vinclozolin treatment levels, no sperm were reported in the vesicular seminalis.

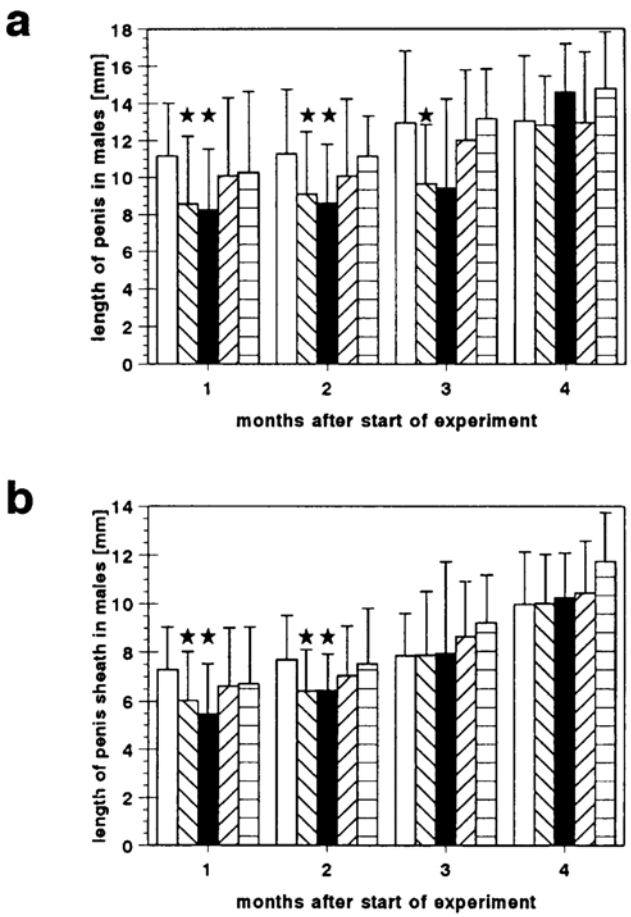

Figure 5. Effects of VZ exposure in *Marisa cornuarietis*. Mean length (± standard deviation; *n*: 11–23) of penis (a) and penis sheath (b) in immature males. Exposure groups by bars from left to right: (□) control; (▨) 0.03, (▨) 0.1, (▨) 0.3, and (■) 1.0 µg VZ/L. Asterisks indicate statistical significant differences to control (H test): ★, $p < 0.05$.

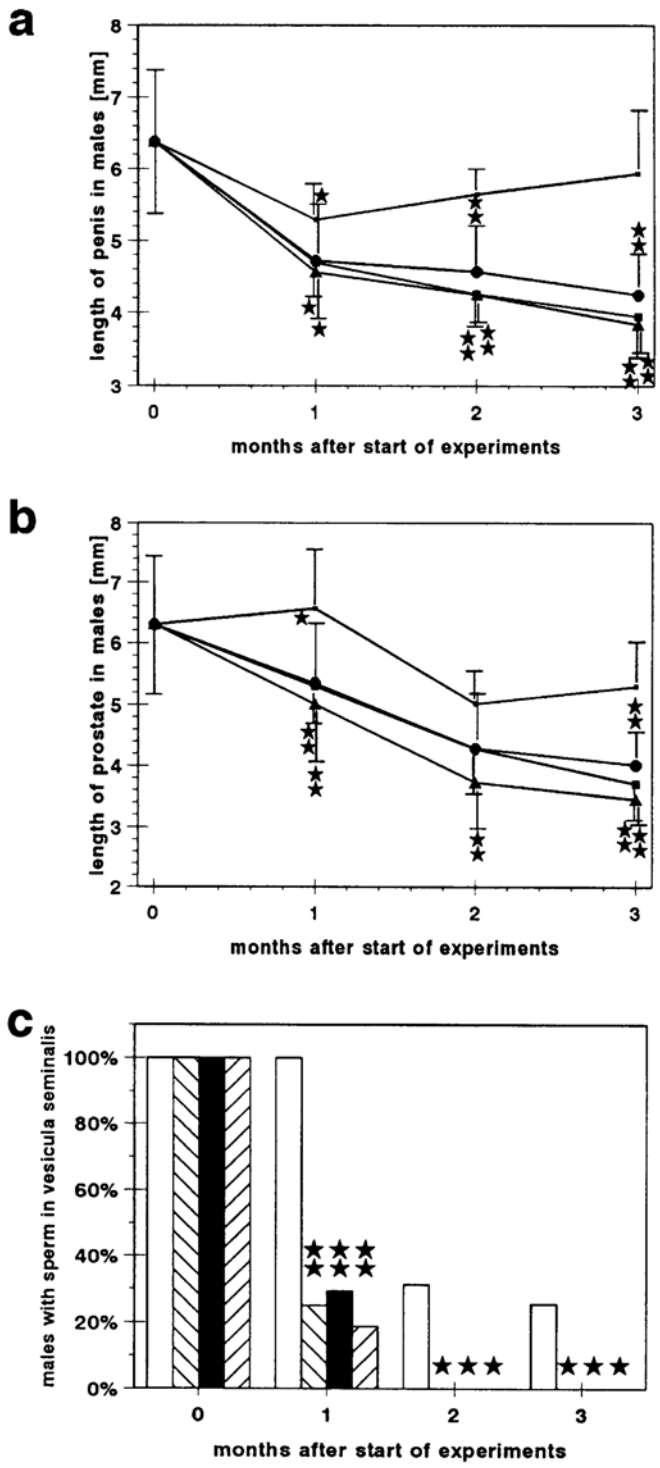

Figure 6. Effects of VZ exposure in *Nucella lapillus*. Development of mean penis (a) and prostate (b) length (± standard deviation; n = 15–23) in adult males. Exposure groups: (•) solvent control, (●) 0.03 µg VZ/L, (■) 0.3 µg VZ/L, (▲) 1.0 µg VZ/L. (b) Relative numbers of males with sperm-filled vesiculae seminalis. Exposure groups by bars from left to right: (□) control; (▨) 0.03, (■) 0.3, and (▨) 1.0 µg VZ/L. Asterisks indicate statistical significant differences to control (in (a) and (b) H test, in (c) χ^2 test): ★, $p < 0.05$; ★★, $p < 0.01$.

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

Rationale for Use: This study provides insights on the potential effect, albeit transient, of vinclozolin in penis length and shaft in *M. cornuarietis*; however, the study showed significant declines in penis length, length of the prostate and males with sperm in vesicular seminalis in N. lapillus over the 3-month study period. By the second and third months of the study, no sperm were reported measured.

Limitations of Study: The source and purity of the vinclozolin used in the study is not stated; as such, it is not possible to determine whether the nominal concentrations reported in the study are intended to represent technical grade active ingredient or formulated product. Exposure was not reported as documented during the study. Animals failed to spawn properly in the study and it is uncertain whether husbandry conditions were potentially related to the poor reproduction. Snail diet during the experiment is not specified.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 106040 Villeneuve, D. L., L. S. Blake, J. D. Brodin, K. J. Green, I. Knoebl, A. L. Miracle, D. Martinovic, and G. T. Ankley. 2007. Transcription of Key Genes Regulating Gonadal Steroidogenesis in Conrol and Ketoconazole- or Vinclozolin-Exposed Fathead Minnows. Toxicological Sciences 98 (2), 395-407.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-04-09

Summary of Study Findings: This study is based on report number 106557. It uses tissue samples taken from fish in that experiment, so all parameters are the same. This study uses PCR to measure the expression of various genes and enzyme expression. It found that a 21-day exposure of fathead minnows to vinclozolin increased pituitary expression of follicle stimulating hormone β-subunit as well as testicular expression of 20β-hydroxysteroid dehydrogenase and luteinizing hormone receptor transcripts. Overall, steroidogenesis-regulating genes were upregulated.

Test tanks were divided into two equal-sized sections with a screen and one male and one female fathead minnow were placed, together with a spawning substrate, on each side of the divider. Fish were exposed to nominal concentrations of 100, 400 and 700 µg/L delivered in continuous flow for 21 -days. Each of the treatments were replicated 5 times. Pituitary and gonadal samples were collected for RNA extraction and QPCR analysis. This particular study focused on analysis of steroidogenesis-regulating gene transcripts in male pituitary and testes

According to the study, exposure to vinclozolin for 21 days had a significant impact on the expression of four of the 11 genes examine in males. In pituitary tissue, mean FSH β expression was significantly elevated in fish exposed to 700 µg/L but there was no effect on LH β expression (**Figure 4**). The FSH expression in the pituitary showed a concentration-dependent increase. A similar dose response was observed for 20 β-HSD and 11 β-HSD expression in the testis tissue. The most sensitive of the 11 transcripts examine in the testis was LHR which was significantly elevated in all three vinclozolin treatments (**Figure 4**).

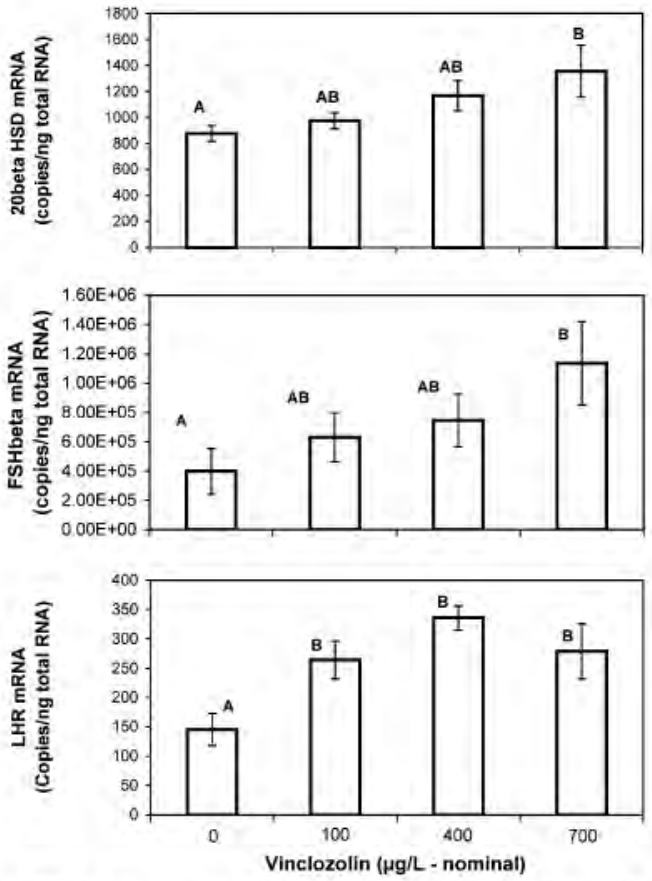

FIG. 4. Mean expression of 20β-HSD, FSH β-subunit (FSHβ), and LHR mRNAs in the testis (20β-HSD and LHR) or pituitary (FSHβ) of male fathead minnows exposed to Lake Superior water (0 µg/l) or VZ for 21 days. Measured concentrations were approximately 60% of nominal (Martinovic *et al.*, submitted for publication). Different letters indicate statistically significant differences among treatments ($p < 0.05$). Error bars = standard error.

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

Rationale for Use: This study provides information on the sublethal effects of a 21-day exposure of fathead minnows to vinclozolin. The NOAEC for this study bases on LHR mRNA induction is <100 µg/L and the LOAEC is 100 µg/L.

Limitations of Study: Study is measuring gene expression and does not report effects on the apical endpoints of growth, survival and reproduction.

Primary Reviewer: TJ Graven, Biologist

Secondary Reviewer Thomas Steeger, Ph.D., Senior Advisor

Open Literature Review Summary

Chemical Name: Vinclozolin

CAS No: 113201

ECOTOX Record Number and Citation: 108669 Zavala-Aguirre, J L., O. Torres-Bugarin, and A. L.Zamora-Perez. 2007. Aquatic ecotoxicology approaches in Western Mexico. Journal of Environmental Science and Health Part A. 42, 1503-1511.

Purpose of Review (DP Barcode or Litigation): Litigation

Date of Review: 8-19-09

Summary of Study Findings: This study examines the effects of vinclozolin on the rotifer *Brachionus calyciflorus*. Experiments were carried out according to the procedures previously reported by Acevedo-Pallares and Zavala-Aguirre[1,2]. Test animals subjects were obtained from the chemistry department at the Universidad Autonoa de Aguascalientes in Mexico. A series of loosely described assays were performed to ascertain the LC_{50} and NOEC of vinclozolin on *B. calyciflorus*.

According to the methods, 24-hr acute toxicity assays were run using 3.12, 6.25, 12.50, 25.00 and 50.00 mg/L to define the LC_{50}.

According to the methods, 96-hr sublethal assay was conducted using 0.185, 0.375, 0.750, 1.500 and 3.000 mg/L. Measurement endpoints included number of asexual females, parthenogenic eggs, sexual females, males and resting eggs. There were 5 replicates per treatment with 10 neonates in each replicate. Test animals were fed with *Nannochloris oculata* algae concentrates ($3x10^6$ cells/mL)

Each assay had both a control and a solvent control group along with several groups of differing vinclozolin concentrations. The estimated values found by these assays were: 24-hr LC_{50} 30.5 mg/L, NOEC 3.125 mg/L, LOEC 6.25 mg/L. The study then appears to somehow combine asexual females, parthenogenic eggs, sexual females, males, and resting eggs into a single endpoint. This endpoint is used to evaluate the effects of varied vinclozolin concentrations (0.185, 0.375, 0.750, 1.500, 3.00 mg/ml) on *B. calyciflorus* populations in a 96 hour sublethal test. The results of this test are not clearly expressed, and seem to show and increase in total organisms at 0.375 mg/ml vinclozolin with no effect seen at any other concentration (**Figure 3**). Although the authors characterize the effect "total population response" as an inverted "U", it could also be interpreted to mean that for whatever reason, the response observed for 0.375 mg/L was aberrant.

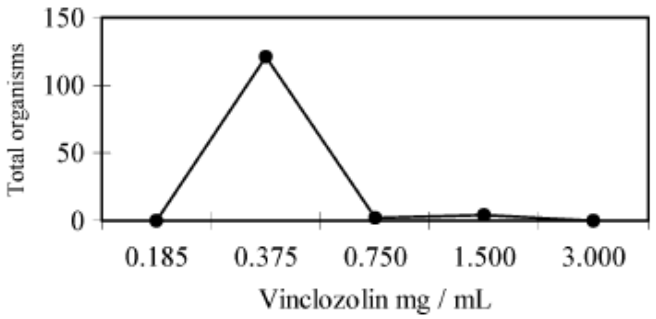

Fig. 3. *Brachionus calyciflorus* total population response to sub-lethal concentrations of the fungicide vinclozolin.

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

Rationale for Use: The study provides an 24-hr LC50 value for the freshwater rotifer *Branhcionus calyciflorus*.

Limitations of Study: Many of the studies methods are unclear and statistics are not provided for much of its data. The rationale for combining the endpoints originally defined into one confusing endpoint is not discussed and as such is unclear. Also, the graph of results needs more explanation than "an inverse U". Chemical percentage is not defined.

Primary Reviewer: TJ Graven, Biologist

[1]Acevedo-Pallares, J.E. Effectos en reproduccion sexual y asexual del rotifero *Brachionus calyciflorus* (Pallas) expuesto a vinclozolin de forma artificial y a sedimentos de lago Chapala (Mex), B.S. Thesis: Uiversidad Autonoma de Guadalajara: Guadalajara Mexico, 2005; 78p.

[2]Acevedo-Pallares, J.E.; Xaval-Aguirre, J.L. Disrupcion endocrina en rotifero *Branchionus calyciflorus* (Pallas) expuesto al fungicida vinclozolin. In Memoria de Congreso de Ecotoxicologia y Quimica Ambiental, Puebla Mexico, April 24-28: 2006; Ramirez-Romero, P. Mangas-Ramirez, E., Wong-Chang, I., Eds: Asociacion Mesoamericana de Ecotoxicologia y Quimica Ambiental: Puebla Mexico: 2006 DE01, 53-54

Degradate (3,5-DCA) Open Literature Reviews

Chemical Name: Vinclozolin (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 ± 4°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 ≤ 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:

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

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_{50}) was:

$$y = 1.16x + 0.15 \quad r = 0.90 \text{ and } n = 12.$$

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)

Compound	EC50 (μg/g soil, t = 7 d)	EC50 (μg/g soil, t = 14 d)	EC50 (mg/L solution, t = 16–21 d)	Log K_{ow}
(Chloro)(methyl)phenols				
Phenol[a]	96 (72–129)	79 (58–107)	20 (8–46)	1.46
Phenol[b]	146 (119–180)	168 (145–195)	14 (7.8–24)	1.46
2-MCP[a]	52 (–)	43 (14–130)	16 (4–64)	2.15
3-MCP[a]	21 (12–37)	7 (3–15)	5.6 (0.5–59)	2.50
2,4-DCP[a]	27 (16–39)	53 (12–299)	2.4 (0.1–9.5)	3.06
3,5-DCP[b]	60 (18–195)	32 (–)	1 (–)	3.62
2,4,6-TCP[a]	19 (12–31)	16 (5–51)	1.8 (0.1–41)	3.69
2,3,5-TCP[a]	17 (8–35)	9 (8–11)	2.0 (1.0–4.0)	3.85
2,3,5-TCP[b]	8.5 (6.3–11)	8.9 (7.5–11)	0.79 (0.6–1)	3.85
PCP[a]	7 (4–15)	8 (4–15)	0.03 (0.01–0.1)	5.24
PCP[b]	2.7 (2.2–3.5)	3.2 (2.7–3.7)	0.03 (0.02–0.04)	5.24
4C2MP[a]	>32, <100	>32, <100	4.0 (2.4–6.5)	2.78
4C3MP[a]	>32, <100	>32, <100	2.3 (0.7–7)	3.10
Catechol[a]	>1,000	>1,000	5.0 (1.3–21)	0.88
β-Naphthol[b]	291 (218–388)	88 (72–107)	4.9 (3.9–6)	2.70
o-Cresol[a]	67 (52–86)	>100	23 (16–31)	1.95
m-Cresol[b]	69 (51–94)	96 (63–147)	50 (42–61)	1.96
Nonylphenol[a]	559 (331–946)	625 (502–>1,000)	>0.1, <0.32	
Chloroanilines				
Aniline[b]	49 (43–56)	56 (49–64)	7.9 (6.9–8.9)	0.90
Aniline[a]	32 (0.4–294)	33 (24–45)	17 (5.2–55)	0.90
2-MCA[b]	>32 (±50)	>32[c] (±50)	31 (27–37)	1.90
3-MCA[b]	17 (13–20)	15 (12–19)	5.9 (5.0–6.9)	1.88
2,4-DCA[b]	32 (24–43)	29 (24–36)	7.0 (5.7–8.7)	2.78
2,4-DCA[a]	24 (17–33)	>10, <32	6.9 (3.4–14)	2.78
3,4-DCA[b]	>10 (almost 10)	>10 (almost 10)	1.7 (1.5–1.9)	2.69
3,5-DCA[b]	16 (13–20)	13 (10–16)	5.0 (4.2–5.9)	2.90
2,4,5-TCA[b]	25 (18–35)	17 (15–20)	1.3 (1.2–1.5)	3.45
2,4,6-TCA[b]	27 (20–36)	23 (20–28)	3.5 (3.1–3.9)	3.52
2,3,4,5-TeCA[b]	47 (40–56)	24 (21–28)	0.39 (0.33–0.45)	3.94
2,3,5,6-TeCA[b]	64 (42–99)	16 (13–19)	0.62 (0.53–0.73)	4.10
PCA[b]	647 (333–1,255)	471 (296–751)	>S_w	
PCA[a]	>1,000	>1,000	>S_w	
Chloro(nitro)benzenes				
MCB[b]	1,000	>1,000	9.3 (7.5–12)	2.84
1,4-DCB[b]	213 (156–290)	248 (212–298)	5.1 (4.2–6.2)	3.52
1,2,3-TCB[b]	5.8 (4.5–7.4)	3.8 (3.4–4.2)	0.028 (0.022–0.036)	4.14[c]
1,2,3-TCB[a]	>1, <3.2	1 (0.2–5)	ND	4.14
1,2,4-TCB[b]	56 (43–74)	48 (41–56)	0.6 (0.53–0.69)	4.02
1,3,5-TCB[b]	115 (93–142)	123 (105–144)	2.0 (1.6–2.6)	4.15
1,3,5-TCB[a]	ND	ND	>0.32, <1	4.15
1,2,3,4-TCB[b]	67 (45–98)	32 (27–38)	0.63 (0.53–0.76)	4.64
1,2,4,5-TECB[b]	4.2 (2.5–7.3)	1.3 (1.2–1.5)	0.07 (0.06–0.09)	4.82
1,2,4,5-TECB[a]	2 (1–6)	2 (1–4)	>0.1, <1	4.82
PCB[b]	228 (93–554)	56 (39–81)	±1.0	5.17
PCB[a]	862 (76–>1,000)	±320	>S_w	5.17
HCB[b]	>1,000	>1,000	>S_w	
C2NB[b]	5.0 (4.0–6.0)	5.4 (4.7–6.2)	1.8 (1.6–2.0)	2.24
C2NB[a]	>3.2, <10	>3.2, <10	2.0 (0.2–22)	2.24
C3NB[b]	12 (10–14)	12 (11–13)	4.6 (4.2–5.1)	2.41
2,3-DCNB[a]	20 (17–25)	12 (9–17)	>0.32, <1	3.05
N compounds				
Ethylenediamine[a]	>1,000	692 (570–840)	208 (–)	−2.04
Dipropylamine[a]	383 (262–559)	370 (297–461)	>100, <320	1.67
Dibutylamine[a]	510 (383–680)	361 (294–444)	52 (–)	2.83
Acrylamide[a]	101 (60–170)	152 (124–186)	6 (0.5–68)	−0.67

continued

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_{50} for 3,5-DCA are 16 and 13 µg/g (mg/kg) in soil, respectively, and the 16 - 21-day EC_{50} 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 ≤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_{50} 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.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin (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)"[1], 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 ± 1^oC in 10 mL glass [jars] containing 50 mL of test solution. Test solution had a hardness of 250 mg/L (as $CaCO_3$) 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.

[1]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-d[b] LC50	48-h LC50 (95%C.L.)	21-d LC50 (95% C.L.)	21-d LRCT r_m	L	96-h EC50 (95%C.L.)	15-min EC50 (95%C.L.)
		P.reticulata	D.magna	D.magna	D.magna		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 (56–65)
2[a]	2-Cl	6.3	0.13 (0.09–0.19)	–	–	–	32 (26–38)	13 (10–19)
3[a]	3-Cl	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[a]	4-Cl	26.0	0.05 (0.04–0.06)	–	–	–	4.1 (3.8–4.3)	5.9 (5.3–6.5)
5[a]	2,4-diCl	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[a]	2,5-diCl	1.7	2.92 (2.15–3.97)	–	–	–	10 (8.1–11)	3.6 (3.2–3.9)
7[a]	3,4-diCl	6.3	0.10 (0.08–0.11)	–	–	–	4.2 (4.0–4.4)	1.74 (1.66–1.82)
8[a]	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-CH_3	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-CH_3	36.3	0.15 —	–	–	–	44 (40–48)	26 (22–30)
14	4-CH_3	10.7	0.20 (0.14–0.29)	–	–	–	138 (130–148)	8.0 (7.6–8.5)
15	2-C_2H_5	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-C_2H_5	27.1	0.42 (0.35–0.51)	–	–	–	22 (20–24)	6.2 (5.8–6.5)
17	4-C_2H_5	29.1	0.09 (0.08–0.11)	–	–	–	6.2 (6.0–6.4)	0.21 (0.20–0.22)

[a] Selected by the EC and the IRC.

[b] Data from Hermens et al., (1984).

TABLE VIII:

QSAR-equations for the aniline compounds.
Lineair relationships[a].

QSAR-equation	log $^1/C$= a log P_{oct}(calc)+b				
C (µmol/l)	a	b	no.	s	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

[a]No.: compounds 1-11 of Table II; s: standard error of the estimate and r: correlation coefficient.

TABLE IX

QSAR-equations for the aniline compounds.
Introduction of $\Sigma\sigma$[a]

QSAR-equation	log $^1/C$= a log P_{oct}(calc)+b $\Sigma\sigma$ +c					
C (µmol/l)	a	b	c	no.	s	r
LC50 14-d P.reticulata	0.24	1.17	-3.04	1-11	0.22	0.970
LC50 48-h D.magna	0.06	-0.77	0.10	1-11	0.52	0.587
EC50 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

[a]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_{50} 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.

Primary Reviewer: TJ Graven, Biologist

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

Open Literature Review Summary

Chemical Name: Vinclozolin (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_{50} (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.

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

Equation	Number of compounds	log(1/LT)=	Correlation coefficient
	All compounds, lethal threshold to Crangon		
1	45	1.03*logP + 2.41	0.778
	Phenols and anilines		
2	33	1.02*logP + 2.48	0.780
3	33	0.74*logP + 1.17*(OH)+ 0.81*(NH2) + 0.16*(CH3)+ 0.42*(Cl) + 0.97*(NO2)+1.09	0.909
4	33	0.57*PI + F + 0.86*R+4.65	0.798
	Phenols		
5	23	0.48*logP + 0.54*(DpH)+2.93	0.960
	Phenols, lethal threshold to Mya		
6	8	0.62*logP + 0.79*(DpH)+1.43	0.972

Remarks:
P = octanol/water partition coefficient
(OH), (NH2), (CH3), (Cl), (NO2) = number of hydroxy, amino, methyl, chloro, and nitro groups in the molecule, respectively
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	Number of compounds: Difference between observed and calculated log(1/LT) >0.9	0.7-0.9	0.5-0.7	0.3-0.5	0.1-0.3	<0.1
1	8	7	10	4	7	9
2	8	1	7	6	5	6
3	3	2	4	11	8	5
4	3	2	3	9	6	10
5	3	1	1	3	7	8
6	0	0	1	0	3	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.

Primary Reviewer: TJ Graven, Biologist

Open Literature Review Summary

Chemical Name: Vinclozolin (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: $CaCO_3$ 210 mg/l, mean dissolved oxygen 7.7 mg/L, mean pH 8.0-8.2, Na^+ 1.19mmol/L, K^+ 0.20 mmol/OL, Ca^{++} 1.46 mmol/L, Mg^{++} 0.73 mmol/L, Cl^- 2.72 mmol/L, $SO4^{--}$ 0.73 mmol/L, HCO_3^- 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 µL/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®. The tanks were maintained at 24 ± 2°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[b]
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

[a]All data are experimental values taken from De Bruijn et al. (1989), while the value for 3,5-dichloroaniline was calculated using π constants derived by the same authors.
[b]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)
$\log 1/\text{NOLC} = 0.81(\pm 0.10) \log P_{oct} - 2.85$	0.970	0.36	(9)
$\log 1/\text{NOEC} = 0.66(\pm 0.05) \log P_{oct} - 2.05$	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 re-adjusting the QSAR formula stated earlier to:

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

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.

Primary Reviewer: TJ Graven, Biologist