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Effects of Oral Uptake of the Chemosterilant 4-Vinylcyclohexene Diepoxide in Wild House Mice, *Mus domesticus*

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ABSTRACT: The chemical 4-vinylcyclohexene diepoxide (VCD) induces depletion of primordial follicles in the ovaries of laboratory mice after intraperitoneal treatment and leads to infertility. We assessed the oral uptake of a range of doses of VCD by wild house mice, *Mus domesticus*, in captivity. In Experiment 1, female mice (n = 8 per group) were presented with a daily dose of liquid emulsion containing different concentrations of VCD in a volume equivalent to 10% body weight for 5 days in the presence of *ad libitum* mouse chow and water. Body weight, body condition, and general health status of the mice were assessed daily. Mice were killed 17 days after cessation of dosing. Internal organs were examined for normality and ovaries were fixed for analysis of primordial follicle populations. Over the 5-day treatment period, 95-100% of mice consumed all their daily dose of VCD emulsion. There were no effects on body weight, general activity, or alertness of the mice. Primordial follicle populations showed dose dependent but variable levels of depletion. In Experiment 2, the highest, most palatable VCD dose (300 mg VCD/kg) or a control dose was presented to mice (n = 24-30 per group), daily for 5 days. At 10 days post treatment, males were introduced for 3 breeding rounds. The numbers of pups/litter and proportions of females producing litters for each breeding round was not different between groups. In Experiment 3, male mice were presented with this same VCD dose; assessment of their reproductive status at 7 and 15 days post treatment indicated no treatment effect on testis and epididymis weights. These experiments indicate that while some follicle depletion was achieved in females with the orally delivered VCD dose, this was insufficient to affect their fertility. Further studies will require presentation of higher doses of VCD and/or extended presentation of this chemosterilant.

KEY WORDS: 4-vinylcyclohexene diepoxide, chemosterilant, fertility control, house mouse, *Mus domesticus*, *Mus musculus*, oral delivery, ovarian primordial follicles

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INTRODUCTION

In southeastern Australia the management of the introduced wild house mouse (*Mus domesticus*) in agricultural production systems is an ongoing and significant problem. They cause major damage to crops in parts of the grain growing regions in most years (Caughley et al. 1994, Caughley et al. 1998, Singleton et al. 2005), with serious mouse plagues (when density exceeds 700 mice/ha) generally occurring every 3-7 years (Singleton et al. 2005). Indeed, the most recent plague during 2010-2011 was reputedly the largest ever experienced and affected around 3 million ha in autumn 2010 and cost around AUD200 million in losses (Rohan Rainbow, GRDC, and Simon Humphrys, Chair, National Mouse Management Working Group, pers. comm.), a far greater economic impact than reported for the 1993 mouse plague (AUD120 million) (Caughley et al. 1994). With recent major changes in the management of cropping systems, whereby farmers do not plough paddocks or burn stubble as frequently, and plant seed by direct drilling, there is increased food and shelter available for mice. Such changes in farming practices are likely to lead to increased frequencies of high mouse numbers (Singleton et al. 2005, Brown et al. 2010).

Mouse plagues are currently managed through the widespread in-crop application of the rodenticide zinc phosphide, with the most recent plagues in southeastern

Australia in 2010 and 2011 generating an unprecedented demand for this poison. Alternative management methods which could be applied when mouse numbers are at low levels and when farmers are not experiencing significant economic impacts need to be developed. Indeed, Brown et al. (1998, 2003, 2004) have shown in cereal-based, dryland farming systems and in irrigated cropping systems that habitat modification during non-plague periods can decrease mouse numbers and increase yields. If mouse numbers could be reduced further by decreasing their reproductive output in conjunction with this practice of farm hygiene, low mouse numbers could be maintained for longer periods and reduce damage to crops. Theoretically, fertility control, if able to be delivered efficiently and effectively, could maintain mouse numbers at low levels (Chambers et al. 1999a,b, Davis et al. 2003). Predictive models for outbreaks of house mice have been developed and can be used with some success provided there is current information on rainfall and mouse abundance (spring trapping data) (e.g., for Victorian Mallee; Pech et al. 1999, Kenney et al. 2003). Being able to predict when mouse plagues might occur would allow farmers to decide whether to intervene with pre-emptive control; the threshold at which farmers would apply control would depend on the balance between costs of control and savings in reduced damage to crops (Davis et al. 2004, Kaboodvandpour and Leung

2012). Fertility control has potential to be an additional tool for pre-emptive control that is efficacious, cost-effective, and humane.

In this laboratory-based study, we assessed the effects of a chemosterilant, 4-vinylcyclohexene diepoxide (VCD), on the reproductive function of wild house mice. VCD is an industrial chemical used as an intermediate and reactive diluent for diepoxides and epoxy resins. Toxicological studies conducted by the National Toxicology Program (NIH 1986, NIH 1989, Chhabra et al. 1990) indicated that VCD has an ovary-specific action. In mice and rats, VCD specifically induces depletion of the non-regenerating primordial follicle population of the ovary and thereby accelerates the normal rate of atresia (cell death) of ovarian follicles (Mayer et al. 2002, Mayer et al. 2004, Lohff et al. 2006, Haas et al. 2007, Sahambi et al. 2008, Mark-Kappeler et al. 2011). Treatment of males with VCD temporarily affects spermatogenesis in mice (Chhabra et al. 1990) but not Sprague Dawley rats (Tran, Blome, and Hinds, unpubl.).

The majority of the earlier studies in the mouse and rat were conducted using intra-peritoneal (IP) injection treatments with VCD. Delivery via oral gavage causes follicle depletion but requires approximately 3-fold more VCD than via the IP route. This is most likely due to VCD being susceptible to accelerated hydrolysis into inactive products in the acidic environment of the stomach. We have shown that oral gavage of female Sprague Dawley rats with VCD (250mg/kg, 500mg/kg, and 750 mg/kg) induces a dose- and time-dependent depletion of ovarian follicles, and a significant reduction in numbers of pups produced in successive breeding rounds (Hinds, Tran, Blome, Mayer, and Dyer, unpubl.). Compared to rats, mice appear to be more sensitive to VCD (Borman et al. 2000), although in both species VCD breakdown is very rapid with the half-life in blood being 5 minutes, and with more than 95% of the inactive products being excreted in urine (Salysers 1995). The challenge is to induce >90% depletion of follicles within 2 weeks of dosing to achieve reduced fertility or sterility at the onset of the breeding period (Haas et al. 2007, Sahambi et al. 2008).

The objectives of the present study were to determine the effects of oral delivery of different doses of VCD on gonadal function of wild mice and then assess the effects of an optimal dose on the breeding outcomes of females.

METHODS

Wild house mice were obtained from an out-bred colony maintained by Dr. L. Leung, University of Queensland, Gatton campus. At 5 weeks of age, mice were air-freighted to Canberra and housed individually in cages (330 × 160 × 120 mm, length × width × depth) containing a polypipe for shelter, a running wheel, litter, nesting material, breeder rodent chow (Gordon's Speciality Stockfeeds, NSW, Australia), and water *ad libitum*. The temperature of the animal room was maintained at 22-24 C and the day length was set to 12h light:12h dark (lights on at 0600h and off at 1800h). Mice were checked daily to assess general body condition and weighed at least once per week before any experiment.

Experimental Design

Experiment 1: Effect of oral uptake of emulsions containing a range of VCD doses on female ovarian follicle depletion

Adult females (12-15 g; n = 8 per treatment group) were presented with emulsion (10% body weight by volume) containing a range of doses of VCD (150, 225, 300, 375, and 450 mg VCD/kg) each afternoon for 5 consecutive days. Water and rodent chow were also available *ad libitum*. Consumption of emulsion (% consumed), body weight, and general condition were recorded daily. Control animals received emulsion containing no VCD. The emulsion comprised water, vegetable oil, emulsifier, sweetener, with or without the active ingredient and was produced on a Microfluidics high pressure homogenizer (Microfluidics Corp., Westwood, MA, USA). Two weeks later, mice were anaesthetised and killed for collection of reproductive tissues and assessment of the weight and gross appearance of other internal organs. Ovaries were removed, weighed, and fixed in Bouin's fluid for 24 hrs, and processed for routine histological analysis. One ovary from each animal was serially sectioned (3µm) with every 10th section mounted. After staining with Gill's haematoxylin and eosin, the primordial and primary follicles were counted on each section. The number of each follicle type per ovary was estimated according to the formula derived by Gougeon and Chainy (1987) and numbers were compared between control and treated animals to determine if depletion had occurred due to treatment dose.

Experiment 2: Effects of oral delivery of VCD on fertility of female wild house mice

Using the most effective and palatable dose (300 mg VCD/kg), defined from the results of Experiment 1, two groups of adult females (control n = 24, treated n = 30 per group) were established. VCD emulsion or control emulsion (10% body weight by volume) and rodent chow were provided for 5 consecutive days with consumption of emulsion determined daily. Each female was then paired with another female and held for 8-10 days before a mature male was added to their cage. Rodent chow and water *ad libitum* were provided during the rest of the experiment. Females were weighed twice weekly thereafter and monitored for production of pups. Males were removed when both females in the cage were obviously pregnant. After births were observed (gestation in mice is 19-21 days), litter sizes were recorded, and pups were removed, sexed, weighed, and euthanased. Once both females in a cage had given birth, a different male was added and the cycle repeated. After 2 successive cycles, it was noted that not all pairs of females were producing litters. For the third round of mating, females were held individually with a male, after which all animals were euthanased and reproductive and other internal organs collected.

Experiment 3: Effects of oral delivery of VCD on the male reproductive tract

While effects on males are not essential for effective fertility control, if spermatogenesis is disrupted this has

the potential to further disrupt overall reproductive success in mouse populations and could enhance the efficacy of the method. Therefore, the effects of the dose (300 mg VCD/kg) used in females in Experiment 2 was assessed in males. Adult males (control n = 8, treated n = 21) were presented with emulsion and standard rodent diet for 5 days. Consumption of emulsion and body weight and condition were recorded daily. Half of the animals in each group were killed on Day 7 and the remainder on Day 15 after the end of the treatment period. The reproductive tissues (testes and epididymides) were removed, weighed, and assessed histologically to determine effects on spermatogenesis.

RESULTS

Experiment 1: Effects of oral uptake of emulsions containing VCD on female ovarian follicle depletion

Mice receiving control emulsion consumed all of that emulsion (100%) daily for 5 days (Figure 1). Consumption of emulsions containing VCD was dose-related, with lower doses being more palatable: for the three lower doses (150 mg VCD/kg, 225 mg VCD/kg, 300 mg VCD/kg), consumption was greater than 90% from Days 2-5, while that for the two highest doses (375 mg VCD/kg and 450 mg VCD/kg) was less than 60% for the first 2 days and increased thereafter (Figure 1). The body weights of all mice were maintained during this period.

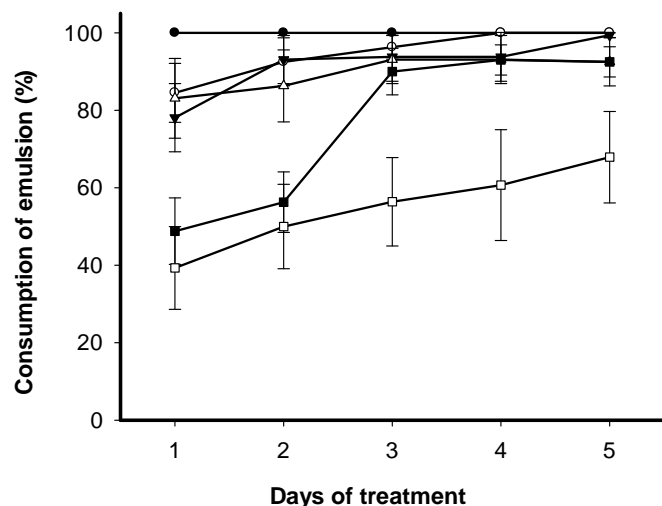


Figure 1. Uptake of emulsions (%; mean \pm SEM) over 5 days by wild house mice (n = 8 per group) receiving either control emulsion (○) or emulsions containing different doses of VCD (□ = 150 mg/kg; △ = 225 mg/kg; ◇ = 300 mg/kg; ▽ = 375 mg/kg; ◊ = 425 mg/kg).

All internal organs (spleen, kidneys, liver, heart) appeared grossly normal and their weights were not different between the control and treatment groups. The reproductive tracts were similar in appearance and all ovaries appeared to contain follicles and *corpora lutea* or *corpora albicantia*. There were no differences in mean combined ovarian weight between the control and treatment groups.

Analysis of the follicle numbers in the ovaries collected from the animals which received 375 mg VCD/kg and 450 mg VCD/kg days was not undertaken because emulsion consumption was highly variable over the treatment period in these groups. Primordial and primary follicle estimates were therefore made only for animals which consumed at least 90% emulsion over 4-5 days: control animals (n = 8), 150 mg VCD/kg (n = 7), 225 mg VCD/kg (n = 7), and 300 mg VCD/kg (n = 7). Initially we estimated follicle numbers for each ovary for the control animals and found no differences in the mean follicle number between the ovaries of an individual animal. Therefore, the results for only one ovary for each control and treated animal are reported here. The estimated number of primordial and primary follicles was variable both between individuals within a treatment group and between the different treatment groups. A dose-dependent depletion of primordial but not primary follicles was observed (Figure 2).

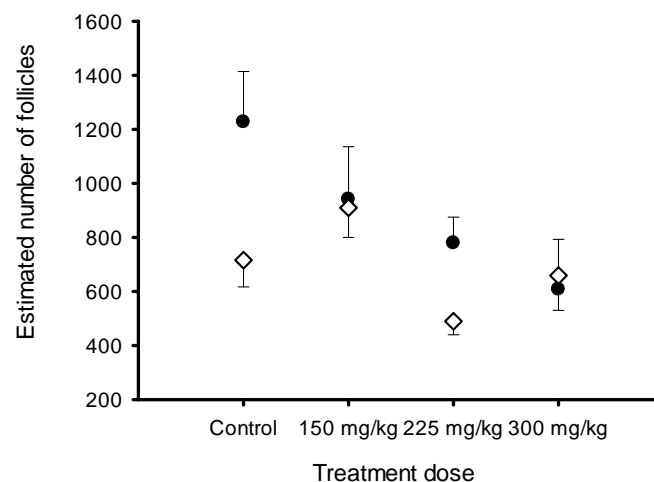


Figure 2. Estimated number of primordial follicles (◇) and primary follicles (□) (Mean \pm SEM) in a single ovary for animals consuming more than 90-100% emulsion daily for 5 days. Controls (n = 8); 150 mg VCD/kg (n = 7); 225 mg VCD/kg (n = 7); 300 mg VCD/kg (n = 7).

Experiment 2: Effects of oral delivery of VCD on fertility of female wild house mice

Control emulsion was all consumed each day for 5 days by the control mice. Consumption of the VCD-containing emulsion (300 mg VCD/kg) was more variable: 3 females consumed 0-25% of their emulsion while 4 females consumed between 25-75% of their dose during the 5-day period. The remaining animals (n = 23) consumed >75% of their emulsion each day for 5 days. The fertility of the latter 23 animals has been compared to that observed in the control females.

After the first round of breeding, the output of the control and treated groups was very similar: 13 of 24 control females produced a litter while 14 of 23 treated females produced litters. Neither mean litter size nor time from introduction of the male to birth was different between the groups (Table 1). Similar results were observed for Round 2. For Round 3, females were

Table 1. Breeding outcomes (litter size and time to birth) over 3 successive rounds for control and treated female wild house mice.

	Round 1		Round 2		Round 3	
	Control	Treated	Control	Treated	Control	Treated
Litter size ^a	5.8 ± 0.5 ^b	5.7 ± 0.6	6.9 ± 0.8	6.2 ± 0.7	6.9 ± 0.4	6.5 ± 0.5
Number of females in group producing litters	13 / 24 (54%)	14 / 23 (61%)	10 / 23 (43%)	11 / 21 (52%)	21 / 23 (91%)	19 / 21 (90%)
Time to birth since pairing with male	25.9 ± 1.0	25.9 ± 1.6	26.1 ± 1.4	24.9 ± 1.4	21.9 ± 0.3	24.6 ± 0.4

^aValue is only for females which produced litters. ^bValues are Mean ± SEM.

Table 2. Body weight, liver and reproductive tissue weights for control and treated males killed 7 or 15 days after 5 days of emulsion uptake.

Treatment	n	Body weight (g)	Liver (mg/g body weight)	Testes (mg/kg body weight)	Epididymides (mg/g body weight)	Seminal vesicles (mg/g body weight)
Control	6	16.1 ± 0.5*	48.3 ± 2.8	9.6 ± 0.4	2.9 ± 0.1	6.2 ± 0.9
VCD for 5 days; killed 7 days later	7	17.1 ± 0.8	49.7 ± 2.0	10.0 ± 0.5	3.0 ± 0.1	6.3 ± 0.5
VCD for 5 days; killed 15 days later	7	14.4 ± 0.7	46.2 ± 1.1	9.7 ± 0.3	3.2 ± 0.1	6.2 ± 0.8

*Values are Mean ± SEM.

individually paired with a male and overall breeding success in each group increased. There were no differences in mean litter size or time to birth between treatments in Round 3 (Table 1). Sex ratios of pups and their body weights were similar between control and treated litters for all rounds.

Experiment 3: Effects of oral delivery of VCD on the male reproductive tract

Control animals consumed all of their emulsion daily for 5 days; however, not all treated males consumed emulsion containing VCD (300 mg VCD/kg). Of 21 males offered VCD emulsion, 4 males consumed none of their offered dose on any day, and 3 other males consumed a small portion of their emulsion on 2 or 3 days only. These treated animals (n = 7) were removed from the experiment and were not included in further analyses.

Mean organ weights were not different between the respective groups of males (Table 2). In the absence of any decreases in tissue weights, histological analyses of the reproductive tissues (testis and epididymis) were not undertaken.

DISCUSSION

Female and male wild house mice consumed emulsions containing the chemosterilant VCD for periods up to 5 days. A range of concentrations of VCD was presented to females and consumption declined as VCD concentration increased. There were no treatment effects on body weight or general behaviour during the 5-day treatment period or thereafter, and all internal organs and tissues were grossly normal at the end of each experimental period. A dose-dependent decline in primordial follicle numbers but not primary follicles was observed in treated females, but this depletion did not result in decreased fecundity or fertility.

The outcome of the breeding trials indicated no short- or long-term effects of the selected orally-delivered dose

of VCD on the fertility of female wild house mice. Consumption of VCD emulsion did not affect litter size, number of females producing pups in each successive breeding round, or the time to birth after the introduction of a male compared to control females. Treatment of males with this selected VCD dose had no short-term effects on reproductive tissue weights.

Earlier studies in laboratory mice where VCD was presented intra-peritoneally (Haas et al. 2007, Sahambi et al. 2008) showed that it is essential to induce >90% depletion of follicles within 2 weeks of dosing to achieve reduced fertility or sterility in the first breeding round. In our study, therefore, it is not surprising that there were no effects on the fertility of the treated mice, as we observed only approximately 50% depletion of primordial follicle numbers.

From our study it is not clear whether the uptake of the VCD across the gut was insufficient; whether VCD was rapidly metabolised once in circulation and thus did not reach the ovarian tissues; or if the treatment duration was too short. Further studies are required to improve the efficacy of VCD as a chemosterilant of house mice. This would require the presentation of higher doses of VCD in a formulation which does not reduce palatability; protection of the VCD formulation to enhance efficiency of uptake and to reduce metabolic breakdown of VCD in circulation; and treatment over a longer period or for successive periods to enhance ovarian follicle depletion. In principle, and with further research, this chemosterilant alone or in combination with other reagents that impair follicular development (e.g., triptolide) (see Dyer et al. 2014) has the potential for managing wild mice when their populations are low.

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