PROJECT REPORT

Project Title: Comparison of 20,25-diazacholesterol, 22-azacholesterol, and aromatase inhibitor as orally delivered contraceptive agents for rodent control.

Research Agency: National Wildlife Research Center

Principal Investigators: P. Nash and L. Miller

Budget: $30,000

Background: Many varieties of rodents cause damage in agricultural and urban settings. In some cases a non-lethal population control method, such as contraception, would be useful. Because most rodent are prolific breeders with short live spans, contraceptive controls may have a visible effect on populations in a short amount of time.

Objectives:
1) Determine which of three compounds (20,25-diazacholesterol, 22-azacholesterol, and aromatase inhibitor) is most effective in inhibiting reproduction using the house mouse as the test animal.
2) Compare the three compounds for possible negative effects on general health and well being.

Summary and Results:

Rodents are associated with a variety of agricultural and structural damage throughout the US. Pocket gophers and ground squirrels cause significant damage to alfalfa crops (Case 1989, Whisson et al. 1999). Pocket gophers, ground squirrels, and voles are associated with damage in orchards (Hazen and Poché 1992, Giusti et al. 1996). Rodents are also vectors of diseases that can be transmitted to humans, such as plague, Q-fever, Rocky Mountain spotted fever, and relapsing fever (Smith 1992, Townzen et al. 1996). Methods of control usually involve lethal measures and repellents. However, when lethal control occurs in the public eye, resistance to such control measures can occur, necessitating the development of additional nonlethal methods.

Population modeling shows reproductive inhibition may be a more effective management strategy than lethal control for species like rodents that have high reproductive and mortality rates (Dolbeer 1998). An integrated program that uses both lethal and reproductive control could potentially reduce the population even more quickly than either method used alone.

DiazaCon

DiazaCon (20,25-diazacholesterol dihydrochloride) prevents the conversion of desmosterol to cholesterol by inhibiting the Δ_{24}-reductase enzyme (Emmons et al. 1982, Yoder et al. 2004). Cholesterol is needed for the production of pregnenolone, the precursor hormone to progesterone, estradiol, and testosterone. Progesterone and estradiol are needed for follicular development; testosterone is necessary for sperm

production. Reducing cholesterol reduces reproductive steroid hormone synthesis, thereby decreasing reproduction (Yoder et al. 2004).

DiazaCon has been tested on several rodent species with success. Mice treated with 10-30 mg DiazaCon/kg body weight for either 10 consecutive days or 10 consecutive days followed by once weekly treatments exhibited a 61% and 91% reduction in reproductive rate (pups/female). The greatest decrease in reproductive rate was observed > 1 month post-treatment. However, no reproductive inhibition was observed in a subsequent dose-response experiment that compared dose levels of 0.1, 1, 10, and 100 mg DiazaCon/kg body weight (Nash, unpublished data).

Prairie dogs treated with 30-45 mg DiazaCon/kg body weight on 10 days spread out over a 3 week period exhibited a 41% decrease in plasma cholesterol concentrations and a 47% decrease in reproductive rate (juveniles/adult). Treatment of prairie dogs was not started until just prior to the breeding season, indicating that earlier treatment with DiazaCon would have a greater inhibitory effect (Nash et al. 2006).

Rats treated with 30 mg DiazaCon/kg body weight for 10 consecutive days exhibited a 74% decrease in plasma cholesterol concentrations, but no significant reduction in reproductive rate. However, this may have been due to the study not being carried out long enough to see the maximum reduction in reproductive rates. A second study with rats compared treatment with 10 mg DiazaCon/kg body weight for either 10 consecutive days or 10 consecutive days followed by once weekly treatments. Rats in this study exhibited only a 27% decrease in plasma cholesterol concentrations in the group receiving DiazaCon for 10 consecutive days. Rats in the treatment group receiving DiazaCon for 10 consecutive days followed by weekly treatments did not have reduced plasma cholesterol concentrations. This dose level was lower than any effective dose reported in the literature, and indicates that a minimum dose of 30 mg/kg is needed to affect cholesterol concentrations. A third study with rats compared treatment with 100 mg DiazaCon/kg body weight for either 10 consecutive days or on 3 days over a 10 day period. Rats had similar plasma cholesterol concentrations, although rats in the 10 consecutive days group had slightly lower concentrations. Because no there was no simultaneous control group, a percent reduction in cholesterol concentrations could not be determined.

AzaCon

The chemical structure of AzaCon™ is identical to cholesterol except for a single nitrogen substitution for the hydrocarbon at the 22 position. AzaCon™ prevents the cleavage of the side chain of cholesterol, thereby inhibiting production of pregnenolone (Lu et al. 1981). Because pregnenolone is the precursor to the reproductive steroid hormones testosterone and progesterone, reproduction is also inhibited.

Aromatase Inhibitors

Aromatase inhibitors are used to treat estradiol-dependent breast cancer (Harper-Wynne et al. 2002). Testosterone is converted to estradiol via the aromatase enzyme in the thecal cells of the follicle. Inhibition of aromatase should reduce estradiol concentrations, thus reducing follicular development, leading to reduced reproductive rates. Arimidex® is a commercially available aromatase inhibitor used to treat breast cancer, and was shown to decrease reproductive rates in rats treated with 0.02-0.1

mg/kg (AstraZeneca Pharmaceuticals, 2002). Because use of Arimidex® itself as a reproductive inhibitor would be cost prohibitive, a mimic compound (Estragone) was synthesized at the National Wildlife Research Center for experimentation.

Centchroman

Centchroman is a selective estrogen receptor modulator (SERM) that is available in India as a weekly nonhormonal contraceptive for women. It is believed to work through two mechanisms: (1) It creates an asynchrony between ovulation and transport of the oocyte through the fallopian tube so that the oocyte reaches the uterus before it is receptive to implantation, and (2) It prevents the uterus from building up a sufficient lining for implantation to occur.

PMHI

Pipecolinomethylhydroxyindane (PMHI) affects reproduction by increasing production of melatonin from pinealocytes, and may also affect the testes directly (Fang and Anderson 1976, Maji et al. 1990). The increase in melatonin decreases GnRH production, and mimics what occurs in the fall in long-day breeders. Research shows only 1-2 doses of PMHI are needed to suppress reproduction, and has been used successfully in seasonally breeding rats to decrease reproductive rates.

Scope of Research

Research was divided into two phases, with initial tests occurring in mice and subsequent testing occurring in rats. Initial tests with mice were to be conducted to determine the efficacy of DiazaCon, AzaCon, and Arimidex as reproductive inhibitors. Because mice do not depend on estrogen for follicular maturation and embryonic development (Guo et al. 2004), further studies with the same compounds were to be conducted on rats. Because several new reproductive inhibitors became available prior to the beginning of the study (Estragone, Centchroman, PMHI), these were also tested.

METHODS
Mouse Studies

Trial 1. Each treatment group consisted of 5 cages of 5 females. Commercially available rolled oats were mixed with molasses to form bait to administer a daily target dose of 10 mg/kg for DiazaCon and AzaCon, and 0.02 mg/kg of Arimidex and Estragone. Three times this amount was provided so that all mice group housed in a cage would have a higher probability of receiving at least the target dose. Bait was provided for 10 consecutive days, and after 10 days of treatment a male was placed in each cage. Beginning approximately 21 days after introduction of a male, cages were monitored for the birth of young.

Trial 2. Each treatment group consisted of 5 cages of 5 females. Commercially available rolled oats were mixed with molasses to form bait to administer a daily target dose of 75 mg/kg for DiazaCon and AzaCon, 5 mg/kg Estragone, and 2.5 mg/kg Centchroman. Arimidex was not tested in this trial. For DiazaCon, AzaCon, and Estragone, bait was provided for 10 consecutive days. After 10 days of treatment, a male was placed in each cage. For Centchroman, bait was provided twice weekly for two weeks, then weekly for two weeks, after which a male was placed in the cage.

Treatment continued on a weekly basis throughout breeding and pupping. Beginning approximately 21 days after introduction of male, cages in all treatment groups were monitored for the birth of young.

Rat Study

Female rats were divided into the 5 following treatment groups: (1) Control, (2) 75 mg DiazaCon/kg body weight, (3) 25 mg Estragone/kg body weight, (4) 5 mg Centchroman/kg body weight, and (5) 150 mg PMHI/kg body weight. Bait was prepared such that 5 g oats with 5% molasses by weight contained the appropriate compound and dose as calculated using the average pretreatment body weight of rats in the study. Female rats were dosed daily with DiazaCon or Estragone for 14 consecutive days. Female rats were dosed with Centchroman twice a week for 2 weeks, then once weekly for the remainder of the study. Female rats were dose with PMHI for 5 consecutive days. Rats were housed individually for 14 days post-treatment prior to the onset of breeding, at which time females were bred to untreated males for 19 days.

RESULTS

Mouse Studies

Trial 1. There was no significant effect of treatment on reproduction (P = 0.5798), but this may have been due to a relatively small sample size. Arimidex appeared to have a slightly inhibitory effect during week 4 as compared to the control (Figure 1). The overall reproductive rate for AzaCon was 2.8 ± 0.6 pups/female compared to 3.5 ± 0.7 pups/female in the control group. Overall reproductive rates were 4.1 ± 0.7, 4.1 ± 0.8, and 3.6 ± 0.7 pups/female in the Arimidex, DiazaCon, and Estragone groups, respectively (Figure 2). By week 8, all of the treatment groups had lower reproductive rates than the control group. Whether this effect is due to a delayed response of the reproductive system to the contraceptives, or is a chance occurrence could not be determined because the study was stopped at that point.

Trial 2. There was no significant effect of treatment on reproduction (P = 0.7394). The overall reproductive rate for DiazaCon was 7.1 ± 1.4 pups/female compared to 8.1 ± 0.7 pups/female in the control group. The overall reproductive rates were 9.1 ± 1.7, 8.7 ± 1.1, and 9.5 ± 0.8 pups/cage in the AzaCon, Centchroman, and Estragone groups respectively (Figure 3). It is possible that breeding was not carried out long enough to observe reduced reproduction. A prior mouse study indicated a delayed response of the reproductive system to DiazaCon, and the Trial 1 results may indicate a similar response to other contraceptive agents.

Rat Study

There was no significant effect of treatment on reproduction (P = 0.1400). The overall reproductive rates were 11.8 ± 0.8, 13.8 ± 0.5, 11.4 ± 0.9, 12.9 ± 0.5, and 12.6 ± 0.7 pups/female in the control, DiazaCon, Centchroman, Estragone, and PMHI groups, respectively (Figure 4). Rats may be similar to mice in that there is a delayed response of the reproductive system to certain contraceptive agents. Breeding was not carried out beyond one cycle in this study.

DISCUSSION

Ground squirrels are seasonal breeders as opposed to rats and mice which are continuous breeders. The results of these studies indicate rats and mice do not make good laboratory models for seasonal breeders. Prairie dogs responded well to DiazaCon treatment even though treatment was implemented late. The response of ground squirrels to DiazaCon may be closer to that of prairie dogs, therefore dose-response tests should be conducted on ground squirrels. Although reproduction cannot be measured in the lab for ground squirrels, enough research has been conducted with DiazaCon to predict the effect on reproduction from the desmosterol and cholesterol concentrations (Johnston et al. 2003). Additionally, if an oral GnRH vaccine becomes available, it may provide a feasible alternative that requires fewer doses for efficacy. California ground squirrels treated with a single injection GnRH immunocontraceptive vaccine resulted in a 91% reduction in lactating females the first year, and a 96% reduction in lactating females the second year. Testicular development was reduced by 89% by the second year, and the number of offspring were reduced by 66% the second year (Nash et al. 2004).

Further research is needed to fully understand the inconsistent results achieved with mice and rats. One possibility is that the strain of lab rat or mouse is important. Some previous studies achieved successful reproductive inhibition using Arimidex and PMHI on laboratory rats (Fang and Anderson 1976, AstraZeneca Pharmaceuticals 2000). Because these studies do not mention the strain of rat used, their results cannot be duplicated. For this reason, one recommendation is to trap mice and rats from the wild to eliminate strain differences.

It is also possible that mice and rats are less susceptible to reductions in plasma cholesterol concentrations at particular times of the year, even though they breed continuously. In the wild, there are certain times of the year when it is more advantageous for rodents to breed because there is greater availability of food and cover. Although, rodents typically have access to food and cover year round, at some point in their evolutionary history they did not. As a result, there may still be a seasonal component to rodent breeding where reproductive output is “protected” more than during periods when food and cover would be less available. Studies using PMHI on seasonally breeding rats were successful, whereas the results of these studies indicated PMHI was ineffective for a continuously breeding rat. Because of these potential problems, reproductive inhibitors that target gonadotropin-releasing hormone (GnRH) or cause testicular lesions may be more appropriate for continuously breeding mice and rats.

Finally, results from prior studies indicate reproductive inhibition may not take effect until > 1 month after the end of treatment. Future studies with mice and rats should carry breeding out at least 6 months after the end of treatment to determine whether this holds true for a given contraceptive.

RESULTS

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Figure 1. Reproductive rates throughout the study period for mice treated with either 10 mg/kg DiazaCon or AzaCon, or either 0.02 mg/kg Arimidex or Estragone in Trial 1.

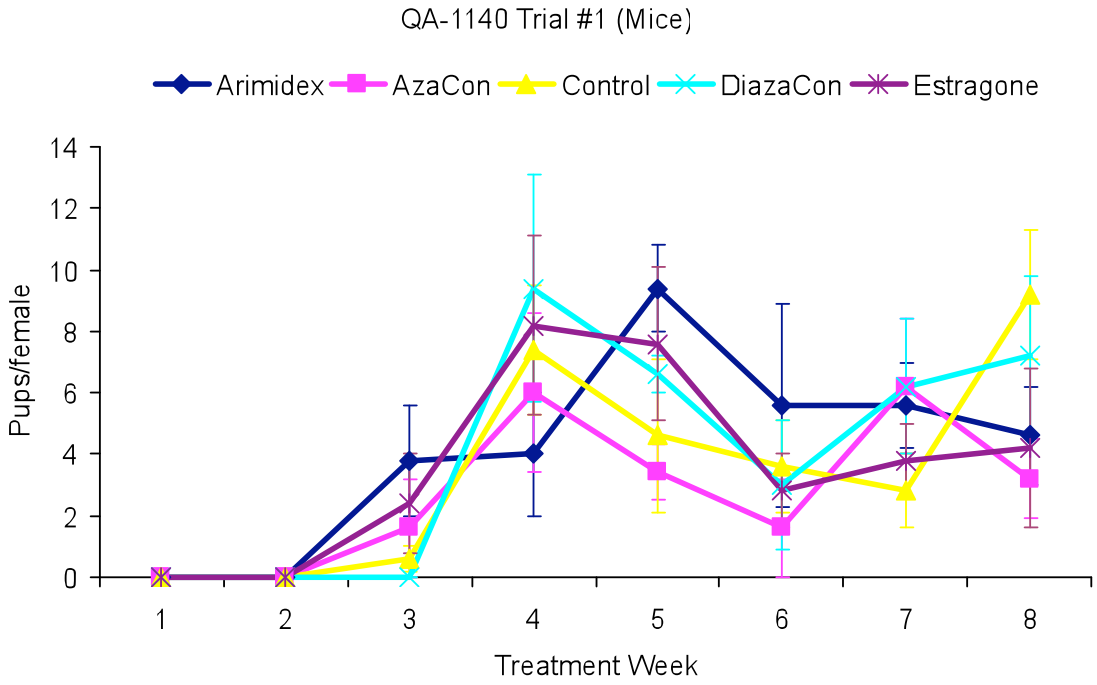


Figure 2. Overall reproductive rates for mice treated with either 10 mg/kg DiazaCon or AzaCon, or either 0.02 mg/kg Arimidex or Estragone in Trial 1.

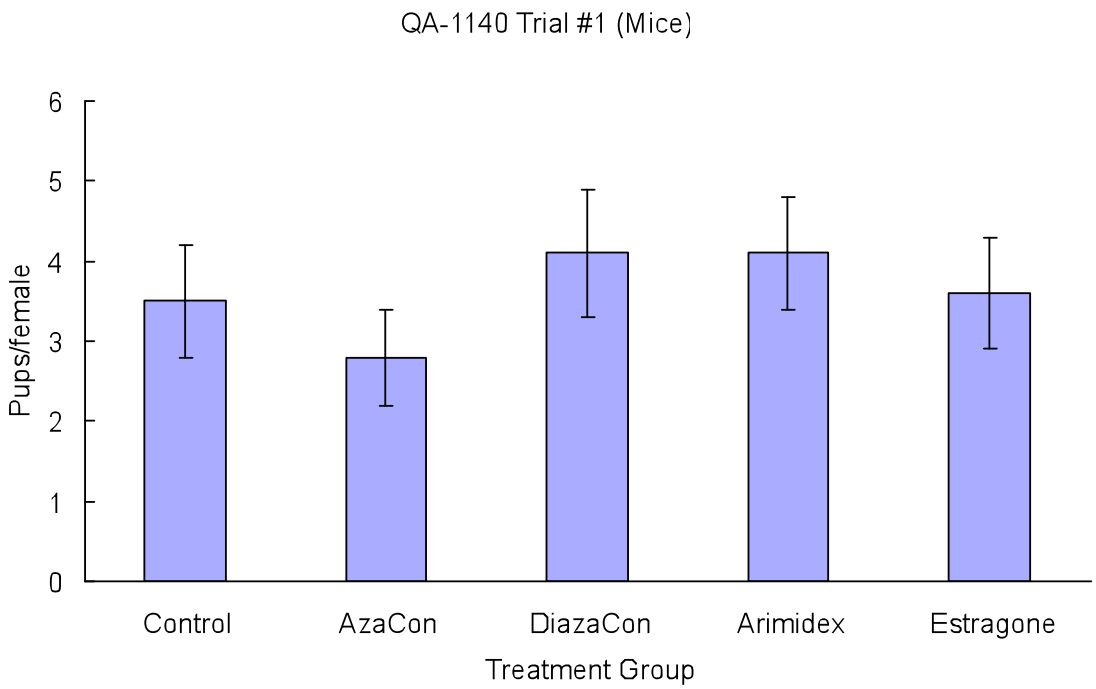


Figure 3. Overall reproductive rates for mice treated with either 75 mg/kg DiazaCon or AzaCon, 5 mg/kg Estragone, or 2.5 mg/kg Centchroman in Trial 2.

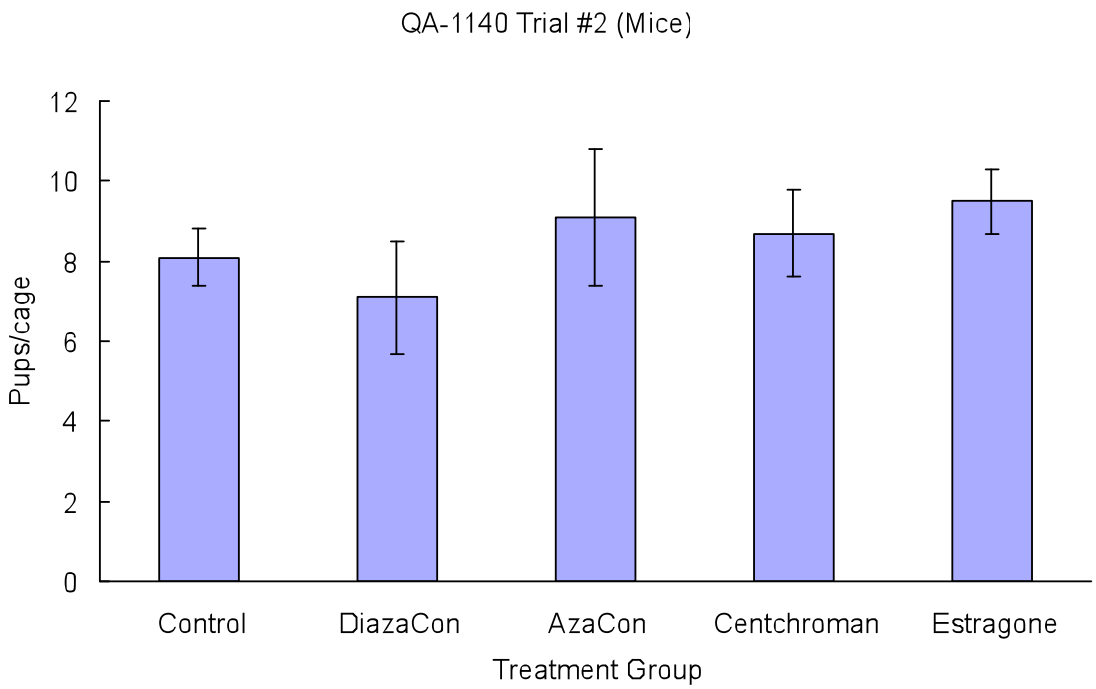
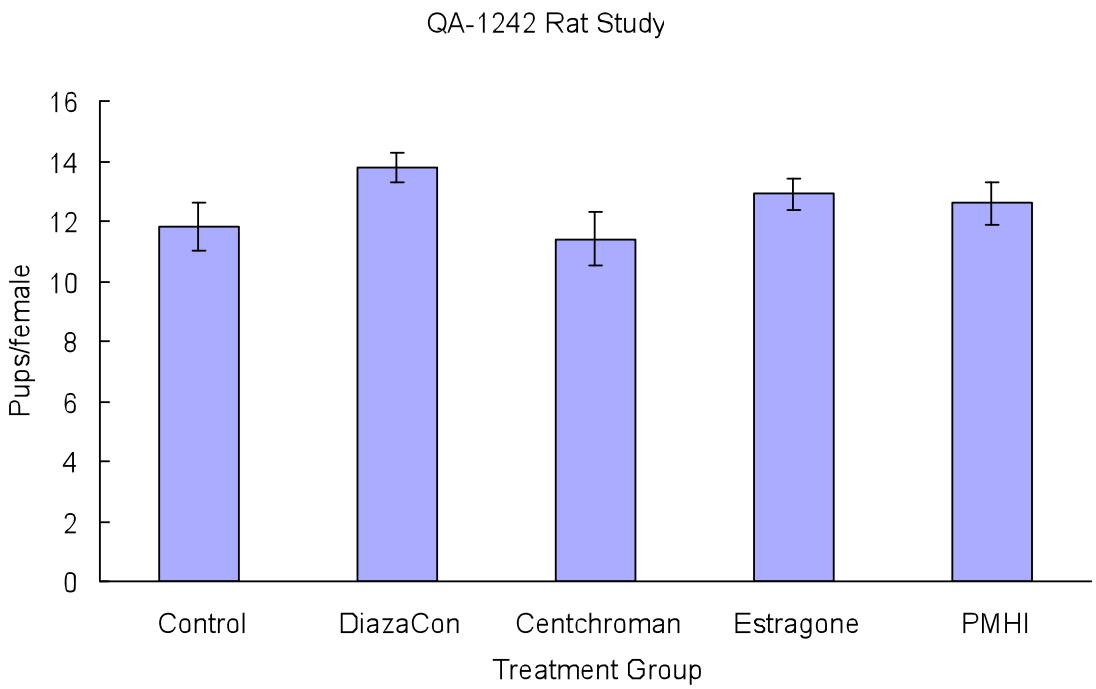


Figure 4. Overall reproductive rates for rats treated with either 75 mg/kg DiazaCon, 25 mg/kg Estragone, 5 mg/kg Centchroman, or 150 mg/kg PMHI.



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