WILDLIFE CONTRACEPTION: TARGETING THE OOCYTE

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Abstract: The USDA's National Wildlife Research Center (NWRC) has successfully researched and developed a number of chemical and immunologically-based wildlife contraceptives. Diazacon™ interferes with cholesterol metabolism and disrupts steroidogenesis, while nicarbazin (registered as OvoControl-P® and OvoControl-G®) disrupts the structure of the vitelline membrane of bird eggs. Immunologically-based agents act to stimulate targeted antibody production. GonaCon™ causes the host's immune system to bind gonadotropin releasing hormone, preventing ovulation, while SpayVac™ prevents fertilization of the post-ovulatory oocyte. This kind of target specificity can be highly advantageous. A number of oocyte-only control contraceptive strategies are currently being researched at the NWRC. 4-Vinylcyclohexene diepoxide (VCD) is an industrial chemical which is specifically ovotoxic, depleting the ovarian oocyte pool with repeated exposure. Research into VCD efficacy as well as the comparability of a similar diepoxide, ERL 4221, in rats and pigs is in progress. Immunological inhibition of recently discovered oocyte-secreted proteins which regulate follicular development in mammals is also of interest. Two such proteins, growth differentiation factor 9 (GDF9) and bone morphogenic protein 15 (BMP15), are highly specific targets for the suppression or elimination of folliculogenesis. These oocyte-specific strategies may offer new, effective alternatives for wildlife contraception.

Key Words: 4-vinylcyclohexene diepoxide, 20,25-diazacholesterol, Diazicon™, bone morphogenic protein 15, contraception, ERL 4221, GonaCon™, gonadotropin releasing hormone, growth diffentiation factor 9, invasive species, nicarbazin, oocyte, porcine zona pellucida, SpayVac™.

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INTRODUCTION

For the last 15 years, the National Wildlife Research Center (NWRC) has been involved in the research and development of chemical and immunological contraceptive agents to control overabundant wildlife. Chemical agents inhibit or diminish reproduction by exerting an effect which is not mediated by the organism's immune system. These chemicals can be natural unmodified compounds, synthetic non-natural compounds, or biologically-produced molecules whose structures have been modified to alter their normal interactions in the body. Immunologically-based agents act by eliciting a response from the immune system in the form of antibody production directed at a reproductively important target such as a hormone. These molecular targets do not, by themselves, possess the immunogenicity necessary to stimulate the immune system, so the molecule or a small, immunologically active portion of it (an epitope) must be linked or conjugated to a much larger molecule that the immune system will detect, identify as foreign, and produce antibodies against. Ideally, antibodies to the target compound are produced as a by-product of this process. The selection of this larger carrier molecule can be as important as the identification of the appropriate epitope of the target molecule. Normally, the conjugate is dissolved in a saline solution and aggressively mixed with an adjuvant (a mixture of surfactant and mineral oil) to form a thick emulsion. When injected, the appropriate adjuvant potentiates the immune system, enhancing the response to the conjugate without causing serious localized tissue damage. The adjuvant also serves to immobilize and extend the release of the conjugate, maximizing its immunogenicity.

Recently, increasing attention at the NWRC as well as other institutions worldwide has been given to the application of contraceptive agents and strategies to control escalating numbers of introduced or invasive species. Additionally, the NWRC is currently researching new approaches to wildlife contraception utilizing both chemical and immunogenic agents whose actions focus on the pre-fertilization oocyte. Hopefully, the use of these

agents will compliment the chemical/immunogenic products already developed by the NWRC for the contraception of problem wildlife, particularly invasive species.

EXISTING CONTRACEPTIVE STRATEGIES

DiazaCon™

20,25 Diazacholesterol dihydrochloride (CAS# 1249-84-9) was originally developed as a cholesterol-lowering drug for use in humans (Sachs and Wolfman 1965) and later investigated to determine its efficacy in reducing the cholesterol content in egg yolks for human consumption (Singh et al. 1972). The chemical 20,25-diazacholesterol dihydrochloride (Figure 1) is an analog of cholesterol, in which carbons in the 20 and 25 positions have been replaced with nitrogen. It acts by mimicking cholesterol and inhibiting the Δ^{24}-reductase enzyme which converts desmosterol to cholesterol during the process of steroidogenesis (Ahrens et al. 1965, Ranney and Cook 1965). This results in an increase in desmosterol and a corresponding decrease in cholesterol, which in turn reduces the concentrations of steroid sex hormones (e.g. testosterone and estrogen) that are critical for proper reproductive function. Used under a variety of synonyms and trade names such as SC-12937 and ornitrol, 20,25-diazacholesterol has been investigated as a contraceptive in several bird species (Johnston et al. 2001a). Under the new trade name DiazaCon™, the NWRC has continued extensive research using 20,25-diazacholesterol as a contraceptive in birds and mammals.

Yoder et al. (2005) summarized a series of laboratory experiments performed at the NWRC using DiazaCon™ as a contraceptive in quail *(Coturnix coturnix)*, ring-necked doves *(Streptopelia risoria)*, brown headed cowbirds

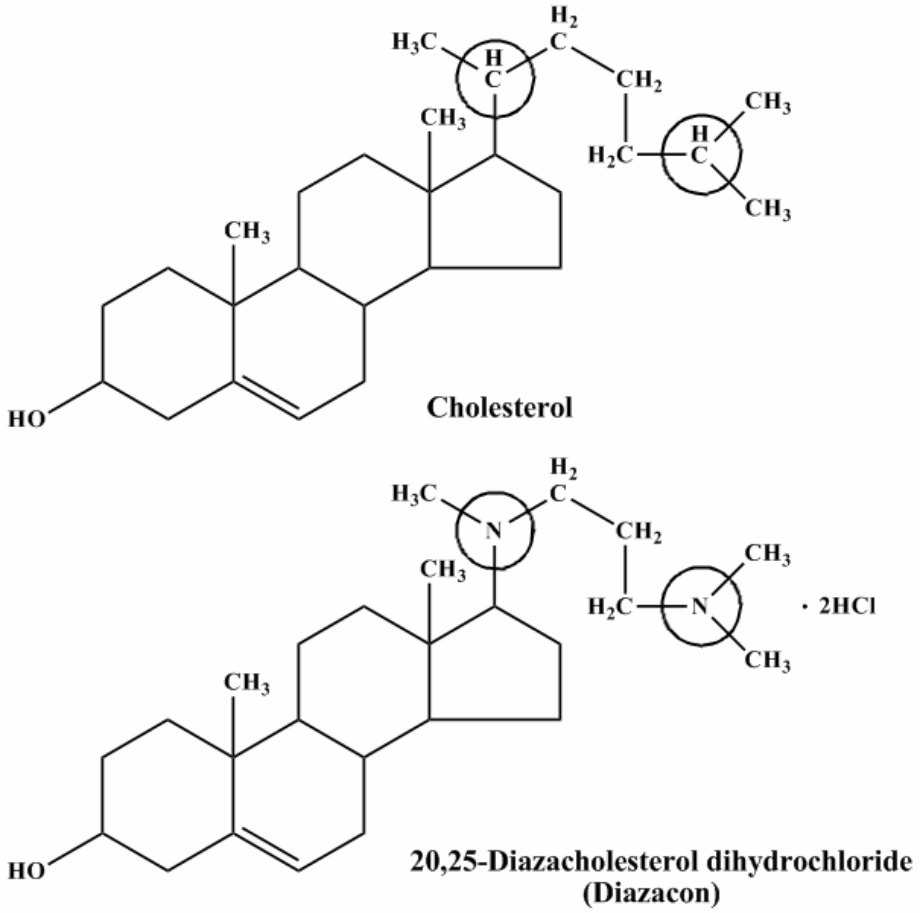

Figure 1. Structural comparison of cholesterol and 20,25-diazacholesterol (Diazacon™). Circled carbons in cholesterol are substituted with nitrogens in Diazacon™.

(Molothrus ater), monk parakeets *(Myiopsitta monachus),* American crows *(Corvus brachyrhyncus),* and mallards *(Anas platyrhynchos).* Serum levels of desmosterol and cholesterol as well as egg production (when possible) were assessed. In all species, treated birds showed substantially higher levels of serum desmosterol and reduced levels of cholesterol in treated birds compared to controls. The direct effect of DiazaCon™ treatment on egg production and hatchability could not be assessed in cowbirds or crows, but treatment dramatically decreased egg production and hatchability in monk parakeets, mallards, and quail. Treated quail also exhibited decreases in female serum progesterone and male testosterone levels of 42% and 37%, respectively (C. Yoder, unpublished data). Increased serum desmosterol levels with a concomitant decrease in serum cholesterol concentrations may serve as a useful indicator of decreased reproductive potential in situations where adults can be captured and blood samples taken, but nests are inaccessible or adults will not breed in captivity.

The use of 20,25-diazacholesterol as a mammalian contraceptive has also been explored. Spermatogenesis was inhibited in both bandicoot rats *(Bandicota bengalensis)* injected with 20,25-diazacholesterol at 100-200 mg kg^{-1} (Hikim and Chakraborty 1986, Hikim 1987) and in mice *(Mus musculus)* given 28 doses of 10, 20, or 30 mg kg^{-1} (Singh and Chakravarty 2003). Nash et al. (2007) found both a 47% decrease in reproductive success and significantly increased serum desmosterol and decreased serum cholesterol in individual free-ranging black-tailed prairie dogs *(Cynomys ludovicianus)* given daily portions of about 18 g of a treated oat baits containing about 45 mg DiazaCon™ for 10 days.

The contraceptive potential of DiazaCon™ is currently being investigated in a number of invasive species. Native to southern South America, the monk parakeet is now widely distributed globally. Between the late 1960s and early 1970s, more than 64,000 monk parakeets were imported into North America (Lever 1987). Parakeets were released both intentionally and unintentionally and stable populations currently exist in as many as 15 states in the United States (US, Spreyer and Bucher 1998). Monk parakeets have also become established throughout the Caribbean, Japan, Israel, and much of Europe. Although the parakeet may pose a threat to local agriculture, the primary problem associated with this species in the US is the construction of large communal nests, woven together out of twigs and branches. These nests are often built in electric utility substations and transmission lines and can cause short circuits and resultant power outages (Avery et al. 2002, Yoder et al. 2007). In the state of Florida, where the population of monk parakeets may exceed 100,000 individuals, the threat to power generation and transmission equipment is of serious concern. DiazaCon™ administered through gavage to captive monk parakeets elicited increased desmosterol serum levels with associated decreases in serum cholesterol as well as significantly decreased egg production in studies conducted by the NWRC (Yoder et al. 2007).

The rose-ringed parakeet *(Psittacula krameri)* is another invasive parrot which has been widely exported for the pet trade. The species native range covers much of northern and central Africa as well as the Indian sub-continent, but rose-ringed parakeets have established self-sustaining populations in the United Kingdom (UK), Japan, Iran, South Africa, and the US (primarily in California and Florida). Large flocks of parakeets can cause extensive damage to orchards and croplands.

The UK's Central Science Laboratory (CSL), in cooperation with the NWRC, is currently researching the use of DiazaCon™ for the control of this species. The CSL is also exploring DiazaCon™ as a means of controlling burgeoning populations of the eastern grey squirrel *(Sciurus carolinensis).* Introduced in the late 18th century from the eastern US, the grey squirrel has rapidly expanded its range and has displaced the native Eurasian red squirrel *(Sciurus vulgaris)* throughout much of the UK.

Nicarbazin

Nicarbazin is an equimolar mixture of 4,4′-dinitrocarbanilide (DNC) and 2-hydroxy-6,6-dimethylpyrimidine (HDP, Figure 2). Commonly used as a treatment for coccidiosis in broiler chickens (Ott et al. 1956), nicarbazin consumption by laying or breeding hens causes a reduction in egg laying and hatchability (Jones et al. 1990, Hughes et al. 1991). Although the exact mechanism of nicarbazin action is unclear, it may prevent ova from maturing (Baker et al. 1957), probably by affecting the structural integrity of the vitelline membrane (the membrane which separates the egg's yolk from the albumen), allowing yolk and albumen to mix (Cunningham 1977, Chapman 1994). Decrease in egg production may be due to a lack of proper yolk deposition in the maturing follicle (Luck 1979). Yoder et al. (2006) found that

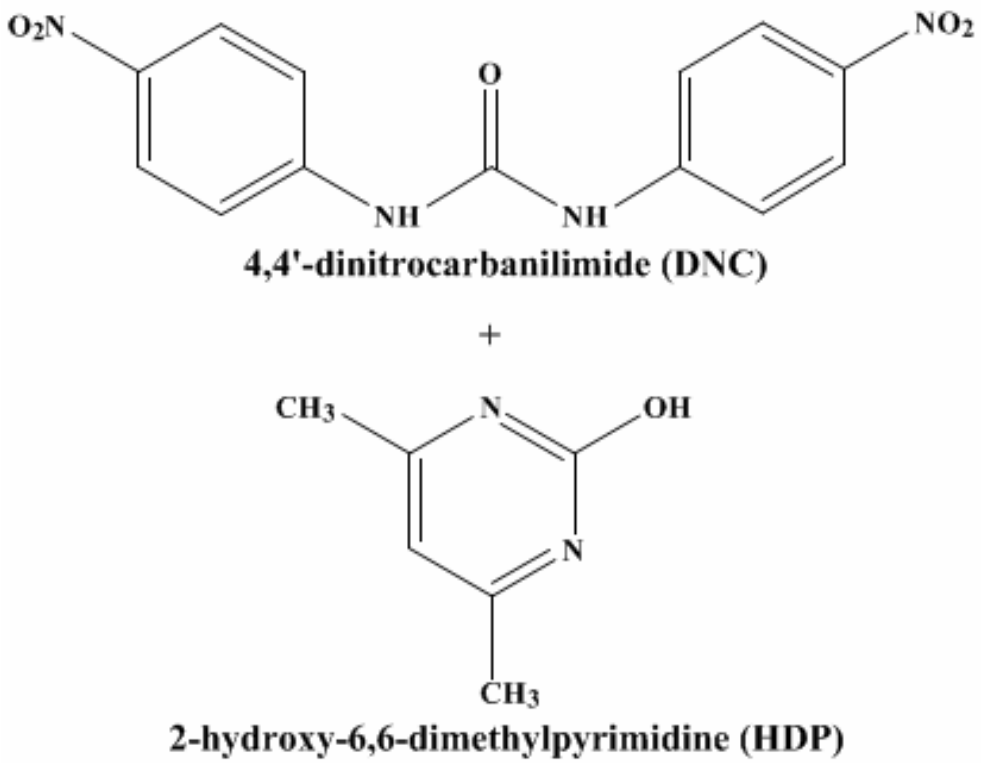

Figure 2. Chemical structure of nicarbazin, an equimolar mixture of 4,4'-dinitrocarbanilimide (DNC) and 2-hydroxy-6,6-dimethylpyrimidine (HDP).

nicarbazin increased lipoprotein lipase activity and acted as a calcium ionophore in vitro, suggesting possible mechanisms for the effects observed in vivo.

By decreasing the number of eggs layed and overall egg hatchability, nicarbazin's potential as an avian contraceptive has been extensively investigated. In tandem with the development of a palatable, nicarbazin-containing bait, analytical methods have been developed to confirm the presence of DNC in avian serum, egg yolk and albumin, and eggshells following ingestion of treated feed or baits (Johnston et al. 2001b, Primus et al. 2003, Stahl et al. 2003).

Sherwood et al. (1956) found a 10-50% decrease in egg fertility from leghorn hens fed nicarbazin in concentrations ranging from 6-700 mg kg^{-1} of feed. Chickens fed varying levels of nicarbazin over 14 days showed positive correlations between amounts of nicarbazin consumed and residues in plasma and eggs (Johnston et al. 2001b), while reproductive success as measured by both hatchability and decreased reproduction rate decreased as amount of nicarbazin consumed increased. Yoder et al. (2006) demonstrated a positive dose response relationship between increasing levels of nicarbazin consumed and DNC residues in plasma, eggs, and feces of treated mallards.

A series of studies conducted at the NWRC led to the development of OvoControl G®, a 2500 ppm nicarbazin bait developed jointly by NWRC and Innolytics, LLC (Rancho Santa Fe, CA). When given to captive Canada geese *(Branta canadensis)*, OvoControl G® was both well-accepted and effective in producing plasma DNC levels in excess of those found to be minimally necessary to affect reproduction in geese (Bynum et al. 2005, 2007). In field studies, free feeding of OvoControl G® to 63 pairs of nesting Canada geese at 10 sites (5 control, 5 treated) in Oregon resulted in a 36% decrease in egg hatchability when compared with control sites (Bynum et al. 2007). OvoControl G® was subsequently registered with the US Environmental Protection Agency (EPA) for use prior to and during the breeding season to reduce hatching success in Canada geese.

In a preliminary study using captive rock pigeons *(Columba livia)* fed a variety of nicarbazin formulations, Avery et al. (2006) found that a 2,500 ppm nicarbazin bait did not significantly reduce egg hatchability, which may have been due to lower than expected plasma nicarbazin residues. A bait formulation of 5,000 ppm produced sufficiently high plasma levels to affect reproduction. In a subsequent study, rock pigeons fed 5,000 ppm baits did not exhibit reduced egg production, but egg viability was reduced by 59%, and productivity tended to be inversely correlated with plasma DNC concentrations. (Avery 2006). These results led to an EPA registration of the nicarbazin-containing bait, OvoControl P®, for use in controlling pigeon populations.

Nicarbazin has been investigated as a possible contraceptive for invasive populations of rose-ringed parakeets in the UK, but birds gavaged with nicarbazin at doses ranging from 8.4 to 18 mg kg^{-1} showed virtually no plasma DNC residues. However, DNC analysis in fecal material yielded high concentrations, suggesting a lack of absorption from the gut. Comparative differences in psittacine digestive physiology may be responsible for the loss of nicarbazin efficacy (C. Yoder, personal communication).

GonaCon™

Gonadotropin releasing hormone (GnRH) is a decapeptide produced by the hypothalamus and transported in the bloodstream to the anterior pituitary where it stimulates specialized cells called gonadotrophs to produce luteinizing hormone (LH) and follicle stimulating hormone (FSH). These gonadotropic hormones are critical for the stimulation and precise timing of ovulation in female vertebrates and for testosterone production and spermatogenesis in males. Prevention of GnRH delivery to the pituitary gonadotrophs and subsequent lack of LH/FSH secretion can partially or completely disrupt these functions, leading to infertility.

By itself, GnRH is not immunogenic as it is both a natural, endogenous peptide which is not identified as foreign, and is simply too small to elicit attention from the immune system. Developed at the NWRC, GonaCon™ is a contraceptive vaccine comprised of the GnRH decapeptide conjugated to either keyhole limpet hemocyanin (KLH), a large protein extracted from the keyhole limpet *(Diodora cayenensis)*, or "blue protein" extracted from the Chilean abalone *(Concholepas concholepas)*. The immunogenicity of the conjugate is increased by emulsifying it in AdjuVac™, an adjuvant also developed at the NWRC. AdjuVac™ is a modification of the Johne's vaccine made more immunogenic by the addition of killed *Mycobacterium avium*, a common bacteria (Miller et al. 2004). In rabbits, AdjuVac™ yielded a good immune response with fewer inflammatory reactions and associated tissue damage when compared with Freund's Complete, a frequently used adjuvant (Powers et al. 2007)

GonaCon™ is an effective contraceptive in a variety of species, including free-ranging California ground squirrels (*Spermophilus beecheyi*), captive Norway rats (*Rattus norvegicus*), domestic cats (*Felis catus*), domestic and feral swine (*Sus scrofa*), wild horses (*Equus caballus*), bison (*Bison bison*), and white-tailed deer *(Odocoileus virginianus)* [Miller et al. In Press A]. EPA registration will be pursued for contracepting female white-tailed deer (Fagerstone et al. In Press).

Feral pigs (*Sus scrofa*) have become invasive in 38 states, with an estimated 2 million individuals in Texas alone and have established wild populations in the UK, Australia, New Zealand, and South America. GonaCon™ was injected into captive domestic pigs in one of three vaccination injection treatments, either 800 μg, 1,600 μg, or a prime injection (400 μg) followed by a booster injection (400 μg). In all pigs, serum antibody titers were highest in the 2 x 400 μg injection treatment, followed by the 1,600 μg and 800 μg treatments. Antibody titers were inversely correlated with fertility and estrus suppression in sows and with testicular size and serum testosterone in boars (Miller et al. 2003). Killian et al. (2003) gave a single injection of either 1,000 μg or 2,000 μg GonaCon™ to penned feral pigs, and found reduced testicular and ovarian size as well as decreased serum testosterone and progesterone, all of which correlated with increasing antibody titers. Pregnancy was reduced by 90% using the 2,000 μg dosage, while 1,000 μg was sufficient to maximize effects in boars. GonaCon™ is currently being used in a research program to contracept feral pigs in the UK (Massei et al. In Press).

Brushtailed possums *(Trichosurus vulpecula)* are native to Australia and have been introduced to both the UK and New Zealand. In New Zealand, possums spread disease and consume native plants, bird eggs, and chicks. Researchers are currently employing GonaCon™ to determine its effectiveness in contracepting possums (Eckery et al. In Press).

SpayVac™

The zona pellucida (ZP) is a glycoprotein coat that encapsulates the ovum, and is essential for oocyte survival and the process of fertilization. In most mammals, the ZP consists of three large glycoproteins, but for the purposes of immunocontraception the principle ZP glycoprotein constituent is ZP3. This glycoprotein contains carbohydrate groups that function as sperm receptors, adhering to specific proteins on the surface of the sperm acrosomal membrane, anchoring the sperm to the ZP surface, and initiating the process of fertilization.

Porcine zone pellucida (PZP) is an extracted, purified preparation from pig ovaries. Injection of PZP into non-porcine species causes an immunogenic reaction, stimulating the production of antibodies directed against the zona's glycoproteins. This in turn prevents sperm from binding to the zona surface, resulting in infertility. Additionally, because the amino acid sequences of the zona glycoproteins are highly conserved between mammalian species, PZP-produced antibodies will frequently cross-react with the ZP of many other species (Killian et al. 2004). Several PZP preparations have been studied, but the use of SpayVac™ (Brown et al. 1997) emulsified in AdjuVac™ adjuvant yielded effective contraception in white-tailed deer for a period of 6 years with a single vaccination (Miller et al. In Press B). These results indicate that the preparation methodology used to produce SpayVac™ provides a "self-boosting" quality to the immunization.

Native species-specific ZP, non-native ZP, and PZP have been used to contracept a wide variety of species. Rats treated with mouse zona pellucida exhibited a decrease in both numbers of litters and pups per litter (Miller et al. 1997). Kirkpatrick et al. (1996) evaluated PZP in 74 species of captive zoo mammals and achieved successful contraception in 27 species from 8 families, including ursids, felids, mustelids, giraffids and bovids. The list of species in which separate studies have shown PZP to provide effective contraception is extensive and includes African elephants *(Loxodontia africana*, Fayer-Hosken et al. 1999), grey seals *(Halichoerus grypus,* Brown et. al 1997), coyotes (*Canis latrans,* Miller et al. 2006), burros (*Equus asinus*, Liu et al. 1989), and baboons (Dunbar et al. 1989). PZP does not appear to be effective in domestic cats (Gorman et al. 2002) which may be due to large differences in zona proteins between the donor and recipient species. Currently, SpayVac™ is used to control overabundant species such as white-tailed deer (Locke et al. 2007, Miller et al. In Press B) and wild horses (Killian et al. 2004), but no applications involving invasive species have been reported.

One of the advantages of the immune response to PZP is the targeting of specific zona glycoproteins which do not occur elsewhere in the body. The PZP response is also inherently sex-specific, as males to do not produce ZP proteins. Likewise, nicarbazin only affects female birds. Although other agents described in this paper may have specific action sites, they can produce more generalized, secondary effects which involve non-target tissues or physiological systems, possibly leading to undesirable side effects. Sex-specificity can be readily achieved for all contraceptive techniques if the desired individuals can be trapped and treated or vaccinated, it would be far more difficult if contraceptive agents were broadcast generally in the environment through baiting. In many, if not most, applications involving wildlife contraception, appropriate baits and baiting techniques will be essential for the efficient deployment of any contraceptive agent. Chemical agents such as DiazaCon™ or nicarbazin are usually better suited to inclusion within a bait matrix and are routinely presented in that form, but for immunologically-based agents, the preservation of immunogenicity following consumption and digestion poses significant developmental challenges. Conjugated agents such as GonaCon™ may prove more amenable to this approach than heterogeneous preparations like SpayVac™. Techniques that facilitate the effective oral delivery of contraceptives to free-ranging wildlife are also under development at the NWRC.

Difficulties related to target specificity might be reduced or eliminated with the continued development of contraceptive agents with highly specific action sites. The advantages of increased target specificity include: (1) selection of the sex of the individual to be contracepted, (2) location of action within the body, and (3) timing of the intervention. A variety of new approaches which specifically target the oocyte are now being researched at the NWRC.

OOCTYTE-SPECIFIC STRATEGIES

4-vinylcyclohexene diepoxide, ERL-4221

As with the previous contraceptive agents discussed, the avenues of research being pursued by the NWRC include both chemical and immunological agents. 4-vinylcyclohexene (VCH)

is a byproduct in the synthesis of rubber, insecticides and plasticizers. In vivo, VCH is metabolized to 4-vinylcyclohexene diepoxide (VCD, Smith et al. 1990a). Initial experiments showed that the administration of VCH to mice reduced the number of primary and secondary ovarian follicles (Collins and Manus 1987), but this effect was later found to be due to VCD following metabolization of VCH (Smith et al. 1990b). VCD ootoxicity is thought to be due to the reduced ability of primary and pre-antral follicles to convert VCD to its tetrol metabolite (Figure 3), resulting in follicular atresia by apoptosis (Hoyer et al. 2001). Intraperitoneal injection of VCD daily for 6 to 15 days at dosages of 80-240 mg kg^{-1} results in significant or near-complete depletion of the ovarian oocyte pool in mice and rats (Hoyer et al. 2001), a phenomenon which has been used to model menopause in humans. VCD-induced oocyte depletion has also been observed in cynomolgous macaques (Appt et al. 2006) and dogs (Miers et al. 2005).

Because of various safety concerns associated with VCD usage, bulk production of the compound was terminated in 2005 (Ellis 2006). Available now only as a specialty chemical, the cost of VCD in large quantities has become prohibitive. For commercial uses, however, VCD was replaced by Cycloaliphatic Epoxide Resin, ERL-4221, Dow Chemical Co. (Figure 4). ERL-4221 is significantly less hazardous, but there are no reports on ERL-4221 efficacy as an ootoxicant. The potential of ERL-4221 as an oocyte-targeted contraceptive tool is currently being investigated by the NWRC in both feral pigs in cooperation with Texas A&M University, Kingsville, Texas, and in comparison with VCD in rats at NWRC headquarters in Fort Collins, Colorado.

Growth Differentiation Factor 9, Bone Morphogenic Protein 15

The control of fertility in general and follicular development in particular is an extremely complex process that is not well understood. A wide variety of biochemical signals mediate communication between the developing oocyte and follicle and the ovarian granulosa cells which surround and support the growing follicle. One group of large, structurally related proteins which have been shown to be extensively involved in this process is the transforming growth factor, β superfamily. The superfamily can be broken down into a number of subgroups, among which are the growth determination factors (GDF) and the bone morphogenic proteins (BMP). These proteins are macromolecules, with some as large as 450+ amino acid units (mers). Some twenty BMP (both cytokines and growth factors) have been identified and are thought to play important roles in embryonic patterning and early skeletal development, while some fifteen GDF have been characterized and have regulatory functions in the oocyte and virtually all developing and differentiating embryonic tissues.

During folliculogenesis, several primordial follicles, each containing a primary oocyte, are recruited for development. The oocyte increases in size and is encapsulated by the glycoprotein zona pellucida coat, and the surrounding granulosa cells

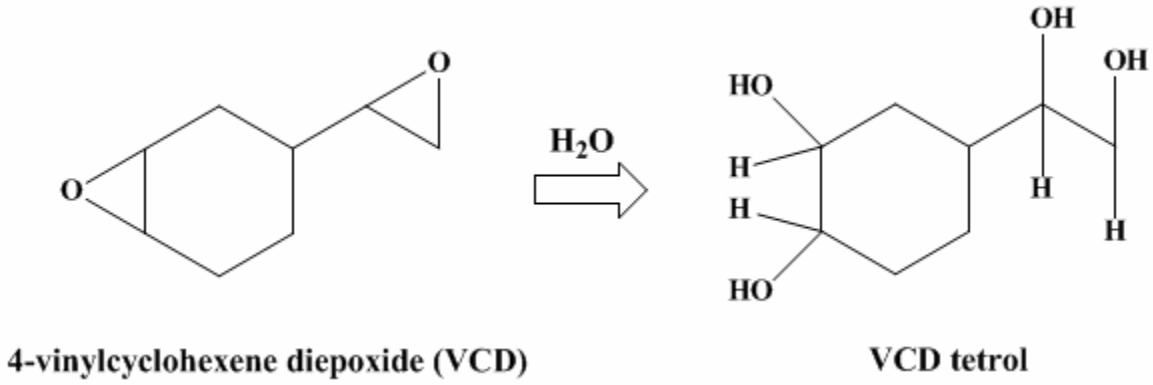

Figure 3. Chemical structure of 4-vinylcyclohexene diepoxide and its tetrol metabolite.

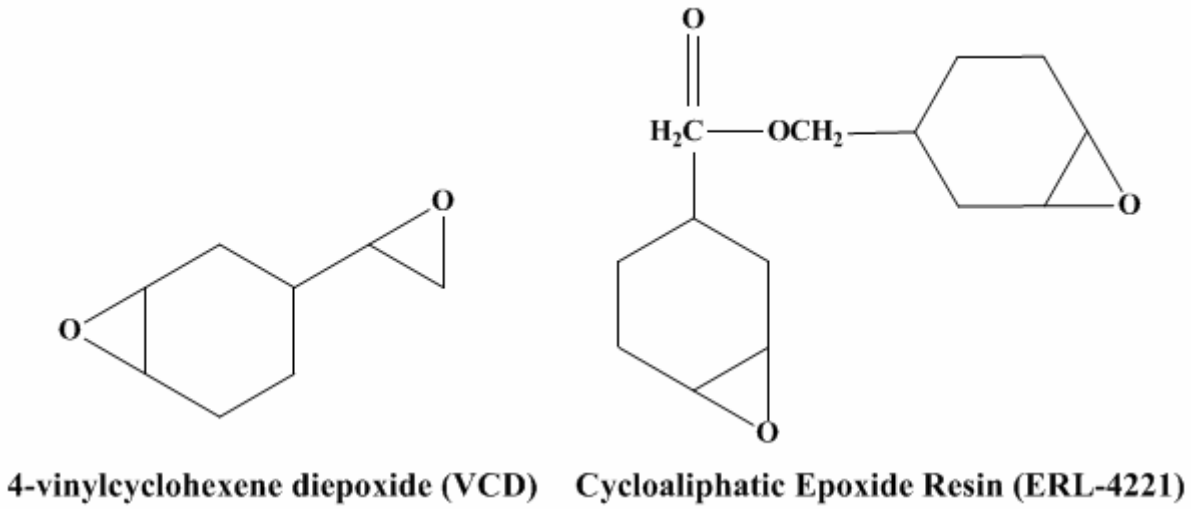

Figure 4. Structural comparison of 4-vinylcyclohexene diepoxide (VCD) and Cycloaliphatic Epoxide Resin ERL-4221.

proliferate, change shape, and begin secreting fluid, marking the formation of the primary follicle. Follicular cells continue to differentiate, while fluid pockets begin to form between the granulosa cells, marking the pre-antral stage of follicular development. These fluid pockets merge, forming a large, liquid filled antral or secondary follicle. The oocyte has become many times its original size, and the granulosa cells which now line the antral cavity have also differentiated into the cumulus oophorous, which attaches the oocyte to the antral wall, and the corona radiata, comprised of support cells which surround the oocyte. Of the follicles which began this process, all but one have died and become atretic. The remaining follicle becomes pre-ovulatory in preparation for ovulation, followed by the resumption of meiosis in the oocyte and the possibility of fertilization.

Oocyte-produced growth factors play a critical role in folliculogenesis, mediating communication between the oocyte and granulosa/granulosa-derived cells. Two of these factors, growth differentiation factor 9 (GDF9) and bone morphogenic protein 15 (BMP15), have been identified as particularly important. They interact to regulate proliferation and differentiation of granulosa cells, probably via production of inhibin and interaction with FSH. In most mammalian species, GDF9 appears to be the dominant factor as follicular development is completely halted in its absence, but both factors are usually necessary for successful folliculogenesis, ovulation, and resumption of meiosis.

Unlike small peptides like GnRH, successful development of a contraceptive vaccine using large macroproteins is made more difficult and expensive because of the sheer size of the molecule and the problems involved in its synthesis. Frequently, however, an epitope of the macromolecule is recognized by the immune system, and can be synthesized and conjugated to a larger antigenic molecule. Using the known amino acid sequences of both GDF9 and BMP15 in sheep, McNatty et al. (2006) synthesized 9 (for BMP15) or 10 (for GDF9) sequential, overlapping 9-15 mer epitopes starting from close to the carboxylic acid-containing (c-terminal) end and progressing approximately 100 mer towards the amine (n)-terminal end of each molecule to compare immunogenicity and contraceptive effectiveness of each epitope. As with GnRH, these epitopes were too small to be immunogenic, so they were conjugated to KLH. Using these conjugates, sheep ewes were immunized with either anti-GDF9 or anti-BMP15 and the epitopes exerting the greatest contraceptive effectiveness were identified. Overall, McNatty et al. (2006) concluded that anti-GDF9 and anti-BMP15 showed promise as contraceptive agents. In cooperation with New Zealand's Victoria University and White Buffalo, Inc. (Hamden, CT), a non-profit wildlife management organization specializing in non-lethal

control of white-tailed deer, the NWRC has obtained and conjugated both the effective GDF9 and BMP15 epitopes described by McNatty et al. (2006) and is currently exploring the use of these epitopes as contraceptive techniques.

In conjunction with the wide variety of contraceptive agents already developed by the NWRC, these new oocyte-specific contraceptive strategies may provide additional tools for the control of invasive species and other problem wildlife.

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