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# Delivering and registering species-tailored oral antifertility products: a review

*Simon Humphrys*[A] *and Steven J. Lapidge*[A,B]

[A]Invasive Animals Cooperative Research Centre, Unley, SA 5061, Australia.
[B]Corresponding author. Email: steven.lapidge@invasiveanimals.com

**Abstract.** Technologies that induce infertility in wildlife are advancing rapidly. This is due largely to our increasing understanding of reproductive physiology, as well as the demand for management techniques that reduce fertility rather than increase mortality. However, transferring wildlife fertility control from the laboratory into landscape-scale utility for free-ranging animal populations will be highly dependent on products possessing oral activity and cost-effectiveness. A significant challenge to the delivery process is providing the international regulators in each jurisdiction with the most relevant data packages they need to assess new products. An essential part of any product registration for free-ranging animals will be the development of species-tailored delivery systems, especially so for non-specific antifertility actives. This review examines the current range of orally deliverable antifertility options, broadly classifies them according to overall risk compared with alternative vertebrate pesticides, outlines a species-tailoring process that reduces identified risks, and encompasses the data requirements for their registration for sale in Australasia, the USA and Europe.

## Introduction

The effect of the introduction of non-native species by man, and the associated environmental impacts, has resulted in the overabundance of some species to the detriment of others. This, along with increased human activity and encroachment on natural habitats, has required the implementation of wildlife-management regimes to preserve impacted agricultural systems and biodiversity, and to reduce the adverse impacts of wildlife–human interactions. A case in point, Australia is host to 56 invasive vertebrate animal species that cause significant economic losses through, for example, predation on livestock, crop damage, and competition for food. It has been estimated that invasive animals, particularly European rabbits (*Oryctolagus cuniculus*), wild dogs (*Canis lupus familiaris*), red foxes (*Vulpes vulpes*), feral pigs (*Sus scrofa*) and feral cats (*Felis catus*), cost Australia at least AU$720 million *per annum* through economic and environmental damage (McLeod 2004). Furthermore, in the last two centuries 22 mammal species have gone extinct in Australia, which accounts for approximately half the mammalian extinctions in the world (Johnson 2006). Managing the damage that invasive vertebrate species cause costs Australian governments and landholders more than AU $60 million annually in control costs, with an additional AU $20 million annually invested in research to find better methods of management (McLeod 2004).

For the past 100 years, management has depended on increasing mortality to reduce local populations, principally through the use of poisons, such as sodium monofluoroacetate (1080) (Braysher 1993), and the biological control agents myxomatosis and rabbit haemorrhagic disease (Williams *et al*. 1995; Cooke and Fenner 2002). The feasibility of manipulating fertility control in free-ranging wildlife populations to similar ends (Bomford 1990; Bomford and O'Brien 1992; Tyndale-Biscoe 1994; Nielsen *et al*. 1997; Sinclair 1997; Curtis *et al*. 1998) has only come to the fore as our understanding of relevant reproductive physiology, our development of antifertility tools and our awareness of the effects of manipulating fertility on population dynamics has developed (Hone 1992; Barlow *et al*. 1997; Davis and Pech 2002; Sibly and Hone 2002). This, along with the perceived humaness of fertility control (Oogjes 1997) has reinforced its potential application in future integrated management of native and invasive wildlife management.

The delivery and integration of effective oral fertility management for large-scale free-ranging wildlife management is not yet a practical reality despite nearly half a century of research (Kennelly and Converse 1997; Fagerstone *et al*. 2006). This is due principally to the need to distribute these across landscapes liberally and repeatedly for effect and the relative lack of species-specificity for currently available contraceptive actives (synthetic steroids, oestrogens, progestins and progesterone antagonists). The current challenge will be to utilise cross-disciplinary application of reproductive and digestive physiology, pharmacology, formulation chemistry and ecology, as well as effective public consultation to identify products that provide long-term contraception or sterility principally in target species through minimal exposures. Aligning these dimensions will ensure that the probability of delivering a product that addresses the ethics of wildlife fertility control, the needs of wildlife managers and the regulatory authorities is maximised. These conditions are encompassed in a 1993 resolution of the American

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Association of Wildlife Veterinarians stating that fertility control may be an acceptable means of population regulation in free-ranging wild animals if the following conditions are met (Fagerstone 2002):

(1) The compound does not affect the health of target species and humans.
(2) A risk assessment is completed delineating potential effects on non-target species.
(3) The application is limited to site-specific, well defined subpopulations or populations.
(4) The application does not alter the gene pool of the species.
(5) Short- and long-term effects on population dynamics, including age structure and behavioural effects, are evaluated through modelling and monitoring.
(6) The program is evaluated by regulatory and wildlife-management agencies before use, with full public participation.
(7) Costs of the fertility control program are borne by the organisations or public that benefit from the program.

The first three conditions indicate the relative importance of efficacy, species-tailoring of products and deployment methodologies. Conditions 4, 5 and 6 may be encompassed and evaluated as part of a regulatory package and will be reflected through approved label claims and directions but will most likely fall within what the authors will term 'product stewardship'. Product stewardship is the appropriate and responsible use of approved products or services and relies heavily on the collective ethics, expertise and skill of the agency or individual applying the product or service to achieve reduced fertility within individuals of a target population. Product stewardship will also encompass the practicalities of treating specific sites and will vary dramatically with the differing conspecifics encountered around the globe. Similarly, the costs and who bears the cost for the fertility control program will differ within different jurisdictions aligned with the expectations of the general public, wildlife managers and government agencies. With the exception of Conditions 4, 5 and 7, these conditions broadly reflect the information that is required to submit a registration application. Registering animal products has commonalities worldwide, whether through the Australian Pesticide and Veterinary Medicine Authority (APVMA), the European Union Directives, the United States Environmental Protection Agency (US EPA), or the New Zealand Environmental Risk Management Authority (NZ ERMA), with data requirements for registering new actives and products somewhat analogous (Table 1). This guidance forms the framework for a discussion around the regulatory studies required for the current suite of actives that are under development, and explores the contribution that species tailoring confers to product efficacy and safety.

## Oral fertility actives: the current options and future possibilities

The different contexts in which fertility control is used to manage wildlife has meant that both reversible contraceptives and sterility-inducing actives have and will continue to be developed in parallel.

### *Contraception – synthetic chemistry*

Risk profile: non-specific, transient exposure, reversible, low environmental risk.

Synthetic steroid hormones, cholesterol mimics (DiazaCon) and nicarbazin comprise the set of contraceptive actives. Of these, nicarbazin (OvoControl-G – EPA registration no. 80224-5 and OvoControl-P), is the only active that confers any targeting as it affects only egg layers (Fagerstone 2002; Yoder *et al.* 2005, 2006). Although effective, their mode of action and pharmacology is dependent on repeated intake of active doses before or during the breeding season (Miller and Fagerstone 2000; Yoder 2000; Fagerstone *et al.* 2008). In this respect DiazaCon and OvoControl use patterns reflect a trade off between achieving efficacy and reducing potential hazards. OvoControl has a relatively short half-life once ingested, and therefore must be supplied throughout the period of egg laying to reduce hatchability (Yoder 2005; Yoder *et al*. 2006). By comparison, DiazaCon is cleared slowly and with repeated intake does bioaccumulate. This means that effective doses can be achieved in most animals with concentrated temporal consumption but also translates into a narrower therapeutic window before undesirable side-effects or environmental residues result (Nash *et al*. 2007; Yoder *et al*. 2007). Such characteristics lend themselves better to captive wildlife (Asa 1997; AZA wildlife contraception centre database 2007), highly seasonal breeders and localised but extreme populations. However, for most free-ranging wildlife these actives are still less than ideal.

### *Contraception – immunocontraception*

Risk profile: non-specific to target-specific, low exposure, potentially reversible, very low environmental risk.

Miller *et al*. (2006) have licensed immunocontraceptive technology that uses recombinant DNA techniques to generate a single chimeric protein containing repeats of GnRH interdispersed among several highly antigenic epitopes derived from *Plasmodium falciparum*, tetanus toxoid and the respiratory syncitical and measles virus (Talwar and Gaur 1987). This chimeric protein has the potential to elicit powerful immune responses to GnRH by proxy, via adjacent antigenic epitopes. Theoretically, this protein could be delivered appropriately via the nasal–pharyngeal route or orally. The target protein for this vaccine is GnRH and is therefore considered non-specific and will require sophisticated delivery mechanisms to limit conspecific exposure.

Australian research into virally vectored immuno-contraception did demonstrate that orally delivered recombinant virus expressing mice-specific zona pellucida proteins could reduce fertility (Redwood *et al*. 2007). However, the magnitude of the genetic manipulation also adversely affected the dissemination of the recombinant virus from infected individuals to naïve counterparts (Redwood *et al*. 2007). This principle applied to laboratory-housed rabbits was less successful, demonstrating a transient depression in fecundity (MacKenzie *et al*. 2006) and the transmissibility of the virus construct used was not tested. Although virally vectored immunocontraception was also considered for fox fertility control, the lack of a suitably species-specific vector resulted

**Table 1. Data requirements for registration of a new fertility-control product**

Requirements for Australia are determined by the Australian Pesticide and Veterinary Medicine Authority (see http://www.apvma.gov.au/industry/MORAG.shtml); those for the United States are determined by the US Environmental Protection Agency (see http://www.epa.gov/pesticides/regulating/data_requirements.htm); and those for Europe are determined by the European Union Directives (see http://europa.eu/scadplus/leg/en/lvb/121178.htm). Note that the European directives for Biocides, Pesticides and Veterinary Medicines Directorates have been combined for ease of presentation

| Australia | Europe | USA |
|---|---|---|
| Chemistry and manufacture | Physical/chemical properties and manufacture | |
| Residues | Maximum residue limit | Residue chemistry |
| Environmental chemistry and fate | Fate and behaviour in the environment | Environmental fate |
| Physicochemical degradation | Persistance | Degradation studies |
| Biodegradation | Metabolism | Metabolism studies |
| Mobility | Mobility | Mobility studies |
| Field dissipation | Spread | Dissipation studies |
| Accumulation/metabolism | Sensitising or bioaccumulative | Accumulation studies |
| Toxicology | Toxicological and metabolic studies | Hazard to humans and domestic animals |
| Acute toxicity | Acute (modes of exposure) | Acute studies |
| Short-term toxicity | | |
| Subchronic toxicity | Subchronic | Subchronic studies |
| Long-term toxicity | | |
| Chronic toxicity | Chronic | Chronic studies |
| Carcinogenicity | Carcinogenicity | |
| Chronic toxicity/carcinogenicity | | |
| | Neurotoxicity | |
| Reproduction/developmental studies | Reproductive toxicity/teratogenicity | Teratogenicity and reproduction studies |
| Genotoxicity studies | Mutagenicity studies | Mutagenicity studies |
| Toxicity of metabolites and impurities | | Metabolism studies |
| | | Re-entry protection |
| | | Pesticide spray drift evaluation |
| Human toxicological data | Human exposure data | |
| Environment toxicology | Ecotoxicological studies | Hazard to non-target organisms |
| Birds, mammals and other vertebrates | All animals considered at risk | Short-term studies |
| Aquatic organisms | Aquatic | Long-term and field studies |
| Non-target invertebrates | Invertebrates | Product performance |
| Non-target native vegetation | Other flora and fauna | |
| Metabolism and kinetics | Toxicological and metabolic studies | |
| Laboratory animals | | |
| Target animals | | |
| Occupational health and safety | | |
| Efficacy and safety | Intended uses and efficacy | |

in the move towards a bait-delivered species-specific immunogen (Hardy and Braid 2007; McLeod *et al*. 2007). Thus, a platform of research exists to inform future progress towards this approach to managing wildlife fertility.

This theme of novel vectors as immunogen-delivery systems is also being taken up by New Zealand researchers who are investigating the potential of transgenic plants (Cowan *et al*. 1999), bacterial ghosts (Szostak *et al*. 1996; Rodger 1999; Jalava *et al*. 2002) and pathogens (Cowan *et al*. 2008) as vaccine vectors for brush-tailed possums. The principle of delivering antigens via these systems has been reduced to practice with antibodies to *Escherichia coli* heat-labile enterotoxin (LTB) detectable in blood and uterine secretions of possums that have eaten transgenic plants expressing the target protein (Mason *et al*. 1998; Tacket *et al*. 1998). Additionally, zona pellucida 3 protein attached to the inner cell membrane of bacterial ghosts by specific anchor sequences, and/or through the use of self-assembling S-layer proteins (Eko *et al*. 1999), demonstrated inherent adjuvant properties that induced humoral and cellular immune responses, which reduced the fecundity of possums in breeding studies (Duckworth and Cui 2004). These technologies have the advantage of being designed specifically for target species and for efficient oral delivery from the outset, compared with those that require complex formulation to aid passage through the gut and translocation across the gut epithelium.

Managing animal populations with minimal inputs will be achieved through sterilisation. In contrast to contraceptives, sterilisation technologies have not benefited from the intense research efforts aimed at regulating human reproduction. This has produced a lag effect that is only now beginning to show signs of advancement and, as such, all sterilisation technologies are at an early stage.

### *Sterilisation actives*

Risk profile: ranges from non-specific to target specific, transient exposure, permanent effect, low environmental risk.

The most promising sterilisation actives currently under development target gonadotrophs in the brain (Yang *et al*. 2003; Qi *et al*. 2004), primordial follicles in the ovary (Hoyer

*et al*. 2001; Mayer *et al*. 2002; Aitken 2006), or aim to immunogenically impair fertilisation (Duckworth and Cui 2004; Ball *et al*. 2006), and it is the potential of these that is explored below. The selective depletion of cells critical for reproduction, be they in the brain or ovary, is a strategy being pursued by several research groups around the world.

Ball *et al*. (2006) are attempting to sterilise through the generation of gonadotrophin-releasing hormone (GnRH) and/or GnRH analogues coupled with cytotoxins that, after systemic administration (injection or ingestion), bind only to cells expressing GnRH receptors to achieve highly specific gonadotroph depletion. This renders the pituitary unable to respond to GnRH, depresses the release of follicle-stimulating hormone and luteinising hormone and effectively disrupts the reproductive axis to effect long-term contraception or sterility (Sabeur *et al*. 2003; Ball *et al*. 2006). Oral delivery of this technology is theoretically feasible, although this will depend on the choice of cytotoxin and formulation such that its integrity is preserved and is bioavailable.

In a similar vein, Aitken (2006) is targeting ovarian granulosa cells via F-pilus attachment peptides expressed by bacteriophage using the same technique developed to target peptides to sperm membranes (Eidne *et al*. 2000). The technology generates vast peptide libraries that can be screened against target cell surface epitopes for their relative avidity. Phage binding to granulosa cells in culture with high avidity can then be treated to successive rounds of panning to further enhance avidity. Phage clones and peptides that are highly species-specific and cell-specific have been developed against cultured murine granulosa cells. Preparations of these phage clones or phage-peptides administered parenterally to mice has significantly reduced ovarian primordial follicle populations and reduced fecundity (Aitken 2006). Oral delivery of phage panned peptides is also a reality as they are relatively small and enteric formulations are commercially available that can aid in the translocation of peptides across the gut epithelium (Mahato *et al*. 2003; Hamman *et al*. 2005). Accordingly, this technology exhibits all the traits required of a field-practical oral fertility product that could be used for wildlife management – exquisite species specificity coupled with oral activity, readily biodegradable and environmentally benign. The qualities of this platform technology are very enticing but the panning process must be repeated for each target species and as yet the approach has been tested only in mice.

Hoyer *et al*. (2001) and Mayer *et al*. (2002), in developing a rodent model of human menopause, characterised a chemical active (4-vinylcyclohexene diepoxide, VCD) that appears relatively more toxic to primordial follicles than other body tissues (Flaws *et al*. 1994). This chemical, when repeatedly injected or ingested over a period of approximately two weeks, causes apoptosis of the squamous epithelial cells, affecting primordial follicle viability. This accelerates ovarian senescence and may ultimately advance sterility in mammals treated with VCD. Before this approach can be practically implemented as a treatment to manage wildlife fertility, VCD must be formulated to achieve sterility with fewer exposures of the target species (preferably only one). This is the present challenge and focus of research at SenesTech Inc., the company that is developing VCD as a sterility inducer.

Undoubtedly, the use of new biotechnologies will be instrumental in the development of orally delivered fertility control. The great challenge will be to marry these increasingly powerful tools with a greater understanding of the ecology of the target species and of conspecifics so that new product packages are tailored to the target species.

## The species-tailoring process

The species-tailoring process is undertaken for two principal reasons: (1) to ensure target species efficacy and non-target species safety, and (2) to reduce the risks for regulators, thereby facilitation of product registration. The species-tailoring process can either be achieved through the vaccine-compound itself or the delivery method. Examples of the former include vectored immunocontraception for foxes, rabbits and mice (McLeod *et al*. 2007; Redwood *et al*. 2007; Strive *et al*. 2007; Tyndale-Biscoe and Hinds 2007; Van Leeuwen and Kerr 2007) or the University of Newcastle's phage bio-panning technique (Aitken 2006). Current orally deliverable actives being investigated, such as GnRH or VCD will, however, require specificity in their respective delivery methods.

Tailoring vaccine delivery requires an understanding of the target species' ecology and niche, particularly in complex communities or between similar species, as depicted in Fig. 1. Niche separation has many aspects, all of which can be utilised to advantage. Habitat (generalist/specialist, terrestrial/arboreal/fossorial), diet (generalist/specialist, omnivore/carnivore/herbivore), morphology as compared with conspecifics (large/small, musculature, dentition, bite force, mouth dexterity, problem-solving ability), sensory perception (relative importance of vision, smell and taste when foraging), physiology (water requirements, digestion/agitation), and spatial/temporal (location of the target species compared with conspecifics in environments) are all characteristics that help isolate a species niche (Bengsen *et al*. 2008). Defining each of these characteristics at the outset of the development process is essential when attempting to tailor vaccine delivery.

Species tailoring is not a new concept. O'Brien (1986) proposed a framework for developing a feral pig bait based on many of the above attributes. This framework formed the basis of PIGOUT development that commenced in January 2004 with the aim of developing and registering a manufactured 1080 feral pig bait (Cowled *et al*. 2006*a*). The manufactured bait had to be highly attractive to pigs, cheap, target-specific and easy to use. Extensive field testing of the product has shown it to be mostly target-specific (Cowled *et al*. 2006*b*), except in America (Campbell *et al*. 2006), and potentially suitable for delivery of disease vaccines or contraceptives (Campbell *et al*. 2006; Cowled *et al*. 2008*b*). For some species, such as canids, the delivery process has been tailored through the use of systems such as the M-44 mechanical ejector and Coyote Lure Operative Device (CLOD) (Busana *et al*. 1998; Berentsen *et al*. 2006). Further levels of selection can be provided through the use of target species attractants and/or non-target repellents.

Perhaps more complex than the development of bait or a delivery device is developing an appropriate baiting strategy. Cowled *et al*. (2008*a*) recently demonstrated the large scale over which some genetically contiguous feral pig populations can

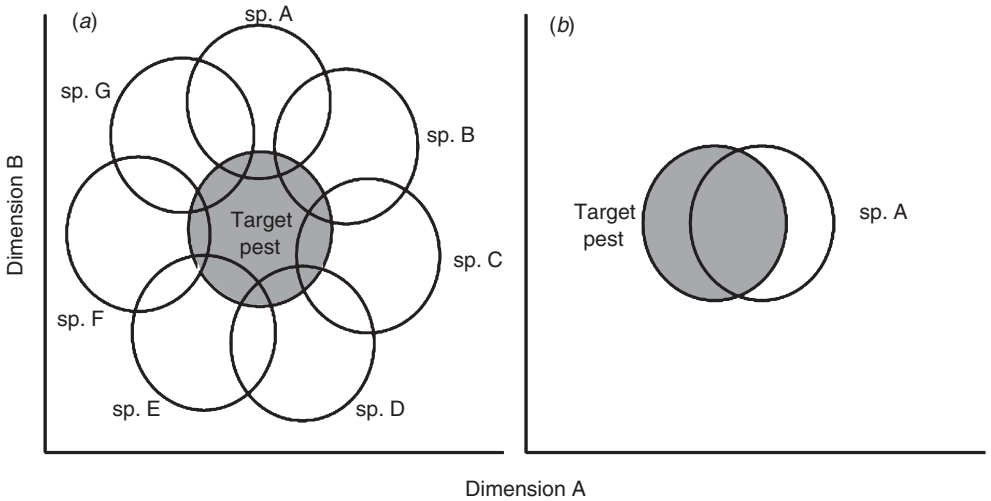


**Fig. 1.** Conceptual diagram illustrating functional overlap in two dimensions between target and non-target species in complex communities. Dimensions represent niche space (Hutchinson 1957). Plot (*a*) illustrates a species-rich community in which many non-target species share a small degree of functional similarity with the target pest. Plot (*b*) illustrates a community in which one species shares a large degree of overlap with the target pest. When extrapolated to more than two dimensions, the target species for fertility control can be seen to be 'functionally crowded' in both of these situations, and therefore difficult to isolate from non-target species. Adapted from Bengsen *et al*. (2008).

occur in the Australian rangelands. Targeting only a small proportion of the population with fertility control, as with lethal control, will likely result in no overall effect due to reinvasion and breeding compensation. Added to the complexity of the scale are questions regarding timing (temporal variation in bait uptake), placement (where in the environment – spatial variation in bait uptake) and distribution (bait density and grouping). Frequency of bait reapplication is also critical. All of these factors should be tested empirically. Moreover, what is appropriate for one habitat may not work in another, when a different suite of non-target species is present.

The above outline is a brief summary of the species-tailoring process, much of which is ultimately defined through trial and error. Regardless of the specific order of steps taken in the process, before attempting registration of a non-specific active each of the following needs to be demonstrated: (1) field efficacy and target specificity in a variety of habitats in which the final product will be used; (2) shelf and field stability of the bait and active under varying environmental conditions; (3) product cost, scale and consistency of production must be amenable to commercialisation; and (4) the logistical benefit and operator safety must be appropriate for the end user. All contribute to the overall species-tailored oral-delivery package for each species, and are essential parts of a successful product registration.

## Registering fertility-control products: pesticides, biocides or veterinary medicines?

Fertility-control products, depending on their mode of action and the target species, have the potential to be classified as pesticides, biocides and/or veterinary medicines. This distinction carries with it a raft of ramifications from a regulatory standpoint, including differing data requirements to approve registrations and, in the USA and Europe, the government agency responsible for evaluating submissions. In many cases the data required are predetermined and based on our understanding of risks associated with conventional pesticides, e.g. insecticides, herbicides and fungicides. Conventional pesticides are, in many cases, used *in toto* and at landscape scales orders of magnitude over and above those used for vertebrate pesticide application. This is particularly so for vertebrate fertility-control actives. This dramatically smaller scale for vertebrate population management rarely constitutes a commercial opportunity; because of this, new product development will depend on the flexibility and discretion of regulators in waiving studies that are less relevant once the risks inherent in the product package (typical end uses, deployment and scale of application) are considered using scientifically informed principles.

In Australia any fertility-control product would be approved and registered by the Australian Pesticides and Veterinary Medicines Authority and in New Zealand by the Environmental Risk Management Agency/Food Safety Authority. In the USA the EPA continues to register pesticides (which would include a fertility-control product used for wildlife and feral animals) but the agency responsibility shifts to the Food and Drug Administration – Centre for Veterinary Medicine if the fertility-control product is applied to zoo, companion or food-producing animals/livestock. In Europe (including the UK) the complexity is multiplied because wildlife products are regulated by the Union Commission and separately by its member countries. For example, pesticides may be approved by the Union Commission under the overarching Biocides Directive (where the application of the pesticide is aimed at population control, e.g. commensal rodent control) or the Plant Protection Products Directive (in the case of controlling animals aimed at reducing browsing damage). However, individual pesticide approvals are also administered in each of the member countries, e.g. in the UK by the Health and Safety Executive,

which administers Biocide approvals, whilst the Pesticides Safety Directorate regulates Plant Protection products. A further tier of potential complication exists in Europe as fertility-control agents based on immunocontraceptive vaccines or fertility-controlling products that modify the physiology of larger mammals are more likely to be considered as Veterinary Medicines and thus regulated by the European Community code relating to veterinary medicinal products, which is then administered in the UK by the Veterinary Medicines Agency.

## The regulatory reality

Undoubtedly, the costs of commercialising new oral fertility-control products will be expensive and complex. As examples, the investigations into virally vectored immunocontraception for rabbits, foxes and house mice (*Mus domesticus*) cost more than AU$15 million over 12 years, without a registration outcome (Lapidge *et al*. 2007). Compounding this increasingly prohibitive commercialisation/registration impasse for wildlife products, current vertebrate pesticide registrations are being discontinued due to the cost and data requirements for their continuation (Jacobs 1992) or their withdrawal as confidence in one or more aspects of their performance is eroded with use (e.g. Baytril for poultry in 2005) (www.fda.gov/cvm/FQWithdrawal.html). This backdrop of increasing data requirements, registration timeframes and costs is already limiting innovation within this field. Addressing this negative feedback on the development of new wildlife management tools is critical if we are to improve animal welfare concurrently with management effectiveness. This aim will require a greater degree of consultation with, and flexibility by, the regulators in order that the dynamics of product usage (risk to human health, product-use pattern, target species, total product deployed over time and environmental toxicity) may be used to scientifically inform the degree of risk that a product represents and therefore what data are essential and that which is less, or not, relevant for new wildlife-management fertility-control products. As an example, the approval of an oral fertility-control product that is deployed within a species-targeted bait, uses a bait station designed to limit non-target/environmental exposure and is transiently applied during only the breeding season should require less data supporting its approval than a pesticide that is used to control rodents locally in food-producing crops and even less data than a broadacre pesticide that is sprayed directly onto food-producing crops. OvoControl product registrations and the current GonaCon registration application are excellent precedents as application of this rationale by the United States EPA put into practice, but still required multimillion dollar investments (Fagerstone *et al*. 2008 – this issue).

## Wildlife fertility product innovation

Product development in this field will predominantly remain with governments or semigovernment organisations working with industry partners (the basis of Cooperative Research Centres in Australia). The high cost of product development and registration, the relatively small scale on which most vertebrate pesticides are used, and the diminutive profit margins will always demand this. The most effective way of further reducing product development costs, times to availability and registration burdens is through global harmonisation of wildlife-management products (Lapidge *et al*. 2007). This remains integral to future availability of species-tailored oral antifertility products.

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