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Workshop on the **Effects of Parasiticides on Dung Beetles**

Report of Proceedings

The Bardon Centre
Brisbane, Queensland

**Mick Alexander and
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Workshop on the effects of parasiticides on dung beetles

Report of Proceedings*

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Introduction

An invitational workshop to review the effects of excreted residues of veterinary parasiticides was held in Brisbane on the 18-19 **September** 2001. The workshop was sponsored jointly by the Australian Pastoral Research Trust and four major animal health pharmaceutical companies (Fort Dodge Australia, Merial Australia, Pfizer and Schering-Plough). The meeting was the first of its kind to be held in Australia and involved representatives from a diverse range of interests, including the CSIRO (Entomology and Livestock Industries), the Universities, State Departments of Agriculture, primary producers, Land Care groups, the animal health pharmaceutical industry, the National Registration Authority for Agricultural and Veterinary Chemicals and Environment Australia (Appendix 1).

Workshop objectives

The purpose of the workshop was:

- to review what is known about the rate and route of excretion of parasiticides commonly used by the livestock industry;
- to consider the evidence that residues excreted in faeces can have an adverse impact on the dung fauna, in particular dung beetles;
- to review the limited information available about seasonal and regional parasticide usage patterns and to examine ways in which more informative data can be obtained;

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- to consider the current requirements for product registration and the procedures used for assessing their likely environmental impact;
- to discuss the possible role of standardised bioassays and ecotoxicological models in the processes of drug registration and assessment;
- to provide a basis for the development of a Decision Support System to enable graziers to make best use of available parasiticides, conserve their efficacy and minimise their environmental impact.

Day 1 was an information day, devoted to formal presentations on each of the above topics (Appendix 2). Issues arising from these presentations provided the framework for the Workshop process, which occupied most of day 2 (Appendix 3).

Précis of Workshop Presentations

The main messages emerging from the presentations were:

- There is widespread recognition among graziers that dung-feeding arthropods, in particular dung beetles, can be important for the maintenance of pasture hygiene and nutrient cycling.
- A high proportion of graziers are aware that some veterinary chemicals have an adverse impact on dung beetle activity but are frustrated by the paucity of easily accessible, objective information about the residue effects of different products and how these vary with time or frequency of application.
- Most results presented are based on laboratory bioassays. Field data on the effects of parasiticides on populations of dung-feeding insects remain limited.
- Some parasiticides are excreted mainly in urine, others almost entirely in faeces. Which route prevails and the rate of drug clearance is determined primarily by the compound's inherent molecular properties, but other factors, including host species, drug formulation, delivery route and pasture conditions can also be involved. Much is known about the excretory pattern of the benzimidazoles and the macrocyclic lactones (MLs) but, surprisingly, there is little information in the public domain pertaining to organophosphate (OP) compounds, synthetic pyrethroids (SP), insect growth regulators (IGRs) or even commonly used anthelmintics such as levamisole and morantel. Several of the benzimidazoles are excreted in faeces (e.g. oxfendazole / fenbendazole) but because they are extensively metabolised and formulated for rapid clearance they are unlikely to have a significant impact on the dung fauna. Macrocyclic lactones are also eliminated in faeces and, depending mainly on delivery route and formulation, the period of excretion may vary from a few days in the case of oral formulations to more than 3 months in the case of controlled-release devices such as those developed for sheep and cattle. The small amount of excretory information available for OPs and the levamisole/morantel group indicates that these chemicals are cleared mostly in urine, whereas the results of insect bioassays suggest that SPs and several IGRs are eliminated largely in faeces.
- During the past 2 decades, numerous studies have been published detailing the harmful effects of ivermectin and abamectin on non-target organisms, particularly dung-beetles and dung-breeding flies. Excreted residues of these compounds, which were the first MLs to be registered for veterinary use, have been shown to be toxic for variable periods after treatment to both fly and beetle larvae and have less pronounced toxicity to some newly emerged adult beetles. Differences in susceptibility between species and between different life stages of the same species are thought to be related, at least in part, to the

- way in which dung beetles ingest and digest their food. For example, adult beetles belonging to the subfamily Scarabaeinae (which includes most of the species of interest to pastoralists) are filter feeders and ingest only the more fluid portion of the dung, with ingested particle size increasing as species size increases. Larvae on the other hand have biting and cutting mouthparts and not only consume 'whole' dung but repeatedly ingest their own faeces so that residues may be re-cycled. These different feeding behaviours may be relevant to understanding the differential effects of drug residues on the larval and adult stages of dung beetles.
- Sub-lethal effects attributed to the ingestion of excreted avermectins include morphological deformities, extended periods of larval development, delayed sexual maturation and reduced fecundity. How this combination of developmental aberrations and enhanced mortality, impinges on the long-term fitness of dung beetle populations, and ultimately, on nutrient recycling in pastures, has not been adequately researched and continues to be a contentious topic needful of resolution.
 - Comparatively little information is available about the ecotoxic effects of more recently registered MLs, namely moxidectin, doramectin and eprinomectin. However, laboratory and field bioassays comparing the effects of each of these parasiticides with those of ivermectin on cyclorrhaphous diptera, staphylinid beetles, and parasitic hymenoptera, ranked their residual toxicities as: doramectin>ivermectin>eprinomectin>>moxidectin. Laboratory data presented for dung beetles indicated that doramectin was less harmful than abamectin to the introduced species, *Onthophagus binodis*, while residues of eprinomectin were found to have a greater effect than moxidectin on the development and survival of another exotic species, *Onthophagus taurus*. Moxidectin appeared to be the least disruptive of all the MLs registered to date, but further comparative studies, based on standardised assay techniques, were considered desirable.
 - Although ecologists have long been aware of the unintended side-effects of parasiticide residues in the faeces of treated livestock, the problem has been little researched except in the case of the macrocyclic lactones. The limited information available for OPs suggests that several of the more commonly used chemicals in this class of parasiticide (e.g. diazinon, fenthion and chlorfenvinphos) are excreted mainly in urine and are thus unlikely to pose a significant threat to the dung fauna. Except for deltamethrin, an SP which is eliminated almost entirely in faeces, there are no excretory data available for this class of compound. However, bioassay data from several parts of the world, indicates that residues of pour-on formulations of SPs such as deltamethrin, fenvalerate, cypermethrin, alphamethrin and cyhalothrin are all secreted in faeces in amounts sufficient to kill adult dung beetles for 1-3 weeks post-treatment. Only flumethrin seems to be relatively innocuous. All of these SPs are in current use in Australia for the control of ticks, lice and biting flies.
 - The fact that SPs are highly toxic to adult dung beetles suggests that this class of parasiticide may be more harmful to the dung fauna than MLs. Output obtained from simulation modelling provided strong support for this supposition. However, a major problem in assessing the potential environmental impact of veterinary chemicals is the complete lack of accessible information relating to the spatial and temporal patterns of drug usage. Currently, there is no governmental agency involved in the routine collection of such data.
 - Simulation models, based on bioassay data, were used to compare the effects of drugs with different toxicity profiles. These showed that the impact of drug therapy would vary with time and frequency of treatment and the biological characteristics of the beetles present. In general, beetles that were able to complete several generations per year were

likely to be more sensitive to the use of parasiticides than those that were univoltine (one generation per year); and parasiticides that killed adult dung beetles were predicted to exert a greater effect on dung beetle populations than chemicals that affected only the juvenile stages. However, major disruption of breeding was also expected when livestock treatment involved the use of sustained-release devices containing highly potent larvicides such as ivermectin.

- Models were suggested as providing regulatory authorities with an objective tool for comparing and assessing the potential impact of new drugs. They were also seen as a means of conserving and optimising the use of parasiticides and as a basis for developing a more holistic and strategic approach to the management of livestock parasites. However, to be most useful, future models should be validated and should aim to include for the effects of insect migration and the possibility of density dependent recovery. They should also be based on data obtained by standardised methods of testing. At the present time, there are no agreed guidelines on test methods, either national or international, to which pharmaceutical companies should adhere when providing data for the regulatory purposes. Standardised, validated bioassays on dung beetles, dung-feeding flies and earthworms were seen as being important in the process of drug registration and assessment. A working group to plan these was proposed

Workshop conclusions and recommendations

Despite the disparate interests of the workshop participants, there was broad agreement that the rural industry's widespread dependence on antiparasitic drugs posed a potential threat to dung-feeding organisms and hence, to the processes of dung degradation and nutrient cycling. This threat was seen as long-term, and was not limited to a single class of drugs. Nor could it be quantified without further biological, ecological and pharmacological research, as well access to detailed information on the seasonal patterns of drug usage in different sectors of the livestock industry.

To progress the findings of the Workshop, it was resolved to form a Coordinating Committee comprising Workshop delegates and members of the National Dung Beetle Steering Committee. The Coordinating Committee's main role will be as follows:

1. to advise and lobby policy makers, including State and Federal Agencies, in respect of the Workshop's main findings;
2. to adopt a similar approach with appropriate funding agencies, e.g. Meat and Livestock Australia, the Natural Heritage Trust, Australian Wool Innovation;
3. to pursue the fulfilment of the Workshop outcomes by enlisting the support and involvement of University research departments and rural research organizations at both State and National level;
4. to ensure continuing community awareness of the important issue of veterinary chemicals; and
5. to develop an action plan identifying organizations, groups and/or individuals with specific responsibility for following up and promoting the Workshop's main conclusions and recommendations.

In order of importance, these were recorded as follows:

1. Chemicals and chemical usage:

The quantitative impact of parasiticide residues on populations of dung feeding organisms remains a contentious and unresolved issue. Synthetic pyrethroids and macrocyclic lactones, particularly the avermectins, are regarded as the two classes of chemical that pose the greatest potential impost on the dung fauna. On the basis of current knowledge, synthetic pyrethroids are considered the most damaging, but further research was needed to confirm this conclusion. Insect growth regulators and organophosphate compounds, for which there are almost no data, were also considered to merit detailed investigation.

Recommendations : Identify specific chemicals for which data are currently lacking or inadequate. Liaise with the NRA and the pharmaceutical industry for the release of any relevant excretory data and/or information on residue effects. Compile and circulate to appropriate research institutions a list of chemicals requiring independent evaluation.

Information on seasonal and regional patterns of chemical usage in different sectors of the livestock industry was regarded as being essential for a proper evaluation of their environmental impact; it was also seen to be important for prioritising research on particular chemicals.

Recommendation : Lobby Government Agencies about the need to conduct regular surveys of veterinary chemical usage.

2. Decision support

The grazing community requires objective and consistent advice about which type of chemical to use at different times of the year. The provision of readily accessible information, detailing the active ingredient, excretion route, withholding period and ecotoxic activity of current and future antiparasitic products was seen as an essential, on-going, service for *all* sectors of the grazing industry. At the present time, this type of information is only available to the cattle industry (see Contracted Report No. 56, Wardhaugh 2001) but does not provide any guidance relating the survival of non-target organisms to the time or frequency of pesticide applications. It was noted that prototype models have been developed to provide this kind of information.

Recommendations : Liaise with NRA, EA and the pharmaceutical industry to expand and regularly update Contracted Report No. 56. Devise appropriate ways of disseminating this information. Evaluate the output of ecotoxicological models and make the results available to graziers.

The above recommendations were seen as an interim measure pending the long-term development of a fully-integrated Decision Support System, which would allow graziers to maximise profitability, whilst managing resistance, minimising pesticide usage, reducing residue problems and sustaining biodiversity.

Recommendation : Form a consortium of interested parties comprising Government Agencies, the pharmaceutical industry, rural research and development organisations and other funding bodies to examine the feasibility of funding the development and validation of a user-friendly Decision Support System for the management of livestock parasites.

3. Standardised parasiticide bioassays

At the present time, there are no agreed protocols, either national or international, for testing or comparing the ecotoxicity of excreted parasiticide residues. This makes for inconsistencies and/or uncertainties in data interpretation and has important ramifications in respect to the processes of drug registration and environmental assessment.

Recommendations : Liaise with NRA, EA and regulatory authorities in the OECD to develop internationally accepted protocols for the scientific testing of the effects of parasiticides on dung beetles and other important dung-feeding organisms, including appropriate indicator species. Standardise future product labelling to include information on the effects on beneficial organisms. Consider the use of ecotoxicological models as an integral and objective part of the registration process.

4. Extension and communication

With the recent advent of a number of Dung Beetle projects in the eastern states, a large proportion of the rural community are seeking information on dung beetles and 'Best Practice' to ensure minimal damage to local beetle populations. Landholders are changing management practices due to the perceived impact various products on beetle populations. It was seen to be important that a communication and extension program be established to train and inform the rural community about the importance of maintaining a healthy and diverse dung fauna and how the fauna might be affected by inappropriate livestock management.

Recommendation : Liaise with State Agencies, Land Care groups, the Queensland Dung Beetle Project and ex CSIRO staff to develop and update a training package which extension officers and livestock industry groups could present within their regions. A package would be similar to 'Farmsafe', Prime Notes or 'Giddy Goanna'.

Post-script

These Proceedings were circulated for comment to each of the 38 delegates that attended both days of the Workshop. Of the 32 that responded, 6 suggested minor changes, mostly of an editorial nature. All except 2 delegates accepted the document as an accurate portrayal of the Workshop and endorsed the recommendations as presented.

Acknowledgments

Special thanks are due to Sharon Murray, CSIRO Entomology, for arranging the accommodation, registration and travel of Workshop participants. Sharon also designed the Workshop brochure and was responsible for organising projection facilities and for providing notes summarising the Workshop discussions. CSIRO Pastoral Research Trust and Fort Dodge Australia were the major Workshop sponsors, but generous support was also provided by Merial Australia, Pfizer and Schering Plough.

Appendix 1

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The Bardon Centre, 17-19 September 2001

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Appendix 2

Abstracts of presentations at the Brisbane Workshop on the Effects of Parasiticides on Dung Beetles

The Bardon Centre, 17-19 September 2001

Introduction of participants and history of the forum

Mick Alexander

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This presentation will briefly introduce the range of participants attending the forum and assign some important ground rules to ensure all participants achieve the maximum benefit from the two days. It will discuss the important history behind this forum, including the community-driven activities and industry and government support being generated. Participants have been invited from a broad cross section of the community including scientific, corporate, community and industry bodies, in order to maximise the sharing of scientific and community knowledge and to generate discussion on future initiatives.

This forum is an important step in the development of community-driven environmental awareness of the need to monitor and manage chemical inputs and their affects upon the grazing ecosystem. In the past four years, five major steps have changed the way the general community views the issue of chemical usage in relation to Dung Beetles and the environment.

- August 1997: Meat and Livestock Australia (MLA) funded the “Springwater Producer Group” to assess the Dung Beetle situation in the Injune district.
- January 1999: MLA and the Taroom Shire Landcare Group funded the South Queensland Dung Beetle Survey.
- December 1999: MLA and the Taroom Shire Landcare Group funded the National Dung Beetle Forum, held at the Bardon Centre, Brisbane, Queensland.
- October 2000: National Heritage Trust agreed to fund the Queensland Dung Beetle Project to assess the spread of Dung Beetles in Queensland and to train landholders in Dung Beetle collection and identification.
- February 2001: In response to the needs to graziers for information on the effects of veterinary chemicals on dung beetles, CSIRO Entomology released “Contracted Report No. 56: Parasiticides registered for use in cattle in Australia – an annotated bibliography and literature guide prepared for the National Dung Beetle Planning Forum”

History of the dung beetle project

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Introduction

The first colonists from Europe arrived in Australia in 1788, cleared the land of much of the native vegetation, and planted improved pastures on which to graze domestic cattle and sheep. The vast quantities of dung produced by the 100 million sheep and 25 million cattle now present in Australia accumulate on the surface of the pastures tying up the nutrients. This dung provided a greatly increased breeding resource for two major

fly pests; the buffalo fly, *Haematobia irritans exigua*, in northern Australia, and bush fly, *Musca vetustissima*, in southern Australia. The Australian dung beetle project, which was the concept of George Bornemissza, aims to introduce beetles of the subfamily Scarabaeinae (Coleoptera: Scarabaeidae) to Australia to correct the ecological balance by more effectively recycling cattle dung. Bornemissza argued that the dung beetle fauna of Australia was adapted to deal with the much smaller dung droppings of marsupial animals living in heath and woodland vegetation.

The native dung beetle fauna

At the onset, an international dung beetle taxonomy expert, Eric Matthews, was recruited to describe the endemic scarabaeine dung beetles in Australia. There are now 315 described species of indigenous scarabaeine dung beetles in Australia. The native scarabaeine species are most common in heath, woodland and open forest, but are rare in open pasture with a few exceptions. Activity of the native species is typically restricted to moist seasons while activity is restricted during dry seasons. Thus, in open pastures with cattle dung, there is clearly a vacant niche in terms of seasonal dung beetle activity, often when the endemic species are not active.

Introductions from Hawaii

Between 1964 and 1970 Bornemissza introduced seven (mostly African) dung beetle species from Hawaii to Australia. Quarantine requirements were much simpler by introducing beetles from disease-free Hawaii. Two species in particular became abundant and spread in a spectacular way across northern Australia. At this time Bornemissza carried out a series of glasshouse experiments in Australia demonstrating clear potential benefits of activity by these beetles in terms of rates of cattle dung burial, control of bush fly, and enhanced plant growth as a result of increased dung burial.

Introductions from southern Africa and Europe

It was decided to expand the program to a continental scale by introducing species from Africa to fill a much wider range of niches across Australia. Key players in this larger program were Doug Waterhouse, Chief of the Division of Entomology, and Mike Jones of the Australian Meat and Livestock Research Corporation. In 1970 George Bornemissza moved to Pretoria in South Africa to set up a laboratory from which overseas beetles were selected and sent to Australia. A quarantine procedure was developed in Pretoria in which eggs were immersed in 3% formalin and placed in sterile peat for the trip. In Australia the eggs were transferred under quarantine conditions to artificial balls of cattle dung. It was only the progeny of the beetles emerging from these eggs that could be released. Mass rearing was carried out to provide sufficient beetles for release in the field in Australia.

Over the period 1970-1979 George Bornemissza and his team was responsible for selecting and introducing beetles from many countries to Australia. However, it became evident as more beetles became established that the impact was not as great as anticipated, and a much greater effort was put into understanding the functioning of the dung faunas in Australia and Africa. From 1979-86 Bernard Doube was responsible for the Pretoria laboratory. More resources were used in a detailed evaluation of dung beetle impact in Africa. At this time work on fly predators increased involving Murray Wallace on mites and Jane Wright on beetle predators. Jane was in charge of the laboratory after Bernard left in 1986. Penny Edwards went to Pretoria to solve some of the dung beetle rearing problems Keith Wardhaugh set up and ran a laboratory at Cordoba, in Spain to select and introduce dung beetles from southern Europe. In Australia releases and evaluation were carried out by Gus Macqueen based in Rockhampton, and James Ridsdill-Smith in Perth. The team was well served by a large number of very capable research support staff exemplified by Hartmut Aschenborn in Pretoria, John Feehan and Ray McInnes in Canberra. Between 1965 and 1985 a total of 57 species of scarabaeine dung beetles were imported to Australia, of which 39 species have been released and 26 species are known to be established. Enhanced dung burial, and reductions in bush fly abundance resulting from the introduced beetles were measured at sites in both western and eastern Australia.

Redistributing and managing the beetles

The Dairy Research and Development Corporation has supported Ian Dadour in the west and Marina Tyndale-Biscoe in the east to crop and redistribute the introduced species during the period 1987-1994. The WA State Government appointed Ian Dadour to improve adoption in the west. John Feehan has moved to the private

sector and has been redistributing dung beetles for farmers and for local authorities through SoilCam. CSIROs Double Helix Club has been active in this area, and dung beetles figure prominently in the activities of many Land Care groups. The latest project is led by Penny Edwards and Mick Alexander.

CSIROs Australian dung beetle project has generated worldwide interest, and stimulated a lot of very good scientific work both in Australia and overseas. In excess of 150 scientific papers have been published by CSIRO staff alone. The project is based on sound ecological principles and it is important that future work is planned equally carefully so as to maximise the future benefits for all Australians from these introduced dung beetles, without jeopardising the benefits already achieved.

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Grazier knowledge and use of veterinary drugs

1 - Dung beetles and veterinary chemicals – some observations on grazier needs and perceptions

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After leaving the CSIRO in 1993, I set up a dung beetle distribution business which, each year, distributes approximately 200 starter colonies of dung beetles to new locations, mostly on beef producing properties in southern Australia.

Some weeks during the summer months I receive up to 50 requests for dung beetle information. I have noticed over the last 2-3 years there is an increase in the number of beef producers asking questions about veterinary drugs. Most farmers realise there is an issue and they are seeking advice as to which products are the most dung beetle friendly.

Another question I am frequently asked is how long do they have to wait after using some products before residues drop to a level which will not harm dung beetles.

In general, the farmers are becoming far more environmentally aware and are more focused on the issues of sustainability, and on the effects that drenches and other livestock related chemicals are having on beneficial insects, particularly dung beetles.

Each year I travel extensively throughout southern Australia. I speak at many dozens of dung beetle presentations, pointing out the important benefits of having many species of dung beetles on beef properties. As I see it, the main need is to provide more user-friendly information, especially information that the average grazier can use to make management decisions. The real need is to have information which relates to the timing of chemical application, its effect on specific beetle species, and the real effect of many drenches and other chemicals, which are widely available today. In my opinion, this would mean a more extensive document, similar to the “CSIRO Contracted Report 56” available to every producer in Australia.

2 - Veterinary drug use by graziers on the northern rivers of NSW

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Cattle on the northern rivers of NSW are frequently treated for buffalo flies, internal parasites and paralysis ticks and sometimes treated for lice, bush ticks and cattle ticks.

To determine the veterinary chemicals most commonly used by northern rivers graziers staff at 16 northern rivers produce stores were surveyed. The staff were asked to estimate the percentage of each veterinary chemical group used for buffalo fly and internal parasite control. They were also asked questions about producer attitudes to dung beetles.

Cattle ticks are a notifiable disease in NSW and when infestations are detected they are eradicated under a regulatory program using amitraz dips, macrocyclic lactone (ML) pour-on or fluazuron pour-on treatments. Flumethrin pour-on treatments are widely used on calves for paralysis tick control.

The survey revealed that ML pour-on products are the most popular round worm treatment largely because of their ease of application. Choice between the different MLs is driven by cost. The benzimidazoles (BZ) have a small market share. Some producers use the levamisole/ oxyclozanide combination drench, Nilzan, to treat both round worms and fluke. The flukacide, triclabendazole is also used. The anthelmintics most commonly used by northern rivers producers are given below with their percentage market share.

Seasonal conditions influence the distribution and population densities of buffalo flies. Consequently treatment intensity varies between years. Insecticidal ear tags are the most commonly used products with synthetic pyrethroid (SP) and SP/ organo phosphate (OP) combined sprays and OP back-rubber applications also used. Macrocyclic lactones are only used for buffalo fly control when they are being used simultaneously for internal parasite control. The buffalo fly treatments used and their percentage market share are given below.

Produce store staff vary widely in their assessment of producers attitudes to the importance of considering the impact on dung beetles when selecting veterinary treatments. This variation is at least partly related to the staff's own attitude. Staff at 11 of the stores surveyed raise dung beetles as an issue with clients and report that at least 40% of producers are aware that some veterinary drugs impact on dung beetles but only 10 to 20% of producers would use this as the deciding factor in drug purchase. However a small number of staff never discuss dung beetle issues with their customers and they report that their customers don't raise the issue with them.

3 - Grazer perceptions, knowledge of and needs for veterinary drugs – Queensland

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A survey of 110 cattle producers and landholders taking part in the NHT Queensland Dung Beetle Project provides data on current use of parasiticides on cattle in Queensland, in terms of target pests, types of chemical used, and frequency of use. The main parasites for which chemical treatments are used in Queensland are buffalo fly, ticks, lice and worms. Most chemicals used are synthetic pyrethroids, organophosphates, amines or macrocyclic lactones. A summary of chemical use in different regions of Queensland will be presented.

Additional data on ‘grazier perceptions’ of the possible impacts of parasiticides on dung beetles comes from a separate survey undertaken at the request of the Management Committee of the Queensland Dung Beetle Project. The survey was designed to provide baseline data on landholders’ knowledge about dung beetles, and entailed a random sample of 104 primary producers. Results indicated that 100% of producers had heard about dung beetles, and 21% indicated that they had changed their management practices since learning about dung beetles. These changes mainly related to using chemicals less frequently, or to using chemicals that are less harmful to dung beetles. It is presumed that the source of information for these landholders on the safety of chemicals for dung beetles is primarily that provided by chemical companies. Anecdotal evidence gathered by project staff involved in field days, agricultural shows and other extension activities confirms that there is widespread knowledge that chemical use can be harmful to dung beetle populations. However, it is also apparent that producers are frustrated by the lack of information on the relative safety for dung beetles for many of the veterinary chemicals currently on the market. There is clearly a need for much more information to be provided by chemical companies, as well as an independent assessment of such information.

Dung beetle feeding

On what and how do dung beetles feed ?

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Dung beetles of the subfamily Scarabaeinae feed on the excrement of vertebrates, principally large herbivores. A very few species are necrophagous or saprophagous. The mouthparts of adult dung beetles are modified for the ingestion of soft, semi-liquid food, an adaptation for feeding on the fluid part of dung. The fluid component of dung contains colloidal particles, micro-organisms, fine particulate matter, and soluble material. It has been demonstrated experimentally that the amount of this material in the fluid (measured as dry matter) determines the ‘quality’ of the dung as food for adult dung beetles (Aschenborn, Loughnan and Edwards 1989). It has recently been shown by Holter, Scholtz and Wardhaugh (2001) that the maximum size of particles ingested by adult dung beetles ranges from 10-50 μm , depending on the species of beetle. These authors also demonstrated that adult beetles do not grind larger dung particles into smaller particles. They concluded that larger particles are filtered out by setae on the mouthparts, and the remaining small particles are squeezed between the molars to eliminate excess liquid before being ingested. In contrast, the mouthparts of dung beetle larvae are modified for biting and cutting, and are adapted for eating the drier dung found in brood balls, including the fibre component. The digestive systems of adult and larval scarabaeine dung beetles also differ considerably. Adults have a long narrow midgut, allowing rapid processing of large amounts of liquid food. Most larvae have a large chamber in the midgut where cellulose fermentation takes place. Thus the fibre in dung can be digested by cellulose-degrading bacteria, which in turn provide most of the food for the larva. Furthermore, larval faeces, with a high bacterial content, are re-eaten several times by the larva.

The differences between larval and adult feeding mechanisms may in part explain some of the differential effects on adult and larval dung beetles that have been found for some veterinary chemicals. For instance it is known that ivermectin is insoluble in water, and has a high binding affinity to organic matter. It is thus likely that more of this chemical will be found bound to large organic particles in the dung than will be in the fluid, resulting in a greater intake by larvae than by adults.

Finally, adult dung beetles in the subfamily Geotrupinae have mouthparts more suited to biting and chewing, indicating that results obtained from tests of veterinary chemicals on adult Scarabaeinae dung beetles may not be applicable to *Geotrupes spiniger*.

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Excretion and impact of veterinary chemicals

Factors affecting the rate and route of excretion of veterinary parasiticides and their persistence in the environment

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Metabolism of veterinary parasiticides is influenced primarily by molecular properties of the chemical class which determine whether faeces or urine is the predominant route of excretion. Within the particular class differences in substituents on the core molecule can affect the balance between these two routes as well as the rate of clearance which in turn is influenced by factors such as host species, formulation, route of administration and dietary conditions. This paper presents a summary of current knowledge of the pattern of excretion of the major classes of anthelmintics and insecticides used in livestock production and to which dung beetles are likely to be exposed.

Macrocyclic lactones are the principal class of compounds used to treat both endo- and ecto-parasite infections of grazing livestock. They are almost entirely excreted in faeces but the rate differs considerably between the various commercially available compounds due to differences in their lipophilicity, rates of hepatic metabolism and clearance in the bile, as well as the formulation and delivery route.

In *cattle*, following *subcutaneous* dosing of ivermectin at 200µg/kg, peak concentrations in faeces may occur from day 1 to days 6 or 8. These variations may be attributable to dietary differences between studies; ivermectin concentrations in the faeces of pasture-fed cattle were lower throughout the 14 days post-injection period and peaked later than in grain-fed animals.

Maximum concentrations of doramectin in cattle faeces occurs 4 days after injection and are almost 2.5 times the peak value for ivermectin; the mean residence time in faeces of doramectin (7.7d) is slightly longer than for ivermectin (6.3d) under similar pasture conditions.

Maximum faecal concentrations of moxidectin occur during the first day after drug administration at values about 30% higher than for ivermectin; excretion of moxidectin in faeces has a mean residence time of 10.7 days. Parent ivermectin, doramectin and moxidectin may still be detectable in faeces 58 days after subcutaneous injection.

Topical, or pour-on, administration of ivermectin to cattle at 500µg/kg results in higher initial concentrations in faeces, but by 5 to 7 days levels are similar to those following subcutaneous treatment at 200µg/kg. By contrast, pour-on treatment of cattle with moxidectin at 500µg/kg results in substantially lower faecal residue concentrations than after subcutaneous dosing and peak levels are not attained until 11 days.

The ivermectin *sustained release bolus* for cattle is designed to deliver ivermectin at 12.7 mg/day for 135 days in animals weighing between 100 and 300 kg. Following administration of this device to calves under pasture conditions faecal ivermectin concentration increases to a peak at 4 days and then declines to a steady state level at 7 days which are maintained until 120 days.

In *sheep*, faecal excretion of orally administered ivermectin is more rapid; by 7 days cumulative faecal residues range from 69 to >95% of the dose, two-thirds of this being recovered during the first 2 days and 61-69% of these residues are present as parent drug.

Doramectin given orally to sheep is excreted rapidly in the faeces, being about 90% complete by the fifth day. Oral administration of moxidectin to sheep results in initial faecal concentrations 10 times higher than those observed in cattle after subcutaneous injection, but by 7 days, drug concentrations in the dung of the two species are similar. At this stage cumulative excretion accounts for 43% of the dose and moxidectin comprises 25% of the total residues in sheep faeces.

Benzimidazoles are still used to treat gastrointestinal helminth infections in grazing livestock, although widespread resistance in sheep nematodes restricts their effectiveness. These compounds are very insoluble in water and are orally administered as an aqueous suspension whence they significantly associate with the particulate phase of digesta. The quantity and type of feed can influence both rate and extent of absorption, and metabolism and excretion is affected by the nature of substitution of the benzimidazole moiety.

Aromatic substituted benzimidazoles (fenbendazole / oxfendazole) are extensively metabolised (hydroxylated) with >70% of dose being excreted in faeces, almost all of this within 3 days of administration. Similarly, over 90% of the dose of the flukacide triclabendazole is excreted in faeces within 7 days, with less than 7% cleared in urine, mostly as hydroxylated sulphoxide and sulphone metabolites.

Aliphatic substituted benzimidazole (albendazole) is rapidly oxidised with >60% of the dose, excreted in urine as the sulphoxide and sulphone within 48h of administration. Over the same period only 20% of dose, mainly as hydroxylated sulphoxide and sulphone metabolites is excreted in faeces.

The unsubstituted benzimidazole, thiabendazole, is rapidly hydroxylated and almost completely eliminated (>75% dose) in urine within 48 hours of administration.

Closantel is a narrow spectrum anthelmintic which is used to treat infections of blood-sucking helminths such as *Haemonchus* and liver fluke. It is relatively poorly absorbed and, depending on feed intake, up to half of the oral dose passes through the gastrointestinal tract and is excreted in faeces within 24-36h of administration. Absorbed drug is almost completely returned into the gastrointestinal tract and excreted in faeces within 7 days of administration, although blood concentrations may be maintained well beyond this period. There is negligible excretion of closantel in urine.

Miscellaneous compounds. There is no information in the public domain describing the quantitative relationship over time for excretion of other anthelmintics such as levamisole, morantel / pyrantel and praziquantel. Once absorbed these compounds are rapidly and significantly metabolised (levamisole to OMPI; praziquantel is hydroxylated and morantel/pyrantel is almost completely fragmented) with little parent drug identifiable in urine or faeces. What little is known indicates that these compounds are predominantly cleared in urine.

Synthetic pyrethroids (SP) are administered to sheep and cattle as a topical or 'pour-on' formulation to control ectoparasites such as lice and buffalo fly. In sheep the SP quickly associates with lipids and organic matter which retains the drug in the wool grease preventing absorption into the bloodstream. High concentrations of SP can occur in wool and while some may wash off into the environment, there is no evidence for excretion in faeces. There is no excretory data for SP in cattle, although there is evidence that lice dwelling at sites too remote from the point of drug application to be contacted by topical diffusion, are adversely affected within 24h

of administration. This indicates that SP is absorbed, but there is no quantitative information available on the clearance over time of topically applied SP in cattle.

The macrocyclic lactones – impact on non-target organisms

Abamectin and ivermectin: non-target research since 1992

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An international workshop was held in Columbus, Ohio in 1992 to review the “Environmental impact of avermectin usage in livestock”. Two questions were of primary interest: 1) Do faecal residues affect non-target species in dung of treated animals? 2) Does dung from treated versus untreated animals degrade at a similar rate? Research indicated that faecal residues could reduce insect activity in dung of treated animals voided days to weeks after treatment. Use of avermectins also was shown to affect dung degradation under experimental conditions in at least some studies. Because of the widespread use of avermectins, particularly ivermectin, implications of these findings to populations of dung-breeding insects and to the degradation of dung in pasture ecosystems generated considerable debate.

Numerous studies since have been published on the non-target effects of abamectin and ivermectin. The current talk provides a brief overview on the general findings of this more recent research, summarizes conclusions from a number of review articles, and lists developments with implications for future research. The talk is not comprehensive, but rather is intended to reflect the current ‘state-of-knowledge’ for this area of research. Articles used in the preparation of this talk are listed below.

These more recent studies support the main conclusions reported in 1992:

- 1) faecal residues can reduce insect activity in fresh dung voided for a period of days to months after treatment. The degree of effect is affected by a number of factors that include endectocide formulation, species of insect, and life stage.
- 2) faecal residues can be directly toxic or have sublethal effects that include altered reproductive success of exposed insects and their parasitoids, and altered patterns of insect colonization in dung from treated animals.
- 3) faecal residues have been associated with reduced degradation of dung under experimental conditions.

These findings suggest that recommended use of abamectin and ivermectin adversely affects populations of at least some species of dung-breeding insects and may delay dung degradation in pasture ecosystems. However, insect activity is only one of a suite of factors (e.g., weather, mechanical disruption, season) that affect dung degradation. Hence, it cannot be assumed, *a priori*, that use of these products will necessarily delay degradation. The extrapolation of experimental results to pasture ecosystems remains a topic of considerable debate.

Developments with implications for future research include: commercialisation of new endectocides, generic versions of existing endectocides, new formulations, endectocide resistance, and apparent discrepancies among studies.

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New macrocyclic lactones – moxidectin, doramectin and eprinomectin

1 - Residual toxicities of doramectin, eprinomectin, ivermectin and moxidectin in cattle dung.

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Doramectin, eprinomectin, ivermectin and moxidectin are registered in Canada for use on cattle to control internal and external parasites. Residues in dung of animals treated with these products affect dung-breeding insects and possibly dung degradation, but not necessarily to the same degree. Here we discuss the results of research that compares: 1) the larvicidal activity of the faecal residues of these products against pest flies, 2) the activity of faecal residues of these products against non-pest species of insects, and 3) degradation of dung from cattle treated with these products.

Larvicidal activity against pest flies. Two experiments were performed to assess the larvicidal activity of faecal residues against pest flies. In the first experiment, groups of cattle (2 animals per group) were treated with a topical dose (500 µg/kg BW) of ivermectin or moxidectin with an untreated group of cattle serving as the control. In the second experiment, groups of cattle (8 animals per group) were treated with a topical dose (500 µg/kg BW) of doramectin, eprinomectin, ivermectin or moxidectin. For each experiment, cattle were housed under similar conditions and maintained on the same diet. Dung from cattle was collected fresh prior to treatment (Week 0) and for nine (Experiment 1) or four (Experiment 2) weeks after treatment. Dung from different pats of the same treatment was homogenized at the time of collection and frozen until use.

Containers of dung were seeded with 50 eggs or newly hatched larvae of horn fly, *Haematobia irritans*, house fly, *Musca domestica*, or stable fly, *Stomoxys calcitrans*. In each experiment, 10 to 20 replicate containers were prepared for each combination of week and species. ANOVAs ($P = 0.05$) subsequently were performed for each treatment to compare the number of adult flies emerging in each container.

Application of doramectin, eprinomectin and ivermectin suppressed horn fly in dung of cattle voided at least four weeks post-treatment, and suppressed house fly and stable fly in dung voided from one to five weeks post-treatment. Direct comparison of these products in Experiment 2, identified doramectin as having the highest level of larvicidal activity. Survival combined for horn fly, house fly and stable fly in dung voided one to four weeks post-treatment was 17 percent for doramectin, 35 percent for eprinomectin, and 35 percent for ivermectin. Application of moxidectin suppressed horn fly in dung voided no more than 1 week post-treatment and had no detectable effect on house fly or stable fly. Complete results of these lab bioassays are reported in Floate et al. (2001).

Activity against non-pest species. Experiments were performed over a three-year period to assess the effect of faecal residues against non-pest species of insects in dung of treated cattle. In 1998, groups of cattle (2 animals per group) were treated with a topical dose (500 µg/kg BW) of doramectin, eprinomectin, ivermectin or moxidectin with an untreated group serving as the control. In 1999 and 2000, groups of cattle (8 and 10 animals per group in 1999 and 2000, respectively) were treated with a topical dose (500 µg/kg BW) of doramectin, eprinomectin, ivermectin or moxidectin. In each year, cattle were housed under similar conditions and maintained on the same diet. Dung from cattle was collected fresh prior to treatment (Week 0) and for six (1998) or four (1999, 2000) weeks after treatment. Dung from different pats of the same treatment was homogenized at the time of collection and frozen until use.

Artificial dung pats (0.5 litre) were prepared for each combination of treatment and week of collection (5 replicate pats in 1998, 12 replicate pats in 1999 and in 2000). Pats were placed on styrofoam plates on a 1-2 cm

layer of sand, then positioned in the field with treatments randomized in a complete block design. Pats were placed in the field in May for one week to permit natural colonization of dung-breeding insects. Wire enclosures were used to prevent mechanical disruption of pats by birds and rodents. Pats and associated plates subsequently were returned to the laboratory and held in separate cages. Adult insects emerging in cages during the next 12-14 weeks were collected, sorted, and identified to species.

MANOVAs, wherein multiple species were analyzed simultaneously, were performed to assess the effect of week on insect assemblages for each treatment group. To reduce false negatives, analyses were arbitrarily restricted to species of insects comprised of 100 or more individuals. If results were significant ($P < 0.05$), ANOVAs were performed on individual species with Bonferroni post-hoc tests to identify differences among weeks. Data were rank-transformed prior to analyses.

In 1998, 13,435 insects were recovered representing 60 species. Eighteen of these species representing 92% of the total insects were included in analyses. In 1999, 7,547 insects were recovered representing 56 species. Fifteen of these species representing 91% of the total insects were included in analyses. In 2000, 25,682 insects were recovered representing 65 species. Twenty-six of these species representing 95% of the total insects were included in analyses.

In 1998, insect assemblages differed among weeks for eprinomectin ($P = 0.006$) and ivermectin ($P = 0.001$). No effect was detected for the control group ($P = 0.134$), doramectin ($P = 0.286$) or moxidectin ($P = 0.187$). For eprinomectin, examination of individual species did not reveal significant differences between Week 0 and Week 1, when faecal residues should have been in highest concentration. For ivermectin, a significant difference was detected between Week 0 and Week 1 for one species.

In 1999, insect assemblages differed among weeks for doramectin ($P = 0.001$), eprinomectin ($P = 0.004$) and ivermectin ($P < 0.001$). No effect was detected for moxidectin ($P = 0.177$). For doramectin, four species were more abundant in Week 0 than in Week 1. For eprinomectin, one species was more abundant in Week 0 than in Week 1. For ivermectin, three species were more abundant in Week 0 than in Week 1.

In 2000, insect assemblages differed among weeks for doramectin ($P < 0.001$), eprinomectin ($P < 0.001$), ivermectin ($P = 0.023$) and moxidectin ($P = 0.004$). For doramectin, six species were more abundant in Week 0 than in Week 1. For eprinomectin, seven species were more abundant in Week 0 than in Week 1. For ivermectin, two species were more abundant in Week 0 than in Week 1. For moxidectin, four species were more abundant in Week 0 than in Week 1.

In each year, there were several cases where species were more abundant in Week 0 than in Week 1, but differences were not significant. This lack of significance was attributed to small sample sizes and large variation in insect numbers among samples. Hence, samples were combined across years to increase the rigour of analyses. This approach added year-to-year variation as a confounding factor, but increased sample sizes from 5 (1998) or 12 (1999, 2000) to 29. For these combined analyses, insect assemblages differed among weeks for doramectin ($P < 0.001$), eprinomectin ($P = 0.043$) and for ivermectin ($P < 0.001$). No effect was detected for moxidectin ($P = 0.096$).

To compare the relative toxicities of the four endectocides for data combined across years, reductions detected for individual species were rated from 1 to 4. Ratings of 1, 2 and 4 indicated significant reductions only for Week 1, for Weeks 1 and 2, and for Weeks 1 to 4, respectively. Ratings of individual species were then combined to obtain a cumulative rating for each treatment. Note that this underestimates the severity of effect, because residual toxicities were not tested for dung voided beyond four weeks post-treatment. For doramectin, reductions were detected for ten species with a cumulative rating of 37. Hence, each species was suppressed for an average of about four weeks post-treatment. For ivermectin, reductions were detected for eleven species for a cumulative rating of 32. For eprinomectin, reductions were detected for ten species for a cumulative rating of 19. For moxidectin, reductions were detected for six species with a cumulative rating of 7.

Insects most susceptible to endectocide residues were species of cyclorhaphous diptera and their hymenopteran parasitoids, but also included feather-winged beetles (Ptiliidae) and species of rove beetles (Staphylinidae).

Note: These results should be considered as preliminary until more extensive ongoing analyses have been completed.

Dung degradation. Experiments were performed over a three-year period to assess the effect of faecal residues on dung degradation. Methodology was identical to that used in studies of insect assemblages with the following exceptions: 1) dung pats were placed directly on soil in native pasture from May to October, 2) pats were not protected by wire enclosures, and 3) treatments of Week 2 and Week 6 were not included in 1998.

In October, pats were removed from the field and separated into 'degraded' and 'undegraded' fractions. Degraded material comprised small dry particles with the consistency of sawdust whereas undegraded material comprised larger more solid portions. Percentage degradation of each pat was measured as $(d/(d+u)) \times 100$ where 'd' = dry weight of the degraded fraction and 'u' = dry weight of the undegraded fraction. ANOVAs ($P = 0.05$) were performed for each treatment group (doramectin, eprinomectin, ivermectin, moxidectin) to assess the effect of week (1998: Weeks 0, 1, and 4; 1999 and 2000: Weeks 0, 1, 2 and 4) on degradation.

In 1998, an effect of week was detected for doramectin ($P = 0.011$), eprinomectin ($P < 0.001$) and moxidectin ($P = 0.020$). For the first two treatments, dung degradation was reduced in Week 1, relative to Week 0 and Week 4. For moxidectin, dung degradation was reduced in Week 1 and in Week 4, relative to Week 0. In 1999, no effect of week was detected. In 2000, an effect of week was detected for moxidectin ($P = 0.009$), reflecting reduced degradation in Week 1 relative to Week 4. For data combined across years, an effect of week was detected for eprinomectin ($P = 0.016$), where dung degradation was reduced in Week 1 relative to Week 0. In no cases did dung from untreated cattle degrade at a slower rate than dung from treated cattle.

These results show that reduced degradation of cattle dung can be a consequence of treatment with endectocide. However, results also emphasize the apparent unpredictability of this phenomenon. Faecal residues of moxidectin had the least effect on insect assemblages, yet apparently reduced degradation of dung voided four weeks after treatment in 1998. Faecal residues of ivermectin were second only to doramectin in toxicity to insect assemblages, but had no detectable effect on dung degradation. These findings are not surprising given that insects are only one of a suite of factors (e.g., weather, mechanical disruption, animal foraging) that affect dung degradation, for which individual roles in the degradation process are expected to differ with year and season.

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2 - Dung Beetles, Doramectin and other Unfinished Research

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A comparison of the ecotoxic effects of excreted residues of Avomec® and Dectomax® were bioassayed using the dung beetle *Onthophagus binodis* Thunberg. A second study using excreted residues of Cydectin® and Avomec were also bioassayed using the same species of dung beetle. Dung samples were collected on 8 occasions in the Dectomax® trials between 1 and 42 days post-injection and on 7 occasions in the Cydectin® trials between 1 and 40 days post injection.

Fewer newly-emerged adults of *O. binodis* survived exposure to dung from cattle treated 3 and 6 d previously with abamectin or 9d previously with doramectin than from dung of untreated cattle. Both compounds induced a range of sub-lethal effects on *O. binodis*. Abamectin residues excreted in dung up to 42 d post-injection had a deleterious impact on ovarian condition, brood mass (egg) production, and larval survival. Doramectin residues only had a deleterious effect on these parameters at 3 and 6 d post-injection relative to dung from control cattle. Results for the Cydectin® study are still being analysed.

The last experiment to discuss concerns the relationship of diet and ivermectin on the survival and reproduction of dung beetles. Previous studies have shown that dung from cattle fed on grain or pasture has no effects on adult mortality but significant effects on F1 survival and progeny size. Furthermore ivermectin is excreted in dung at significantly higher concentrations in faeces of grain-fed cattle compared with pasture fed cattle. The following trial combines the parameters of diet and ivermectin and shows that irrespective of the diet ivermectin has a significant impact on dung beetle survival.

The talk will end with a discussion of the overall potential ecotoxic effects of these compounds in terms of dung beetle activity and strategies for parasite control of cattle in the Australian environment.

3 - The development and survival of the dung beetle, *Onthophagus taurus* when fed on the faeces of cattle treated with pour-on formulations of eprinomectin or moxidectin

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Most antiparasitic agents are excreted to some extent in faeces of treated animals, creating concern over their effect on the myriad of organisms that feed and/or breed in animal excrement. Many of these organisms, e.g. dung beetles, play a vital role in the processes of dung dispersal. They are therefore important for maintaining pasture hygiene and nutrient cycling. The recent registration of eprinomectin and moxidectin for use in Australian dairy cattle is symptomatic of a worldwide trend towards the use of highly potent, broad-spectrum drugs for the control of livestock parasites. Eprinomectin, a novel avermectin, and moxidectin, a milbemycin, are macrocyclic lactones (MLs) active against a wide range of nematodes and arthropods. Hitherto, concerns over drug residues in milk have precluded the use of MLs in the dairy industry, except for treatment of dry cows. Until recently, nematode control has been dominated by drugs belonging to the benzimidazole and levamisole/morantel classes of chemical whereas management of external parasites, such as lice and biting flies, has been affected largely through the use of synthetic pyrethroids and organophosphates. With the entry of two multi-purpose ML parasiticides into the market place, current patterns of drug usage in the dairy industry are likely to undergo significant change. Both chemicals are also registered for use in beef cattle.

Faeces voided by one-year old cattle at 3 – 70 days after treatment with a pour-on formulation of moxidectin had no detectable effects on development or survival of the common dung beetle *Onthophagus taurus*. However, faeces voided by cattle treated with a pour-on formulation of eprinomectin were associated with enhanced juvenile mortality during the first 1 – 2 weeks after treatment. Increased mortality also occurred among newly-emerged beetles fed on faeces collected 3 days after eprinomectin treatment and there was evidence of suppressed brood production among those that survived. This effect was still apparent even after insects fed for a further 10 days on the faeces of untreated cattle. A model simulating the effects of drug residues on dung beetle populations in the immediate vicinity of the treated herd suggests that, in the absence of immigration, a single treatment of eprinomectin is capable of reducing beetle activity in the next generation by 25 - 35%. Effects are likely to be greatest when treatment coincides with emergence of a new generation of beetles.

Earthworms and macrocyclic lactones

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Earthworms play the major role in the disappearance of dung pats in temperate pastures. Even so, studies on the effect of veterinary medicines on earthworms populations are scarce and mostly concerned with laboratory assays. Initial studies (Halley *et al* 1989; and Gunn and Sadd 1994) examined the effects of ivermectin on the epigeic earthworm, *Eisenia fetida*, which is more commonly regarded as a composting worm and is not normally associated with cow dung. The relevance of these studies is therefore uncertain, especially since they produced conflicting results. Halley *et al* (1989) could detect no effect of ivermectin on the development or survival of *E. fetida*, whereas Gunn and Sadd (1994) claimed that the presence of ivermectin was associated with significant mortality and reduced growth rates. It should be noted, however, that the experimental design of the latter study may have biased the outcome of these assays.

The effects of faecally excreted ivermectin and fenbendazole, and their metabolites, on the survival and growth of the common pastureland earthworm *Lumbricus terrestris*, have been studied in the laboratory (Svendsen *et al* 2001). Newly hatched worms were fed with dung voided by untreated cattle or cattle given sustained-release boluses of the parasiticides ivermectin or fenbendazole. Hatchling survival and growth rates were followed until maturity. The survival of worms provided with untreated dung was 100 % whereas survival in ivermectin and fenbendazole groups was similarly high (97 and 91 %, respectively). The first worms were mature 16 weeks after hatching, regardless of dung type, and all worms matured within 24 weeks of hatching. The growth rate of the worms fed dung from cattle given ivermectin boluses was 2.6 mg higher day⁻¹ than that recorded for the control group, whereas the growth rate of worms fed on dung from cattle given fenbendazole boluses did not differ significantly from the control group. Svendsen *et al* (2001) concluded that ivermectin, fenbendazole and their metabolites had no adverse effects on the survival and growth of *L. terrestris* when exposed through dung under laboratory conditions.

Deep-burrowing (anecic) species of earthworm, such as *Aporrectodea longa*, are rare in mainland Australia (Svendsen and Baker 2001). This species, which is common in northern Tasmania, can enhance the incorporation of surface organic matter such as dung and has the potential to colonise pastures in the higher rainfall areas of south eastern Australia. However, in these areas, antiparasitic drugs are widely used in the grazing ruminants and residues of these drugs are excreted in the faeces. Svendsen and Baker (2001) studied the effects of the broad-spectrum anthelmintic, moxidectin in sheep and cow dung, on the survival and growth of *A. longa* in the laboratory. Over a ten-week period, there were no lethal or sub-lethal effects of the drug and its metabolites in sheep and cow dung on *A. longa*. Worms removed more sheep dung than cow dung from the soil surface and hence had a higher growth rate on sheep dung.

Although the volume of information is still small, it seems unlikely that residues of currently used macrocyclic lactones have important adverse effects on the development or survival of earthworms. A small pilot study using excreted residues of the synthetic pyrethroid, deltamethrin, suggest a similar conclusion (Wardhaugh, unpublished data).

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Synthetic pyrethroids, organophosphates, insect growth regulators and other livestock parasiticides

Insecticidal activity of antiparasitic drugs other than the macrocyclic lactones

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Concern over unintended side-effects of drug therapy on non-target organisms is not a new phenomenon. The prospect of insect-free droppings was first raised in the USA in the early 1960s, at a time when the control of livestock parasites was undergoing major change. The 1970s and 1980s saw the introduction of new compounds such as synthetic pyrethroids, insect growth regulators and the macrocyclic lactones, coupled with new methods of drug administration and new ways of manipulating the residence time of parasiticides in the target animal. However, apart from a few sporadic studies by Blume *et al* (1976) in the USA and Lumaret (1986) in France, the entomological and ecological implications of this revolution in animal husbandry past largely unnoticed until the seminal paper of Wall and Strong (1987). Working with an experimental sustained-release bolus containing the highly potent, broad-spectrum insecticide, ivermectin, Wall and Strong concluded that this type of device had the potential to adversely affect the survival of non-target, dung-feeding arthropods and thus seriously disrupt the processes of dung degradation and nutrient cycling. This conclusion spawned a vigorous and at times contentious, world-wide debate that continues to the present time. More importantly, it so focused attention on the environmental effects of macrocyclic lactones, that compounds belonging to other classes of chemical, have been all but ignored.

At the present time in Australia there are more than 200 products available for the control of livestock parasites. These can be divided into 6 main groups (namely the Benzimidazole – Levamisole – Morantel group (BLMs), the macrocyclic lactones (MLs), organophosphates (OPs), synthetic pyrethroids (SPs) and a group of ‘biological’ parasiticides (BIOs) that include insect growth regulators and chitin esterase inhibitors. The BLMs are used for the control of internal parasites, whereas OPs, SPs and BIOs are aimed at ectoparasites control. MLs are used to control both internal and external parasites and are commonly referred to as endectocides.

BLMs

None of the compounds comprising the BLM group appear likely to have a significant impact on the survival of dung-feeding organisms. This is partly because some of the more commonly used compounds, such as albendazole and levamisole, are largely excreted in urine. Of those that are excreted in faeces, only oxfendazole has been shown to exhibit insecticidal activity. Laboratory assays have shown residues of this drug are active against larvae the bush fly, *Musca vetustissima* (Wardhaugh *et al* 1993), but there are no data indicating activity against dung beetles (Wratten *et al* 1993).

OPs

Early work on organophosphates such as dichlorvos, ruelene and coumaphos indicated that all of these compounds had deleterious effects on the dung fauna (e.g. Blume *et al* 1976; Lumaret 1986), especially when used as feed additives. However, none of these OPs are currently registered for use in Australian cattle, and OPS

that are registered (i.e. chlorfenvinphos, fenthion, diazinon, famphur, phosmet, trichlorfon, maldison and propetamphos) are formulated mostly for application as sprays, pour-ons or ear-tags. The effects of spray formulations of dichlorvos and trichlorfon on the survival of the dung beetle, *Onthophagus gazella*, have been tested by Bianchin *et al* (1992). The resultant data are difficult to assess, but it appears that trichlorfon was less harmful than dichlorvos and, compared with several synthetic pyrethroids, neither compound had a major impact on beetle survival.

With the exception of fenthion, which is eliminated mainly in urine (Avrahami and White 1975), there is no information in the public domain to indicate the temporal pattern or route of excretion of any of the OP preparations currently registered for use in Australia. Information dealing with their metabolism during passage through the animal is also lacking. In consequence, we have virtually no information by which to gauge the potential ecotoxicity of this class of drugs. From a grazier standpoint, this knowledge gap needs to be rectified as a matter of urgency.

BIOs

There are numerous chemicals that act as parasite control agents by disrupting insect development, but relatively few of these have been registered for veterinary use in Australia. The two most important for the cattle industry are probably fluazuron and diflubenzuron whereas cyromazine is widely used for fly control in the sheep industry. The latter, when used as an additive in cattle feed is very active against fly larvae, but is reported to have little effect on scarabaeids (Miller *et al* 1981). However, no information is available about the rate or route of elimination of cyromazine when used in sheep.

Fluazuron is a systemic, chitin esterase inhibitor, used for tick control. There are no data in the public domain describing either the excretion profile of this drug or whether or not it is active against dung feeding flies or beetles. Anecdotal information suggests it is an acaricide with no insecticidal activity, but this needs to be confirmed by the manufacturer.

Diflubenzuron, an insect growth regulator, is eliminated in faeces and is active against both fly and beetle larvae. Fincher (1991) found that dung voided by animals treated with a sustained-release bolus of diflubenzuron inhibited the survival of fly larvae for up to 21 weeks post-treatment and those of two species of dung beetle for 7 weeks. Diflubenzuron applied as a dust has also been reported to result in the production of dung toxic to fly larvae. However, no information is available about activity or elimination profile of this drug when used as a pour-on formulation.

SPs

Almost 20 years ago Kunz *et al* (1983) demonstrated suppression of a population of dung-breeding horn fly, *Haematobia irritans*, via area-wide (960 km²) treatment of cattle with fenvalerate-impregnated ear-tags. Accordingly, Schreiber *et al* (1987) examined the possibility that the use of synthetic pyrethroid ear-tags may also result in a reduction in both the abundance and diversity of predatory and coprophagous beetles. No evidence for this was found, leading to the conclusion that such chemicals were unlikely to significantly alter the dynamics of the dung beetle community. However, work carried out in Brazil (Bianchin *et al* 1992, 1997, 1998) and, more recently, in Australia (Wardhaugh *et al* 1998), South Africa (Kruger *et al* 1999) and Denmark (Sommer *et al* 2001), has shown a broad range of SPs to be highly toxic to adult dung beetles. Bianchin and colleagues concluded that toxic effects associated with the administration of the SPs alphamethrin, cypermethrin, deltamethrin, cyhalothrin and flumethrin may persist for up to 18 days post-treatment with the impact varying according to the type of formulation used (ear-tag < spray < pour-on). The study concluded that SPs had the potential to be more harmful to the dung fauna than MLs. Although more recent research on flumethrin (Kruger *et al* 1998; 1999; Sommer *et al* 2001) contradicts the Brazilian research and indicates that this chemical is innocuous to both flies and beetles, findings in relation to deltamethrin and cypermethrin have been substantially confirmed. Wardhaugh *et al* (1998) showed that dung collected from deltamethrin-treated cattle is toxic to adult dung beetles for 7-14 days after treatment and concluded that a single pour-on treatment of this drug may reduce beetle activity in the next generation by more than 70% if the time of application coincides with peak beetle emergence in spring. Two or more successive treatments at three weekly intervals had the potential to drive

beetle populations towards local extinction. Kruger *et al* (1999) have shown that a pour-on formulation of cypermethrin has toxicity characteristics similar to those recorded for deltamethrin and have concluded that the widespread and frequent use of this class of parasiticide poses a serious risk to dung beetle populations.

General conclusion

Knowledge of the insecticidal activity of drug residues in faeces voided by livestock treated with OPs, SPs and BIOs is sadly deficient. Although the available data are limited, SPs, because of their adulticidal properties, appear to constitute the class of chemical which poses the greatest threat to our dung fauna. Much further research will be required before we can develop a Decision Support System for graziers that will maximize parasite control and minimise environmental damage.

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Important veterinary chemicals for which there are no data.

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This presentation will briefly discuss the information presented in a recent review of parasiticides registered for use in cattle in Australia (Wardhaugh 2001). It will discuss the importance of this document in relation to stakeholder decision making as to the application and effect of various chemicals on Dung Beetles. Although much is known about the effects of macrocyclic lactones, information relating to other chemical classes is scarce. From discussions with land holders in the northern region, the National Dung Beetle Steering Committee has identified 7 chemicals that are commonly used and for which additional information is required. These are:

- diazinon, chlorfenvinphos and fenthion (organophosphates);
- deltamethrin and flumethrin (synthetic pyrethroids);
- fluazuron. (chitin esterase inhibitor); and
- amitraz (amine)

Other grazing regions are likely to identify other compounds.

In closing, I will discuss the need for:

- the release of relevant toxicological information that may be contained in the archives of State and National Regulatory Authorities;
- the routine provision of such information in the public domain as each new product is registered;
- and on-going, independent research on the environmental impact of veterinary chemicals.

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Management of parasiticide use

Conserving and optimising the use of parasiticides

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Integrated Pest Management (IPM) involves using a number of strategies to control a pest species, so dependence on one control method is avoided. For the control of parasites in domestic livestock this can include chemotherapy, vaccination, improved nutrition (especially for *at risk* stock), breeding for resilient or resistant stock and pasture/grazing management. The aim of such an approach is to avoid detrimental parasite exposure rather than merely treating stock after they become infected. However, while the overall population and the host burden of parasites are significantly diminished, the above IPM control strategies, with the exception of

chemotherapy, tend to increase the proportion of the parasite population contained in or on the host. In this situation a drug treatment will impose higher selection on the parasite population by comparison with drug treatments applied when an IPM is not adopted. Thus, to avoid the rapid emergence of drug resistance the IPM should aim for a substantial reduction in the number of drug treatments applied and preferably the drugs should have either high efficacy (99.9% or greater) or low efficacy (less than 70%). In simple and general terms the better the pest control program the more likely it will select for drug resistance if it includes chemotherapy, the exception being when the drug treatment on application is 100% effective and it has no lingering activity.

Rotation or application strategies of chemically-unrelated-pesticides, such as rapid or slow rotations and mosaics have been considered to delay selection for resistance. The consensus (though not unanimous) is that the simultaneous use of different drugs (mixtures) is the best approach and this has been advocated against insects (Mani 1985), helminths (Barnes *et al* 1995 and Smith 1990) and pests in general (Comins 1977 and 1986). Though more expensive in terms of drug and labor cost this is recommended for worm control of livestock because a portion of the worm population, infective larvae on pasture, escape selection by the anthelmintic. These provide a source of susceptible genes even if a small number of worms survive the multiple treatment. A potential risk to this combination therapy is when treated hosts graze clean pastures. In this situation the next generation of the worm population comes from the parasites exposed to the combination of drugs.

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Drug registration, regulatory assessment of environmental effects and the need for standardised testing

The registration and label approval process for veterinary chemical products

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Before agricultural and veterinary chemical products can be sold, supplied, distributed or used in Australia, they must be registered and their labels approved by the NRA. A separate approval is also required for active constituents either before, or at the same time as a product is registered. This paper will describe the

assessment process for registration and approval of a chemical product and will cover such aspects as the legislative criteria that must be satisfied, the advice the NRA may seek from external agencies as well as the consultation process with stakeholders.

Assessment of the environmental impact of veterinary chemicals in Australia

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This presentation will outline the history and evolution of the assessment of the potential environmental impact of veterinary drugs in Australia. It will explain Environment Australia's role in this area since it began these assessments in 1986 and explain the philosophy behind the approach that has been taken. It will also discuss the routes through which veterinary drugs reach the environment and the range of possible exposures depending on the formulation and/or the animal to be treated. Particular emphasis will be given to the considerations given to environmental assessments carried out for veterinary drugs used on cattle, including Environment Australia's experience with the assessment of tests examining the impact on dung fauna. Finally the current international moves through the VICH to harmonise the environmental data requirements for the assessment of veterinary drugs will be discussed.

Towards standardised bioassays for dung fauna as part of the registration process for veterinary medicinal products.

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Dung fauna, in particular beetles, flies and earthworms, are an important component of pasture ecosystems. Scientific studies have shown that the use of certain groups of veterinary medicines has adverse effects on the dung-inhabiting fauna. Within the EU testing of such effects is required as part of the registration process for veterinary medicines, through the CVMP Note for Guidance: Environmental Risk Assessment of Veterinary Medicinal Products (EMA/CVMP/055/96-FINAL). The requirement is also included in the draft VICH Phase II guidance. There are presently no available standard regulatory test methods for assessing toxicity of veterinary medicinal products to this non-target fauna. It is important to have standardized testing methods available to fulfil the regulatory requirements and for international harmonisation. Standard testing will also ensure results are comparable within a group of drugs.

Standardisation of bioassays should make use of the considerable number of published laboratory studies in which single species are exposed to dung from treated target animals. Ecologically realistic, reproducible and simple assays should test dung beetle, dung fly and earthworm species that use dung organic matter as a resource for feeding and/or breeding. Endpoints must include lethal and sublethal effects on adult and immature stages. The inclusion of a positive control will ensure an acceptable sensitivity of the test method.

It is suggested that a working group discuss and ring-test methods. The testing will need financing but it is not clear whether such funds are available. Those interested in being involved in such a group should contact the authors.

Modelling the future

Models for comparing and assessing impact of veterinary drugs

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Because of the escalating costs of field trials and the almost inevitable uncertainty of their outcome, attention is turning increasingly to computer modeling as a basis for improved environmental management. For example, in response to growing concerns about the impact of veterinary parasiticides, English Nature, which is responsible for the conservation of English wildlife, is currently exploring the development of computer models to evaluate population responses of dung-dependent invertebrates and likely flow-on effects at different levels in the food chain (Alistair Burn, Senior Pollution Adviser English Nature, pers. comm.). Models based on a sound ecological knowledge can be powerful tools for understanding the likely impact of new control technologies and often represent the only practical and objective way of assessing likely outcomes at the larger temporal and spatial scales. They are also valuable as tools for setting research priorities and, in the context of the current meeting, for comparing the effects of different parasiticides.

This presentation will describe a simple model developed to assess the impact of parasiticide residues on dung beetle populations. The model uses age-specific data on fecundity, development time and survivorship, in a Leslie matrix, to compute mortality in an age-structured population. For univoltine species (i.e. species having one generation per year), egg-to-adult development is assumed to take 80 ± 20 days, followed by a pre-reproductive phase of 10 days. Females lay 2 eggs per day over a 10-week period, and move to a fresh pat every 2-4 days. Adult females have a half-life of eight weeks, during which death rate remains constant. For multivoltine species, egg-to-adult development time is reduced to 40 ± 10 days. Half-life of females is set at 4 weeks, with females laying two eggs per day over a 6-week period. At this stage in the model's development, no allowance is made for sub-lethal effects such as reduced fecundity, or for compensatory effects associated with either immigration or density dependence.

The model will be used to compare single and multiple treatments of drugs with prescribed toxicity profiles similar to those reported in the literature. The reduction of beetle activity associated with a particular treatment will be standardised against that of the untreated control to provide a Relative Activity Index (RAI):

$$RAI_i = n_i / N_i$$

where n and N are the number of beetles surviving at day 'i' in treated and untreated dung respectively. When there is no survival in treated dung, $RAI = 0$; when survival in treated and untreated dung is similar $RAI = 1$.

Drugs that act mainly as larvicides will be compared with those that kill adult beetles and, in all scenarios, we will examine the importance of time of application relative to the onset of beetle emergence in spring. The impact of different drug formulations will be simulated by varying the period of dung toxicity.

Model simulations will show that the impact of particular treatments are not always consistent with simple expectation and may be particularly useful in elucidating more complex situations involving developmental delays and other sub-lethal effects. It is suggested that simulation modelling could provide regulatory authorities with a valuable tool for assessing the potential impact of new drugs.

Towards an integrated model for cattle parasite management in Australia: Estimating population responses of dung beetles to agro-chemicals.

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It is time to adopt a more holistic and strategic approach to the management of livestock parasites in Australia. A key step in that direction is to pull all of the pieces together in a computer simulation model. A current stimulus arises from the many modern agro-chemicals that are broad-spectrum and potentially persistent. This means that there is the potential for extensive non-target effects on other parasite species and on beneficial fauna in dung pads. Products registered for one species may affect other non-target parasites, either by causing unrecognised mortality or by selecting for resistance genes. These effects need to be taken into account when calculating the benefits and costs associated with each product in the design of regionally optimal control strategies. Similarly, the achievement of sustainable production demands that the 'free' services provided to industry by beneficial fauna, in the form of biological control and nutrient recycling for example, be protected.

It is proposed to build on the existing cattle tick simulation model, which has had extensive use in designing tick control strategies over the past 2 decades, by including all of the other major cattle parasites and representative beneficial fauna in Australia. This will enable the impacts of different types of interventions to be assessed using the criteria necessary to achieve sustainability, namely: profitability, resistance management, avoidance of residues and protection of natural biodiversity. A prototype model is used to demonstrate the potential of such a whole-system parasite model in parasite management, with an evaluation of the effects of a number of hypothetical cattle tick and buffalo fly products on populations of dung beetles in northern Australia. It is concluded that such a model will help to answer many of the questions surrounding parasite management in the cattle industry in Australia. It will also help to design regional programs that minimise risks to biodiversity and yet assist industry to manage problems related to cost-effective parasite control and the management of resistance.