

National Registration Authority for Agricultural and Veterinary Chemicals

GUIDELINES FOR ESTABLISHMENT OF EFFICACY AND
MANAGEMENT DATA IN SUPPORT OF APPLICATIONS FOR THE
REGISTRATION OF PRODUCTS TO BE USED IN CONTROL OF THE
BUFFALO FLY (HAEMATOBIA IRRITANS EXIGUA)

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PREFACE

These guidelines have been prepared in order to develop a uniform approach by commercial groups, in the development of data for the registration of products for buffalo fly control. The experimental procedures recommended cover aspects related to in vitro tests, to determine the likely activity of potential products against the fly, and in vivo trials, to establish efficacy against fly infestations on the host animal. Other fundamental registration requirements, relating to detailed documentation of various aspects of environmental, occupational health and safety, animal welfare and product residue issues are not covered in this document. Organisations intending to apply for registration of a new product should consult with the National Registration Authority, Agricultural and Veterinary Chemicals, concerning these matters, and refer also to individual State's legislation eg Queensland's "Chemical Usage (Agricultural and Veterinary) Control Act" 1988. It is recommended that other available guidelines, such as the "Interim Requirements for the Registration of Agricultural and Veterinary Chemical Products" (1993), and its succeeding registration documents, should be consulted before experimental procedures are undertaken.

In accordance with ethical scientific practice, it is expected that results of all efficacy trials conducted should be submitted for consideration by registration authorities. Extenuating circumstances which could explain anomalous results in any of these trials should be noted, in order to protect the manufacturer, distributor, and end user from any problems which may occur under extensive field use conditions.

Results of efficacy trials conducted in overseas countries, can provide useful evidence for registration of a new product in Australia for buffalo fly control. Closely related *Haematobia* species do occur in other areas of the world, particularly in the Americas, and considerable research has been conducted on chemical control procedures in these areas. However differences in ecology and population dynamics of different species are likely, and variations in resistant strains have been established. The number and timing of chemical treatments, to provide optimal fly management, can vary significantly, even over the range of climatic conditions found in the fly infested areas of Australia, extending from northern New South Wales to tropical Queensland. It is important therefore that basic efficacy data, to support the registration of buffalo fly control products in Australia, should be generated under local conditions.

The requirements outlined in these guidelines can be fulfilled by a range of protocols and experimental designs. It is beyond the scope, or intent, of these recommendations to stipulate statistical methods for analysis of such experiments. However it is expected that appropriate advice will be sought, to ensure that experimental protocols are soundly based, and results of trials will be amenable to statistical analysis to establish significance.

INTRODUCTION

In the time that has elapsed since the publication of previous editions of these guidelines, considerable developments have occurred in relation to application methods, modes of action, and the biological targets of new fly control agents. Such innovative new methods, and materials, are likely to have a wide range of disparate effects on either the parasitic, or free living life cycle stages of the parasite. Consequently, because of the various strategies that may be employed to best utilise these differences, strict adherence to these guidelines should not be considered as a requirement for all potential new products.

No attempt has been made for example to set mandatory efficacy standards, as defined by knockdown or periods of protection, as a requirement for registration. Organisations developing new products for buffalo fly control are encouraged to undertake longer term trials, which demonstrate strategies for the seasonal management of buffalo fly populations, in addition to trials designed to define knockdown and protection afforded by single treatments. For potential new products or application methods, designed to provide seasonal control, rather than a single treatment effect, such strategic trials will form an essential part of the registration package. Future submissions, relating to products of this type, will need to include detailed recommendations as to the number, seasonal timing and intervals between treatments, required to achieve a satisfactory and sustainable fly management program in various areas. These recommendations should be based on results obtained in field trials, conducted in accordance with methods outlined in these guidelines. Reviewers will be able to assess the value of any new technology to the cattle industry, and the risks associated with its use in terms of control failure or emergence of chemical resistance, if comparable data is made available in various submissions.

The realistic evaluation of fly control efficacy can not be achieved without the use of host cattle. However the welfare of animals, used in efficacy trials, must be a prime consideration. Proper supervision by qualified personnel must ensure fly infestations do not reach levels causing undue stress, and such trials should not extend for unnecessarily prolonged periods. Individual animals unduly affected should be treated and, where treatments under evaluation are obviously not achieving a satisfactory control level, trials should be terminated.

1. LABORATORY IN VITRO DETERMINATION OF ACTIVITY

Methods are available to determine LC50 values for any potential contact or systemic insecticide. Such methods rely on exposure of adult flies to impregnated filter papers, immersion of flies in aqueous solutions containing 1 per cent ethanol and 0.02 per cent triton X - 100, or incorporating the active chemical in blood meal fed to the flies. *In vitro* evaluations of efficacy can be conducted on a contractual basis at CSIRO, Long Pocket Laboratories and at the DPI, Oonoonba Veterinary Laboratories.

Currently *in vitro* evaluations, as discussed above, are not an obligatory requirement for product registration. However one, or a combination of the above assays, can provide useful information concerning the potential insecticidal activity for an experimental compound against buffalo flies. The tests should not be used as a basis for determining the likely field use concentration for a particular material. Chemicals having a mechanism of action based on interference with growth, moulting, or ovicidal action can currently only be assessed using *in vivo* tests.

2. EVALUATION OF RESISTANCE CHALLENGE

Diminished periods of protection from buffalo fly infestations, following treatment of cattle with some chemicals previously providing effective control, have been reported extensively in Queensland. Evidence points to the emergence of chemical resistance as the most likely cause of this loss of protection. Synthetic pyrethroid (SP) resistance is thought to be widespread in the buffalo fly field population.

SP resistance in field populations was established in *in-vitro* tests by Schnitzerling et al (1982). In the method used by these workers, various concentrations of active ingredient, in acetone solutions, were topically applied to the pronotum of the adult female flies. Other methods commonly used are based on the exposure of adult female flies, to surfaces treated with known concentrations of active ingredient.

Unfortunately characterised susceptible and resistance reference strains of buffalo flies are not cultured in any Australian institute. At present therefore data establishing the likely challenge to a new product, from resistance in the buffalo fly field population, must be based on field observations. These need to be derived from trial sites where substantially reduced protective periods, following treatment with particular chemicals, have been observed. It is in the best interests of both the manufacturer and end user of any new product, to ensure that candidate materials are tested in the field at sites where such control problems, associated with resistance, have been reported.

3. PEN TRIALS. - EFFICACY ASSESSMENT

Assessments of efficacy based on observations made on penned cattle, under controlled conditions, have the advantage of establishing a standard method for comparison of products used for buffalo fly control. Facilities such as those available at CSIRO, Long Pocket Laboratories, are suitable for conducting such trials. Cattle are held individually in temperature and humidity controlled pens and are infested immediately following treatment. Subsequent infestations are carried out on a weekly cycle, repeated until protection is no longer evident. Three treated and three control animals are normally used for each assessment.

Results established in these trials, while not essential for registration of buffalo fly control agents, provide significant supporting evidence for efficacy claims. Dose titration trials, using stalled cattle, can also be used to determine optimal concentration required for satisfactory levels of knockdown, and protective period. Information obtained in such trials, prior to the release of a new material, would form the basis for a useful future assessment of the significance of any suspected resistance, developed following field use.

The methods and facilities, described above, can also be used to determine buffalo fly control likely to be achieved from the use of insect growth regulators, either applied externally or as a feed through. Pupae that emerge from the dung of treated cattle, or subsequent emerging adult flies, can be collected and counted as a basis for efficacy assessment.

4. FIELD TRIALS AND OPTIMAL USE STRATEGIES

Buffalo fly infested areas of Australia cover a wide range of climatic and seasonal conditions. These fluctuations will affect the performance of any product developed for fly control, as well as causing variation in the ecology and population dynamics of the pest. Strain differences, within the fly population, further complicate management strategies. Consequently all registration proposals for materials to be used for buffalo fly control need to be supported by convincing evidence that these products will provide sustainable long term fly control in a variety of locations.

4.1 Sites and timing

In selecting suitable sites, and co-operators, it should be noted that best results will be achieved where a significant fly challenge is expected. Trials may have to be extended, or postponed until the next fly season, if this challenge does not occur. It is recommended that a minimum of four trial sites should be selected from the regions listed in Table 1. At least two of these trial sites should be situated in Tropical Queensland, and one in Central Queensland. The fourth site can be located in the South East, or in one of the above areas. Where it is intended to register the product for use on dairy cattle a fifth site, located on a dairy farm in one of the regions listed, should also be chosen for an efficacy trial.

TABLE 1

Recommended geographic areas and timing for field trials.

Region	Area	Timing
Tropical Queensland	High rainfall area North of Townsville	from November to April
Central Queensland	Bundaberg north to Mackay	from December to April
South East Queensland	Buffalo fly infested area South of Bundaberg	Trials should be conducted between January and April

4.2 Animal selection and maintenance

At least fifty animals, carrying a fly burden in excess of 200 flies per animal, should be available prior to the commencement of any field trial. In addition two holding paddocks, of similar pasture quality and stocking rate, and each capable of maintaining a minimum of 20 animals, will be required for the duration of the trial. These holding areas should be separated by a distance of not less than 500 metres and not exceeding 3 kilometres. In order to ensure a continuous and stable buffalo fly population, animals should be grazed in each of these paddocks for a period of at least three weeks prior to treatment. On three occasions, during the ten days prior to treatment, fly burdens on individually identified animals should be assessed by one observer using binoculars, as described by Bean et. al. (1987). Where infestations are heavy, greater than 200 flies per side, counting in blocks of 50 flies can prove to be useful in minimising errors. Counts should be carried out consistently at the same time of day, preferably between 10am and 4pm, on each occasion. Care should be taken to avoid weather conditions, for example strong winds, which may influence levels of apparent infestation, and likely predilection sites, chosen by the flies on the host animals. Hand feeding of animals during this assessment can prove a useful means of attracting and holding cattle. On the basis of these visual assessments animals should be ranked, and randomly allocated to treatment and control groups in pairs, to ensure an even distribution of buffalo fly infestations between the two groups. A minimum of twenty animals should be maintained in each of these groups. Other animals treated with the experimental material on treatment day, Day 0, can be run with the treatment group. However, care must be taken to avoid contact between the treated and control animals, during the trial period.

4.3 Animal treatment and protective period assessment

Where group allocation is carried out immediately prior to treatment on day 0, control animals, having been separated, should be returned to the grazing paddock before chemicals are applied to the treatment group. Immediately after treatment all animals in the treated group should be kept under observation to determine the 'knockdown period' i.e. reduction of fly numbers to an insignificant level. In addition, using the methods described above, single side counts of all animals in the treated and control groups should be carried out in the paddocks on days 3, 7 and at weekly intervals until re-infestation of the treated group is established. The period of protection against fly re-infestation is

considered to have ceased when the mean fly numbers on the treated group have reached a level of 5 per cent of the mean figure derived from similar counts on control animals. However, variations in fly numbers, moving in from outside sources, can cause aberrant, albeit temporary, increases in fly burdens on all animals. It is important therefore to continue assessment past this initial apparent re-infestation, to ensure a continuous progression of re-infestation towards the control baseline has been established.

4.4 Establishment of a strategic program

Where knockdown and protective period can be established, using the above methods, additional trials, to establish strategic recommendations for management of the pest, provide useful data to support registration applications. Such information also represents a valuable contribution, to ensure proper long term management of the product by the end user.

In those instances where products are designed for seasonal fly management, and the mode of application or action does not produce immediate reduction in fly numbers, proposals for registration will be considered on the basis of results obtained in strategic trials. Field experiments, conducted under this category, should be scheduled to begin at the onset of the buffalo fly season in each area, and should include the same number and location of trial sites, as set out previously in Section 4.1 and Table 1. Prior to initial treatment counts should be carried out, as described above, on a minimum of twenty five adult animals, to establish local infestation levels. These animals should then be monitored with counts at weeks +1, +2, and observations continued at three weekly intervals for a period of three months. Obviously the maintenance of a control herd in heavily fly infested areas may not be feasible over this extended observation period. However evidence must be provided that significant buffalo fly pressure was maintained during the trial period. As a minimum requirement this evidence should be based on counts, made at monthly intervals, on local cattle from the surrounding area.

Products included in this category may involve self treatment of animals with back rubbers etc, application through ear tags, or other sustained release devices, or a strategic series of treatments during a critical part of the buffalo fly season. Control methods based on the use of traps, lures or biological control agents, designed to gradually reduce buffalo fly populations, should also be evaluated using a strategic program. Trials should be designed to ensure firm recommendations can be made as to the seasonal timing, duration, and where applicable, number of treatments required to produce a satisfactory level of control. In some instances these recommendations may need to be varied for different regions within the buffalo fly infested area.

5. SUPPLEMENTARY INFORMATION

In addition to results of efficacy trials, and the development of optimal use recommendations, it is expected that submissions for registration of a chemical formulation for buffalo fly control should also include the following information.

5.1 Animal Welfare

Any contra-indications in relation to animal use should be documented. Adverse effects causing discomfort to particular animal species, or use limitations in relation to age or lactation status should be noted. Where a particular formulation, or application method, has the potential to cause damage to hides, or animal products, this fact should be recorded. Similarly careful observations should be made as to the extent of skin lesions and discomfort caused by fly infestations on host animals.

5.2 Rain fast tests

Chemical formulations used for buffalo fly control are used under a wide range of climatic conditions. The necessity to ensure effective fly control is achieved under these extremes is important. Products intended for application as pour ons, over sprays, backline sprays or by other topical application methods should be tested under artificial rainfall conditions following treatment, to demonstrate effective control in such circumstances. Current recommendations are that animals should be subjected to the equivalent of 12.5 mm of rainfall over a 10 minute period, within two hours of treatment. Fly counts in this test on washed animals should be conducted as outlined in Section 4. A minimum of five (5) treated animals should be used.

5.3 Climatic records

All trial results should include details of weather conditions prevailing during the trial period. Rainfall records and significant influences, such as extremes of temperature, are of particular importance.

REFERENCES

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- Bean, K.G., Seifert, G.W., Macqueen, A. and Doube, B.M. (1987).** Effect of insecticide treatment for control of buffalo fly on weight gains of steers in coastal central Queensland. *Aust. J. Exp. Agric.* 27: 329-334.