Proposal for a ‘standard’ field study for the evaluation of the effects of parasiticides on dung and soil organisms

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Background

Numerous published studies and most authorisation procedures of parasiticidal products have shown that simple laboratory studies typically demonstrate a risk for dung fauna and/or soil dwelling invertebrates. Hence, there is a need for a more advanced standard test in order to evaluate risk under more realistic field conditions. In order to develop and validate such a field test, comparable investigations were conducted under varying conditions at Lethbridge (Canada), Montpellier (France), Zurich (Switzerland), and Wageningen (the Netherlands). Results of this project were published in 2016 in a series of articles in *Environmental Toxicology and Chemistry* vol. 35(8):1914-1977. This poster presents some of the lessons learned and recommendations from a methodological point of view.

Study sites

- Field studies should cover all concerned countries and regions, e.g. temperate/ atlantic and mediterranean conditions.
- Studies should be performed at the time of year when the most relevant dung species are active, typically spring (Figure 1).

Figure 1. The Yellow dung fly (*Scatophaga stercoraria*) is often found in temperate climate zones. It uses fresh dung in fields for mating and oviposition and it is also a laboratory test organism.

Field study design

- Comparison of dung with various concentrations of the parasiticide to uncontaminated control dung.
- Control dung is collected from non-treated animals before medication, and dung from medicated animals is collected at relevant time intervals post-medication (including the dung with the maximum expected substance concentration).
- A positive control made up by control dung spiked with the VMP at the highest EC50 value in the first tier has to be included as well.
- Test dung is placed in the field for colonisation by dung invertebrates and for measurement of functional parameters.
- When frozen dung is used, all pats can be placed simultaneously in the field after thawing. A simple ANOVA statistical design for comparison between treatment groups is sufficient in this case.
- Alternatively, fresh dung may be collected from treated and untreated animals and placed in the field on a daily basis . This requires a more elaborate Before-After-Control-Impact (BACI) design and statistical analysis.
- Sufficient statistical power is needed to distinguish between separate groups at the relevant taxonomic level (e.g., 25 % decline in abundance).

Application and analysis of the test substance

- The recommended type of veterinary application formulation and label rate of the test substance should be used (e.g., injection, oral paste, through feed, pour-on solution).
- Concentrations of the substance need to be verified in all dung and soil samples using appropriate extraction and analytical techniques.
- It is advised to follow the same principles for extraction as outlined in the EMA reflection paper on poorly extractable substances (EMA/CVMP/ERA/349254/2014).

Endpoints

- Endpoints should always include abundance of dung dwelling species and degradation rate of dung pats.
- Abundance of soil dwelling fauna associated with dung pats is included if effects can be expected based on laboratory results.
- Dung pats for the assessment of faunal abundance are either (a) recovered simultaneously one week after placing them in the field, after which emerging insects are collected from them in the laboratory, or alternatively, (b) the dung is left in the field and *in situ* traps are used to regularly collect emerging insects.
- The degradation of dung pats is measured as loss of mass (preferably organic matter) over a time span reliant on the degradation rate in the experimentation area.
- Pitfall traps baited with manure must be placed in the field during the experiments in order to monitor the natural activity of dung insect species in the experimentation area.
- When soil fauna is included, soil samples from below the dung pats are taken for analysis of earthworms and/or micro-arthropods using established ISO methods.

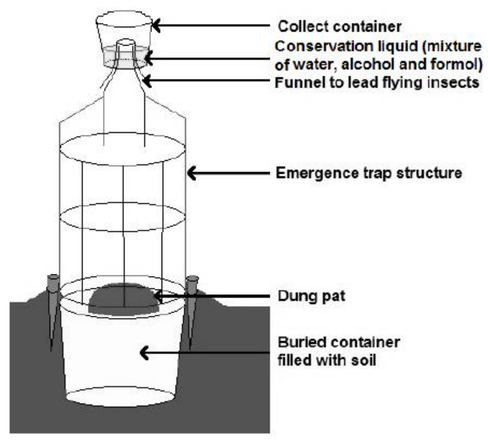

Figure 2. Example on an on-site emergence trap (reproduced from Tixier et al. (2014) ET&C 35(8): 1953-1958.

Figure 3. Field experiment conducted near Wageningen in the Netherlands. Dung pats are placed under cages in order to avoid disturbance by birds and other animals including domestic animals. The white plates seen between the cages are dung-baited pitfall traps for monitoring the presence and relative abundance of the natural dung insect fauna in the experimentation area during the course of the field experiment.

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