Standardisation of dung organism tests: Update of the work of DOTTS

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Background

According to testing requirements for veterinary medicines as agreed-on by VICH initiative (International Cooperation on Harmonisation of Technical Requirements for Registration of Veterinary Products) (2005) the potential effects of these pharmaceuticals on dung organisms have to be assessed. However, since there are no internationally recognised guidelines for laboratory testing for effects of veterinary medicines on dung flies and dung beetles, the Dung Organism Toxicity Testing Standardisation (DOTTS) Group developed a draft guideline for the testing of the flies *Scathophaga stercoraria* and *Musca autumnalis*. In 2005, the draft was ring-tested by members of the DOTTS group using ivermectin as a model substance. First results of this ringtest are presented. In addition, proposals are made to modify the existing draft guideline in the light of the experiences gained in the ringtest. The modified draft has been transformed into OECD format and is currently evaluated by the member states. In this presentation an overview of the activities of this SETAC advisory group is given, highlighting the importance of voluntary work for the further progress on the scientific as well as the applied level of ecotoxicology.

Performance of the ringtest with dung flies

The ringtest currently running follows the draft protocol and is characterised as follows:

Participants:	7 European contract laboratories
Schedule:	Autumn 2005 – Spring 2006
Test plan:	Performance: two runs with two fly species and one drug
Species:	First *S. stercoraria*, later *M. autumnalis*
Model substance:	Ivermectin (technical)
Concentrations:	0.64, 2.0, 6.4, 20.2, 64.0 µg ivermectin/kg wet weight (ww)

Description of the test with dung flies

A standardised bioassay procedure for *Musca autumnalis* De Geer (Diptera: Muscidae), and *Scathophaga stercoraria* L. (Diptera: Scathophagidae) has originally been proposed by J. Hughes (Inveresk). A slightly modified version is used as the basis of the ringtest. Its main properties are as follows:

Name: Determination of Developmental Toxicity of a Test Chemical to Dung Flies
Species: *Musca autumnalis* or *Scathophaga stercoraria (Fig. 1 + 2);* 10 larvae (less than 12 hours old; *M. autumnalis*) or 10 eggs (*S. stercoraria*) per vessel
Substrate: Fresh cattle dung; dried, grounded and re-moistened (moisture: 65 – 70%) dung is also possible. Characterization: moisture, pH and Nitrogen content acc. to ISO methods.
Vessels: Glass or plastic beakers (250 – 300 mL; diameter ca. 5 cm and height ca. 11 cm) filled with 100 g (w.w.) dung.
Conditions: Temperature: 26 ± 2°C (*M. autumnalis*), 20 ± 2°C (*S. stercoraria*); light cycle of 16 h light and 8 h dark
Duration: Termination 5 days after emergence of the last adult in the control
Parameter: Number of hatched larvae (if started with eggs), daily recording of emergent adults; morphological abnormalities
Residue analysis: Not required routinely
Design: Range-Finder; NOEC, ECx (24 – 30 vessels), limit test
Validity criterion: Control emergence > 50% (*M. autumnalis*) or 70% (*S. stercoraria*)
Reference substance: Ivermectin (details fixed after the ringtest)
Experience: Some tests performed (but few results published) for the registration of veterinary drugs
Comments: Other species like *M. vetustissima* are possible. This fly is found throughout Australia and is closely related to the 'narrow fronzed' strain of *M. sorbens*, which is widespread in south-east Asia.

This test is designed to estimate the developmental toxicity of a test chemical to the dung dwelling life stages of dung-dependent dipteran species. A positive control and negative controls (water and, if required, solvent) are included as comparisons. *M. autumnalis* and *S. stercoraria* are considered to be suitable indicator species for estimating the developmental toxicity of parasiticides on dung dependant Diptera for the following main reasons: Collectively, the species cover a wide geographic range. *M. autumnalis* and *S. stercoraria* are widespread in Europe, Asia, Africa and North America. Both species are multi-voltine, do not undergo obligate diapause and are easy to culture. They have a short life-cycle which makes it possible to determine effects on development and survival in the laboratory.

Fig. 1: *Scathophaga stercoraria*

Fig. 2: *Musca autumnalis*

Results of the ring tests dung flies

In total 11 definitive tests (7 with *S. stercoraria* and 4 with *M. autumnalis*) plus two range-finding tests were performed so far. The results (EC50, NOEC and LOEC values in µg/kg ww) of seven valid definitive tests are shown in Table 1. With one exception (a test with a "wild" strain) all test results with S. stercoraria were close to each other. As an example, the effect of ivermectin on the developmental rate of *S. stercoraria* is shown in Fig. 3. There is a clear influence of the test substance on the emergence time of this species (but not on *M. autumnalis*).

Tab. 1: Results of laboratory tests with two species of dung flies and the veterinary parasiticide ivermectin and two dung types (all data given in µg a.s./kg dung ww).

Code	Dung	Species	EC50 (emergence)	NOEC (emergence)	NOEC (develop. rate)
ECT1	fresh	*S. sterc .*	10.2	4.0	0.25
ECT2	fresh	*S. sterc .*	14.3	6.4	0.64
ECT3	dried	*S. sterc .*	25.7	16.0	1.00
GAB1	fresh	*S. sterc .*	21.5	6.4	0.64
COV1	fresh	*S. sterc .*	67.5	20.2	2.00
ECT4	fresh	*M. autu .*	4.0	1.11	> 30.0
IBA1	fresh	*M. autu .*	7.7	3.33	> 30.0

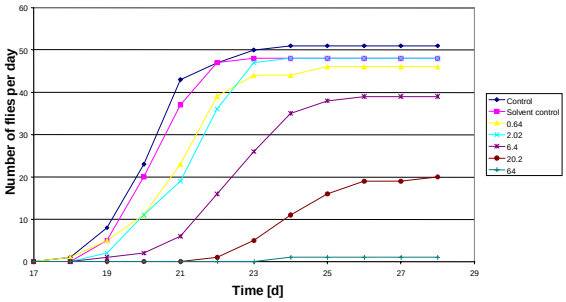

Fig. 3: Effect of ivermectin on the developmental rate of *S. stercoraria*

In comparison to other dung organisms, both species can be cultured very easily. Commercial sources exist for both species. No problems occurred with delivery of the insects (information and help on handling and breeding is available from both sources). No obvious problems occurred when using the test protocol (version November 2005). However, some improvements were identified and have been incorporated in the new version (March 2006). Due to their ecological differences both species will be recommended in the draft guideline.

The results of the ringtest are in good agreement with other studies with ivermectin. Results from a Canadian working group (Floate & Coghlin) using the DOTTS protocol but an ivermectin formulation support the findings reported here. The EC50 (emergence) for *S. stercoraria*, determined here as 10.2 – 25.7 µg/kg ww, was estimated as 15 µg/kg ww. by the Canadians. Basically, the same can be said about the results from the (few) tests with *M. autumnalis*, in which an EC50 (emergence) was determined as 4.0 – 7.7 µg/kg ww in the ringtest, while in a GLP test performed by Huntingdon (but with a formulation) the EC50 (emergence) was 5.65 µg/kg. It seems that this species is slightly more sensitive to ivermectin than *S. stercoraria*.

Conclusion and Outlook

Despite the limited information (e.g. only one substance has been tested in the ring test) it is recommended to standardise the dung fly test according to the current version of the draft guideline (version March 2006) based on the reproducible data obtained.

In addition, two further draft guidelines, covering the beetle species *Onthophagus taurus* and *Aphodius constans* are in preparation. According to the current work programme, ring-testing with these species will start later in 2006.

Contact

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