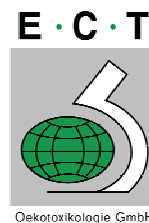


Development of a laboratory test for the investigation of the effects of veterinary pharmaceuticals on dung beetles



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Introduction

Various investigations have shown that antiparasitical drugs can have strong side effects on organisms living in or from dung (e.g. Edwards et al 2001; Lumaret & Errouissi 2002). Therefore, according to the requirements of the European Union as laid down in the EMEA/CVMP-guidance paper these substances have to be tested for dung insect toxicity (EMEA 1998). However, no standardised test guideline is available up to now. Therefore, the German Federal Environmental Agency (Umweltbundesamt (UBA)) started a project entitled „Development of a standardised laboratory test with dung beetles for testing the ecotoxicity of veterinary drugs“, which is performed in close co-operation between ECT GmbH and the University of Montpellier.

The dung beetle species *Aphodius constans* will be used for the new test system. In detail, the following work steps are planned to be done:

- development of a cultivation method;
- performance of laboratory tests with five antiparasitics spiked to the dung as well as tests with three antiparasitics in dung coming from treated cattle;
- clarification of important test details (especially the homogenised inclusion of chemicals to dung substrate);
- formulation of a draft test guideline according to OECD standards.

Experiences gained during the development of a test with dung beetles introduced to Australia (*Onthophagus taurus*, *Euoniticellus fulvus*) will be taken into consideration (Wardhaugh 2002). The work described here is done as part of the activities of the SETAC-affiliated working group DOTTS (Dung Organism Toxicity Test Standardisation).

The Test Species

The dung beetle *Aphodius constans* (Coleoptera: Aphodiidae) was selected as test species due to the following reasons (Figures 1, 2 and 3):

- it is widely distributed in Europe (e.g. in Southern France, it is very common);
- its entire egg, larval and pupal development takes place in dung;
- the beetles can relatively easily be reared in the laboratory; e.g. due to their small size (4.5 – 6 mm), the ability to use different dung origins (cattle, horse, sheep) and the short developmental time;
- several studies testing the effects of antiparasitics on these beetles have already been performed.

Figure 1: Larvae and pupal stage of *Aphodius constans*



Figure 2: Adult beetle *Aphodius constans*



Figure 3: Adult beetle on a dung pat



Figure 4: Typical habitat of *Aphodius constans* (Southern France)



The Test System

The design of the test system to be developed will follow as much as possible existing standard methods which are used for the assessment of other chemicals like pesticides (including GLP rules (Good Laboratory Practice)). The main characteristics are summarised in the following table:

Name:	Chronic toxicity to dung beetle larvae
Guideline:	OECD (under development)
Species:	<i>Aphodius constans</i> (10 larvae per vessel)
Substrate:	Artificial soil or field soil (including vermiculite) plus field collected cattle dung (moisture: 65 – 70%) (4 cm layer in small glass vessels)
Conditions:	Temperature 20 ± 2°C
Duration:	Six weeks
Parameter:	Number of larvae, pupae and adults; biomass, size of adults, behaviour
Residue analysis:	Not required routinely
Design:	Range-Finder; NOEC, ECx (24 – 30 vessels), limit test
Validity criterion:	Minimum number of emerging adults: > 80%
Reference substance:	Ivermectin
Experience:	Developed for veterinary drugs; several compounds have been tested with beetles collected in the wild

The performance of this test will be documented in digital film format. As part of the test development process, the nominal concentrations of one test compound (Ivermectin) will be confirmed analytically. In addition, the homogenous distribution of test substance spiked to the dung will be optimised by using colour tracer and/or radiolabelled compounds.

Outlook

It is expected that within the next two years a draft test guideline will be available. In the meantime, tests with spiked dung (e.g. Ivermectin, Moxidectin, a benzimidazole, a pyrethroid and an organo-phosphate) and naturally treated dung will be performed. Any progress will regularly be presented via the DOTTS group.

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