DOTTS – NEWSLETTER

No. 4 March 2004

Dear colleagues,

As announced in NEWSLETTER No. 3 this issue will mainly cover the ringtest(s) to be performed in the immediate future. Therefore, Boris Rosenkranz and myself tried to summarise the information on this topic in this issue, i.e. mainly by

- putting together the former resolutions of DOTTS concerning the performance of ringtests (e.g. from the first two "newsletters" = meeting reports);
- attaching the newest protocol versions of the "ready" tests;
- summarising the discussions on the fly test protocol;
- compiling the information from the general e-mail discussions within the last weeks;
- and, finally, proposing a schedule how to proceed.

In this context I may remind you that the test methods on dung organisms have been accepted by the National Co-ordinators of the OECD Testing programme. While this is a big encouragement for DOTTS it does also mean that we have to deliver in the foreseeable future the respective draft methods. Actually, OECD representative Jukka Ahtiainen was already asking last week how progress is and whether we can send him some "working drafts".

Again, as said in the last NEWSLETTE, please do not hesitate to send me your comments, critics, information … it will appear in the next issue!

Best regards,

Jörg Römbke

P.S. The e-mails for Glyn Vail came back as “undeliverable”. Does somebody have a more recent e-mail address? Thank you!

1. Resolutions of DOTTS concerning the performance of ringtests

1.1 Huntingdon (2002):

Several participants mentioned in their talks or the contributions to the discussion that ring-testing of any new method is necessary. There was also an agreement that the fly protocols are so elaborated that ring-testing will be the next step while more experience is needed for the beetle tests. From a practical point of view details like the availability of test chemicals were discussed.

1.2 Hamburg (2003):

After some internal discussion about the draft standard fly test protocol within the group a proposal made by Boris and Keith was taken up and decisions concerning the ring-test with flies were agreed on. According to the minutes the plan was as follows (in Italics: citation from the Hamburg minutes; in normal text: the status today):

At the moment three species are proposed for testing (Musca autumnalis (EU pest species and dung degrader), Scatophaga stercoraria (dung species) and Musca vetustissima. (Australian species). It is unlikely that Australian species could be imported to EU for testing, and vice versa, but it should be possible to have a single study protocol, with optional species. The following labs agreed to test one species: IRI, GAB, RCC, IBACON, Kevin Floate (Australian sp. only), HLS, ECOTOX, CSL, ECT, Covance.

According to Boris latest mail dated February 24, 2004 the following laboratories (given in alphabetical order) agreed to perform the test with one species: A&A Canada, CSL, ECT, GAB, HSL, Ibacon, IRI, RCC and Springborn. ECOTOX will not participate.

It was proposed to generate a limited data set on Musca autamnalis, and Scatophagus to determine the most appropriate species for wider ring testing. (IBACON, IRI, GAB, HLS). Based on the outcome of this work a single species will then be selected for the whole group to test. Preliminary work should be completed within 6 months, with the objective of starting the ring testing with the wider group. Proposed Ivermectin and moxydectin formulations will be used. Ibacon will buy a sample of each and distribute to the participating

labs, so all preliminary work is conducted on the same samples.
Action: Boris Rosenkranz to purchase samples and distribute.
Due to problems getting the test substances the whole process was delayed. However, Boris got ivermectin as well as moxydectin formulations early this year. THANKS TO MERIAL AND FORT DODGE! Within the next days the substances (first moxydectin, second ivermectin) will be distributed among the laboratories listed above (if someone new is interested to participate on the ring test, you are of course welcomed). Ibacon will carry out a pre-test with Ivermectin. Bruce and Boris will clarify whether a propylene glycol : glycerol formal vehicle is needed for dung fauna studies, and whether this vehicle can cause toxic effects. The four institutions performing the range-finding tests will start already at end of March. Results of these tests are expected for moxydectin at the end of April and for ivermectin in late May. The main test with the nine laboratories will be done in the second half of 2004.

In addition, information on the process of method standardisation was added to the Hamburg minutes. In the meantime, a new report was published by OECD which probably will form the basis of our own activities:

OECD (2003): Draft Guidance Document on the Validation and International Acceptance of New or Updated Test Methods for Hazard Assessment. OECD Environment, Health and Safety Publications, Series on Testing and Assessment No. 34

2. Test protocol versions

Attached to this mail are the following test protocols:

- Draft Protocol: Determination of Acute Toxicity of a Test Chemical to Dung Flies.
- Laboratory Culture Method for *Musca autumnalis* De Geer (Diptera: Muscidae)
- Working paper on protocol for rearing and testing *Onthophagus taurus* and/or *Euonicellus fulvus* – for comment, discussion and revision

Please note that there is no ringtest planned for the beetle test due to the fact that resources are not sufficient to perform fly and beetle ringtests at the same time. In addition it is probably more efficient to wait until an

Aphodius-protocol is available. However, work with this test is still on-going at Huntingdon. For a recent description have a look at the ENVIRPHARMA homepage where Katie presented a poster (www.envirpharma.org).

3. Summary of the information on the fly test protocol

Boris compiled the information concerning the draft fly test protocol with many comments from several colleagues (see below in different colours and in italics). This is in particular interesting for people not very familiar with this test. Actually, it is not clear to the very last detail whether all remarks have been built in – but anyway, the draft test protocol is a fine basis for the ringtest. Since these issues can be checked while performing the range-finder test, there is good chance to proceed quickly to the definitive test.

4. Compilation of the information from the general e-mail discussions within the last weeks

Since the beginning of the year (and after receiving word that the test substances are finally available), a discussion started on how to secure an intensive and reproducible exposure of the test organisms to the test substance. Issues like the homogenous distribution, the type of dung (fresh or grounded) and so on were raised, mainly due to the fact that the proposed test substance ivermectin is highly toxic at very low concentrations – which means that it is difficult to secure a homogenous distribution of this very small amount to be mixed in the dung substrate. The original questions as raised by Boris and myself were distributed via e-mail on January 30, 2004 (text in italics; due to the fact that some new members joined DOTTS only recently).

After a long quiet time, some agreement on the test performance is necessary before we can start the dung fly ring testing. During the efforts to get some test material, I had some discussions and helpful notes from the industry, which we should include in our draft protocol. One point are the nominal concentrations of the range finding test. Larry Parker of Fort Dodge pointed out that PPB is probably a more relevant level than PPM. This align with preliminary results testing dung flies and beetles using ivermectin, where the NOEC and EC50 was in a range of 25-100 mcg/kg dung. Another comment concerns the procedure, if organic solvents are used in spiking faeces. One

hour is not sufficient for evaporation. When spiking with acetone solutions, it would be better to flatten the faeces and dry overnight, then reconstitute with water. Otherwise control mortality is unacceptable. However, if an evaporation time of more than 8 hours is necessary for flattened dung in order to be sure that the solvent is gone, the dung itself will have changed its properties.

Furthermore we need an arrangement, how we spike the dung. The background is a discussion with Bruce Halley of Merial. We came to the conclusion that 200 mcL of a ivermectin suspension is enough for 100g dung (see attached sheet). When we do this with fresh dung, I see a problem since it is impossible to distribute this drop homogeneously in the dung. Taking the need of the use of a solvent (including the duration of its evaporation) and the small amount to be homogenized into consideration it should be discussed whether dry and ground dung is an alternative. In this case it is possible to use a higher amount of solvent in order to have a better distribution: long evaporation times are also not a problem since the dung was already dried beforehand.

As we want to establish a worst case scenario when spiking a test substance in the laboratory, such a procedure would be the opposite toa "field relevant" test in which the test substance is applied to, e.g., cattle and afterwards the treated (fresh) dung is tested. Both procedures are currently used as part of the development of an Aphodius test system. In case of water soluble test substances we can apply our test material when adjusting the water content in order to meet the test species needs.

After getting many different opinions and a nice discussion (thanks to Dave, Jackie, Jörn, Keith, Kevin and Peter!) Boris and myself tried to compile the information provided as follows (again, given in italics):

As has been seen within the last weeks, there are still discussions for the procedure of the draft protocol. When we know which fly shall be used in the ring test, we can polish some issues and write a protocol only for one species. However, the following issues can be fixed:

- *the target amounts for the ring test can be changed to PPB in the case of ivermectin and other highly toxic substances;*

- *a mixing time of 10 min is okay for the homogeneous distribution of the test*
  item in spiked dung.
- *an unresolved issue is the evaporation time when using a solvent like Acetone, but it seems that an increased period of 4 – 8 hours is recommendable (any drying of the dung during this time has to be avoided by re-moistening);*
- *the validity criterion of emerged adults in the control, set to 70% so far, might be too high (50% was also proposed). It might be appropriate to postpone this issue until the results of the ringtest are available;*
- *the use of dried ground dung (in addition to fresh dung tested after application of the test substance to animals) in this ringtest has been rejected by the group. However, the question of standardisation has to be taken up later on. Further data on the homogenous distribution of test substances in dung are generated currently at IRI and ECT, using radio-labelled material as well as colouring agents. In addition ecological differences between flies and beetles have to be taken into account.*

5. Proposal for a schedule how to proceed

The following schedule for the fly ringtest is proposed:

March 2004: Distribution of the test substances by IBACON (Boris)

April 2004: Range-finding ringtest (four laboratories)

June 2004: Evaluation of the test results and formulation of the definitive test

Until the end of 2004: Performance of the definitive test and assessment of the test results

Within 2005, the performance of the ringtest with dung beetles is planned.