



DOTTS – NEWSLETTER

No. 10

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Dear colleagues,

the focus of this newsletter will again be on the ring test with dung flies. On behalf of Boris Rosenkranz and me we would like to present you the results of several range-finder tests which were performed at IBACON and ECT within the last months. These tests were performed following the draft protocol agreed-on by a small ad-hoc working group at a meeting in Roßdorf last August (this draft “Fly Protocol – Version 3” was distributed within DOTTS as part of the last Newsletter (No. 8; September 27, 2004)). In the same Newsletter we announced that the protocol as well as the concentration range for the test compound Ivermectin will be checked in a pre-ringtest, i.e. by IBACON and ECT.

The original idea of this pre-test was to compare the sensitivity of the two potential fly test species (*Scatophaga stercoraria* and *Musca autumnalis*). Unfortunately, it was not possible to do so, since we could not get enough *Musca*-individuals to build-up own cultures or to perform individual tests. Therefore, the results presented here refer all to *S. stercoraria*.

In total, six tests have been performed in two blocks with slightly differing concentrations (Table 1). In the two ECT tests named S2 and S3, in one case fresh dung was used while in the other case the dung was pre-treated (dried, grounded and re-wetted) before spiking of the test substance. In all other respects the test performance was identical and did follow the draft protocol-version 3.

Table 1: Results of the pre-ringtest with *Scatophaga stercoraria*

All numbers given in µg/kg dry dung	Number of flies		Rate	Remark
	NOEC	EC50	NOEC	
DOTTS1 SC1 - ECT	> 31.6	> 31.6	1.00	

DOTTS1 SC1 - IBA	> 31.6	> 31.6	10.0	
DOTTS2 SC1 - ECT	4.00	16.0	4.00	
DOTTS3 SC1 - ECT	4.00	19.4	4.00	
DOTTS2 SC1 - IBA	No NOEC /EC50 calculated		4.00	Not valid
DOTTS3 SC1 - IBA	3.33	10.00	3.33	

Our experiences can be summarised as follows:

- The draft test protocol worked fine; therefore we recommend to use it as it is for further testing (one open issue was raised by Keith Wardhaugh who asked why measure light intensity – what do other people mean?).
- All tested animals were provided from the culture of Wolf Blanckenhorn (Zürich).
- Five out of six tests were valid (i.e. the emergence in the control was higher than 70%). In the sixth test this percentage was 55%.
- While in the first two tests the concentration range of Ivermectin was not optimal (the highest concentration of 31.6 µg/kg d.w. was too low), the range used in the four later tests covered the endpoints (0.25, 1.00, 4.00, 16.00, 32.00, 64.00 µg/kg dung d.w.).
- Therefore, for the definitive ringtest the following concentrations are recommended:
0.64, 2.0, 6.4, 20.2, 64.0 µg Ivermectin/kg d.w. (i.e. using a spacing factor of 10)
- In the four later tests, the test results are very similar (often only one concentration step apart on the case of NOECs and less than a factor of 2 deviating in those tests for which an EC50 could be calculated), even when including the non- valid test. However, it is not real clear why the first two tests showed smaller effects.
- No difference was found between the reaction of the flies in fresh dung and pre- treated (i.e. dried, grounded and re- wetted) dung – but this is just one data set. Further work is currently under way in order check the suitability of pre- treated dung.
- After a preliminary evaluation of literature, it seems that our results are comparable with other tests in which the effects of Ivermectin on *S. stercoraria* were studied (Strong & James 1993). In this paper, effects

were stronger than in our tests, but differences in study design, test substance, endpoint etc. may explain this fact.

Taking all our experiences into consideration we recommend starting the main ringtest, using *S. stercoraria*, the Draft Fly Protocol – Version 3 and five concentrations covering a range between 0.64 and 64.0 µg/kg d.w.

As mentioned above, originally we wanted to compare two fly species, but this was not possible. Our brief literature review did not reveal valid test data from *Musca* – so, if anybody tested the effects of Ivermectin on this species it would be extremely important to provide the group with these results a.s.a.p. in order to compare the sensitivity of these two species. If such data cannot be provided and considering that we already spent some time on the whole issue of getting *Musca*, the two of us recommend not to wait any longer but to start the definitive ringtest with *S. stercoraria*.

However, such a recommendation should firstly be discussed within DOTTS. So, please send me your opinion how to proceed until mid- April (April 17, 2005). Actually, we are in a hurry because OECD would like to discuss the situation concerning dung organism testing at its next meeting (end of April, start of May) – in any case before our next regular DOTTS meeting which is planned for the next SETAC-Europe conference (see below for details).

For dung beetles the development of two protocols is possible and, if two species should be covered, probably necessary (one with the tropical species *Onthophagus taurus*, the other with the temperate species *Aphodius constans*). Actually, nearly no progress has been made with the tropical species since culturing problems as well as the low demand for these animals impede all testing so far.

In the case of the test with *Aphodius constans*, the project sponsored by the German UBA is nearly finished. Five compounds have been tested several times according to a protocol which could be used as a starting point for ringtesting. Ivermectin was one of the compounds used. Despite the fact that the assessment of the test results is not finished yet it seems that the

sensitivity of this beetle species is in the same range as that of the dung fly *S. stercoraria*.

However, the test species *Aphodius constans* cannot be bred in a mass culture; thus any ringtest has to rely on animals which are taken from the field and then kept in the laboratory until they have laid eggs. The larvae hatching from these eggs can be used for testing. Again, this protocol (a draft according to OECD rules is currently written) has to be distributed and discussed within the DOTTS group before a recommendation concerning further progress can be made. According to the schedule of the UBA project a report will be written within the next two months. After approval by the Sponsor we will distribute the main results within DOTTS.

Within the last weeks, the several agencies engaged in the registration of pharmaceuticals as well as OECD have approached me asking for progress. A small status report, more or less giving the same information as provided in our Newsletters (including the latest version of the fly draft test protocol) has been sent to K. Grein (EMEA London), J. Riego-Sintes (ECB Ispra) and A. Gourmelon (OECD Paris). As soon as I have a feedback from these institutions I will inform you.

Finally, I would like to list some technical issues relevant for DOTTS members:

- The membership has nearly not changed, but due to changes in addresses a new list is attached to this Newsletter.
- The next DOTTS meeting will take place during the SETAC-Europe annual meeting to be held in Lille (France; May 22- 26, 2005). A room – named Goya) is booked for Wednesday (May 25) between 17.00 and 19.00. Please bring any material interesting for the group with you.
- As usually, the agenda will cover organisational issues as well as a short report about our activities in 2004, but clearly the most important point will be the fly ringtest.
- Be also aware that at the same conference there is a whole session dedicated to veterinary pharmaceuticals running (Wednesday (May 25)

between 08.30 and 12.20. Several members of DOTTS will present their work in talks and posters during this session.

- Last but not least: What is the opinion of the group concerning a closer affiliation with SETAC? Actually, I do not know many details what this would mean in practice (discussions about this topic will run in Lille too), but I would like to know your opinion about such a step.

As always, please do not hesitate to contact me if you have any comments, questions etc.

Best regards,
Jörg Römbke