

Veterinary Antibiotics in Terrestrial Plant Tests – Effects of a more realistic exposure way via manure

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1. Introduction

Effects on the environment caused by veterinary products are assessed according to manuals of the EMA and VICH. In Phase II of the environmental risk assessment effects on terrestrial plants are tested since residues of active substances can reach agricultural areas because manure and slurry from treated animals are used as fertilizer. Effects on terrestrial plants are examined according to the OECD guideline 208 “Seedling Emergence and Seedling Growth Test” [1]. However, this approach often results in strong effects, leading to an unacceptable risk to terrestrial plants. One possibility to refine the risk is to conduct a modified approach considering a more realistic application form (manure enriched soil). So far no concept exists for performance, of such modified test design.

In the frame of a research project initiated and funded by the German Federal Environmental Agency (UBA), a special terrestrial plant test for veterinary pharmaceuticals – especially antibiotics – with a more realistic exposure scenario via manure application was developed.

The research project comprehends: i) development of methods of preparation, acclimatization, incubation, and application of manure in a plant test, ii) tests investigating necessary technical background (e.g. suitable plant species, suitable manure concentration), iii) tests according to the OECD 208 standard test design and modified test designs, considering an application of the test substance via manure. To ensure a significant evidence of the studies regarding universal validity, main tests were conducted with six plant species and eight replicates. The studies were conducted with pig and cattle manure and two representative veterinary antibiotics. The test design considers additional effects of manure to the test substance (e.g. adsorption) as well as transformation/metabolization of the test substance in manure by investigating the influence of i) the duration of anaerobic incubation in manure, ii) the way of application, and iii) the incubation conditions (anaerobic/aerobic) on the effects of the antibiotics in plant tests.

2. Materials and methods

The techniques for manure preparation, anaerob acclimatization (according to the new EMA guideline on determining the fate of veterinary medicinal products in manure [2] under anaerobic conditions at $20 \pm 2^\circ\text{C}$ in the dark for at least 21 days), application of the test substance into the manure, anaerob incubation of the manure and application of the manure into the test soil were developed and checked successfully.

Manure was sampled from manure storage tanks. Prior to collection the liquid manure was homogenized by mixing in the respective manure tank. Liquid manure was collected from the tank and filled into containers up to approximately $\frac{3}{4}$ of maximum container volume. Containers were closed but allowed gas to expand. Prior to further processing manure was stored at 8°C for a maximum of two months under anaerobic conditions. Prior to the start of the acclimation period, the dry matter content of the manure was adjusted to standardized values. The recommended dry matter content in pig and cattle manure is 5% and $10\% \pm 1\%$, respectively [3,4]. Thereafter, manure was processed using a mixer (a food processor) in order to obtain a fairly stable phase. To prevent introduction of oxygen, mixing was conducted in an extensively sealed beaker flooded with nitrogen. Thereafter, the dry matter content was checked. Subsequently, the processed manure was directly filled into the acclimation container which was used for the acclimation. The acclimation containers were filled up to approximately $\frac{3}{4}$ - $\frac{4}{5}$ of maximum container volume. After flooding the container with nitrogen, it was closed but allowed gas to expand. The acclimation was carried out for 21 days at 20°C in the dark. Manure was characterized by key parameters.

Pretests verified that a maximum manure concentration representing 85 kg N/ha (1/2 maximum amount allowed per year in Europe) should not be exceeded to ensure an unimpaired germination, emergence and post-emergence survival.

The quantity of test item required to obtain the desired concentrations was mixed with a portion of manure (22 g fresh mass manure per 1 kg test substrate) in 100 – 150 glass beakers. The operations were carried out in a way that the sludge has minimal contact with oxygen. The spiked manure was applied directly to the soil or incubated under anaerobic conditions at $20 \pm 2^\circ\text{C}$ in the dark for half-maximum and maximum storage duration, corresponding to 26.5 and 53 days (pig) and 45 and 90 days (cattle), respectively. The spiked manure was added to a portion of test soil in a ratio of 22 g fresh mass manure to 200 g dry mass soil, and mixed thoroughly. The pre-mixture was added to the rest of test soil (800 g dry mass) and mixed thoroughly. The final mixture was placed into the test containers and the respective amount of water needed to adjust the test substrate to 40 % water holding capacity was added.

The plant tests were conducted according to the OECD 208 guideline “Seedling Emergence and Seedling Growth Test”. Modifications were only applied on the test item application. Plant species applied were *A. cepa* (onion), *A. sativa* (oat), *S. lycopersicum* (tomato), *B. napus* (rape), *P. vulgaris* (common bean), *S. alba* (mustard), and *T. pratense* (red clover)), representing monocotyledons, dicotyledons, and legumes. Concentration-response tests with five test concentrations were conducted according to i) the standard test design without manure in four replicates and ii) modified with manure application with a standard control (without manure), manure control, and five test concentrations in eight replicates. Emergence rate, post-emergence survival, shoot length and shoot fresh mass were determined at least 14 days, but not longer than 21 days after 50 % of the seedlings have emerged in the manure control.

3. Results and discussion

3.1. Techniques

The techniques for manure preparation, anaerobic acclimatization (according to the new EMA guideline “transformation in liquid manure”), application of the test substance into the manure, anaerobic incubation of the manure and application of the manure into the test soil were developed and checked successfully.

3.2. Influence of application via anaerobic manure

Application via manure itself has no influence on the effect of the antibiotics on the plants vitality. EC values from tests according to the standard test design were in a comparable order of magnitude when compared with tests where the antibiotics were applied via manure without incubation.

Anaerobic incubation even after half-maximum storage duration (26.5 days for pig manure and 45 days for cattle manure) decreases effects of the antibiotics seriously.

The variance of the replicate results - and therefore the strictness of the calculated EC values – in the modified test design was comparable to the that in the standard test design according to OECD 208.

4. Conclusions

The techniques and methodology of the modified test design are appropriate to obtain reliable results for veterinary pharmaceuticals under more realistic conditions.

5. References

- [1] OECD 208 (19 July 2006): OECD guideline for testing of chemicals – Terrestrial Plants Test: Seedling Emergence and Seedling Growth Test.
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