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Technical Protocol: Transformation of Biocides in Liquid Manures

Summary

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Technical Protocol: Transformation of Biocides in Liquid Manures

Summary

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Summary and conclusions

Innovative concept of laboratory testing

Within the current authorization procedure "TNSG for data requirements for active substances and biocidal products (2000)", biocides applied for disinfection purposes or insect control in animal houses are to be studied on ultimate biodegradability under anaerobic conditions. Due to the lack of an established simulation test in liquid manures, the biocidal guidance documents recommend a screening test according to OECD 311 using digested sludge of a municipal wastewater treatment plant under optimized boundary conditions of an anaerobic digester. From the scientific perspective, therefore, fate and behavior of biocides can be hardly transferred from sludge in a digester to liquid bovine or pig manures in a tank because of significantly diverging boundary conditions. Therefore, the Biocide Project has been targeted at the development of a real simulation test on the transformation of biocides in bovine and pig manures as well as on fate in manured soils. Thus, these research activities consequently advanced the technical protocol already developed for veterinary medicinal products (VMP) within the frame of the Manure Project.

Transformation of biocides in reference manures

This technical protocol focused on the reproducible sampling of excrements of cattle and pigs individually kept in experimental stables under standard feeding conditions. These excrements were thoroughly matrix characterized by the following parameters: dry substance content, total organic carbon, pH value, redox potential, dissolved oxygen content, ammonium and total nitrogen content as well as biological oxygen demand. The excrement samples were conditioned for 21 days to reach strictly anaerobic conditions and to transform readily degradable manure inherent substances. Then, reference-manure samples were prepared by adding tap water to adjust typical dry substance contents of 10 % for bovine manure and 5 % for pig manure.

These reference-manure samples were used for transformation tests of two biocides, namely imazalil and cyanamide, applied for disinfection or insect control in animal houses and liquid manures, respectively. Both test substances were spiked as ^{14}C -labeled radiotracers to the reference-manure samples. The batches were then incubated in the dark at $20 \pm 1\text{ }^{\circ}\text{C}$ up to 100 or 177 days. Different extraction techniques, i.e., direct ethyl acetate treatment and lyophilization followed by methanol extraction, were applied for imazalil and cyanamide spiked manure samples. As shown by mass balances and metabolite screening, the extractable fractions containing only the parent compound dominated the transformation tests on ^{14}C -imazalil in bovine and pig reference-manures revealed by $\text{DT}_{50} > 177$ days. Thus, the concentration of this imidazole biocide did not considerably decrease within the 177-day incubation periods. Mineralization, extractable and non-extractable residues amounted to 0.1 %, 90 % and 15 % in bovine and 0.1 %, 77 % and 21 % in pig reference

manure.

Within the 100-day transformation test on ^{14}C -cyanamide in bovine reference manure, mineralization, extractable and non-extractable residues finally reached 16 %, 30 % and 35 %, respectively. DT_{50} values were 2 days. Decreasing mass balances indicated furthermore the release of ^{14}C -methane out of ^{14}C -cyanamide under strictly methanogenic conditions. Corresponding data within the 175-day transformation test in pig reference manure were 16 %, 51 % and 18 %. DT_{50} values were also 2 days.

Fate monitoring in manured soil

On the basis of the long-term transformation tests in reference manures, test-manure samples containing 7-day aged ^{14}C -imazalil or ^{14}C -cyanamide residues were prepared. Those were mixed with soil samples in order to monitor sorption and transformation in manured soils. In comparison to the conventional standard application of the test substance, the impact of the manure matrices could be clearly shown. Thus, K_{OC} values of both test substances were slightly reduced after test-manure application, however, mobility tendencies did not considerably change. Hence, ^{14}C -imazalil was classified as slightly mobile, while ^{14}C -cyanamide was moderately mobile.

The transformation of both test substances in soil was affected by the test-manure application, too. After standard application of ^{14}C -imazalil, mineralization, extractable and non-extractable residues amounted to 10 %, 47 % and 36 %, resulting in the DT_{50} value was 83 days. In contrast, the metabolic dynamics were enhanced by bovine and pig test-manure application. Mineralization, extractable and non-extractable residues amounted to 6 %, 24 % and 65 % after bovine and 9 %, 26 % and 55 % after pig test-manure application. DT_{50} values were 29 and 48 days, respectively.

The transformation tests on ^{14}C -cyanamide were dominated by mineralization, particularly after standard application. After the 100-day incubation period, mineralization, extractable and non-extractable residues amounted to 86 %, < 0.1 % and 3 %, respectively. After test-manure application, considerable amounts of non-extractable residues were also formed. At the end of the incubation periods, however, ^{14}C -carbon dioxide was released out of these fractions, too. Amounts of mineralization, extractable and non-extractable residues were 38 %, 0.3 % and 28 % after bovine and 63 %, 0.2 % and 18 % after pig test-manure application.

Evaluation of the reference-manure concept

Following this concept, bovine and pig reference-manure samples can be introduced into reproducible laboratory testing on transformation of VMP and biocides in manures and on sorption and transformation in manured soils. The needed excrement samples are to be preferably taken in experimental stables of animal husbandry. Taking into account that, besides cattle, one group of fattened pigs under study within the Manure Project was kept in a conventional farm and mainly fed by potato refuse instead of barley or wheat, reproducible

excrement sampling should be possible in every stable so far the administration of VMP to the test animals and the application of biocides in the stable could be excluded. Furthermore, defined diets of cattle and pig nutrition may contribute to a minimum parameter variation of excrements.

On this basis, the transformation of VMP and biocides can be monitored in reference manures at defined dry substance contents within an appropriate frame of time and costs. This principle of practicability and feasibility cannot be realized when, instead of one reference manure, at least four different tank manures of one animal species were alternatively taken for laboratory testing. The latter approach would quadruplicate the experimental expenditures of the reference-manure concept. Further analytical expenditure would be necessary for additional matrix characterization tests and screening analyses of the tank manures for interfering VMP and biocide contaminants based on complex extraction and clean-up procedures as well as LC/MS/MS analyses. Following the reference-manure concept, those additional screening analyses are not necessary because the excrements to be taken are operationally free of any VMP and biocide contamination. Thus, this concept will save time and costs and, nevertheless, will gather an appropriate data pool for the environmental risk assessment of the authorization procedure of VMP and biocides. Due to those advantages, the reference-manure concept should be more likely acceptable for stakeholders at research institutions, industry and authorities.

Furthermore, the reference manure concept may initiate laboratory testing of sorption and transformation of VMP and biocides in manured soils. Thus, on the basis of transformation tests in reference-manures, test-manure samples, containing short-term aged residues of VMP or biocides, can be reproducibly prepared and used to simulate the manure application already under laboratory conditions. Numerous laboratory tests on sorption and transformation of VMP and biocides in manured soils emphasized the need of studying fate and behavior of those substances under the impact of the manure matrices in order to understand better complex concentration determining processes in soils under field conditions. Then, those latter aspects are relevant for the prospective evaluation of VMP and biocides in order to advance the environmental risk assessment.

The reference-manure concept has been developed by research activities performed within the Manure Project and the Biocide Project from 2005 to 2009. Due to this limited research period, the compiled data pool is inevitably limited but, it is the most comprehensive one until today resulting in a draft version of the first technical protocol. Since there is an increasing demand for transformation tests in liquid manures, the application of this technical protocol will contribute to gather further experiences and to advance these methodological approaches. There is further need of research because only bovine and pig liquid manures

have to be taken into account until today. Thus, there are definite lacks for transformation tests of VMP and biocides in poultry manures due to more different animal husbandry systems and more different manure compositions and storage conditions. There is the need of more harmonization of the experimental and analytical procedures that could be gained by interlaboratory tests resulting in technical guidance documents or guidelines.