WORKSHOP DOCUMENTATION

8TH INTER BALTIC BIOGAS AREANA

"Antibiotics in Manure and Digestate"

January 10th-11th, 2019

Weimar, Germany

Figure 1 – Group picture. Experts and participants from Germany, UK, Sweden and Switzerland at MFPA Weimar

Funded by:

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Organized by:

MFPA

Institute for Biogas Waste Management & Energy

Background and goal of the workshop

The use of antibiotics has become an important part of today's animal husbandry practices. Although the goal is to protect animal health, the use of such large amounts of antibiotics is already having the opposite effect in areas where large amounts are used.
The workshop was organized as part of a Thuringian project called ABIOTEC, which aims to improve practical analysis of antibiotics as well as manure treatment and utilization. The project represents a cooperation between local agricultural authorities, farmers and scientific institutions.
The workshop's aim was to explore measurement methods and challenges with respect to the use of veterinary antibiotics and reflect on strategies for dealing with the problems associated with their use.

TAKE HOME MESSAGE

- A large percentage of the veterinary antibiotics, which are used therapeutically and preventively in livestock, are released into the environment. There they often persist for long periods of time.
- The antibiotic compounds are transported from the agricultural areas to surface water and in some cases even ground water – thus posing a significant risk to human health.
- Even though some antibiotics are no longer detectable in the soil or water, they have often been degraded or transformed to other substances that have been proven to be equally as hazardous.
- The possible effects of antibiotics in the environment are clear because they are already being observed. However, the question of exactly which antibiotics and amounts (limit values) can be released into the environment before the effects are found is not clear.
- There are several areas in which further research is necessary in order to be able to assess the effects of veterinary antibiotics on the environment and consequently for human and animal health. Until this is achieved it is critical that mechanisms are put in place to monitor amounts and paths of antibiotics that are used in animal husbandry so that correlations can be made and solutions found.

FROM THE WORKSHOP…

Antibiotics in Manure and Digestate and their fate in the environment.

Prof. Alistair Boxall from the University of York kicked off the event as keynote speaker. He was able to give a quick – but nevertheless thorough – overview of the situation including the pathways of antibiotics into the environment, persistency, transport as well as potential for leaching (to groundwater) and effects on organisms.
He noted that studies have been done on the toxicity of the molecules, but that this form of risk assessment does not significantly reflect the actual impact. The danger of long-term exposure is resistance and later ineffectiveness of the antibiotics when they are needed.

The areas in which he sees research priorities are:

- Better understanding of manure storage and management practices
- Measurement of a broader range of active ingredients
- Methods and for measuring a broader range of manure matrices and conditions
- Further information on metabolites (degradation products) and other transformation products of commonly used veterinary antibiotics
- Better understanding of manure effects on persistence and transport

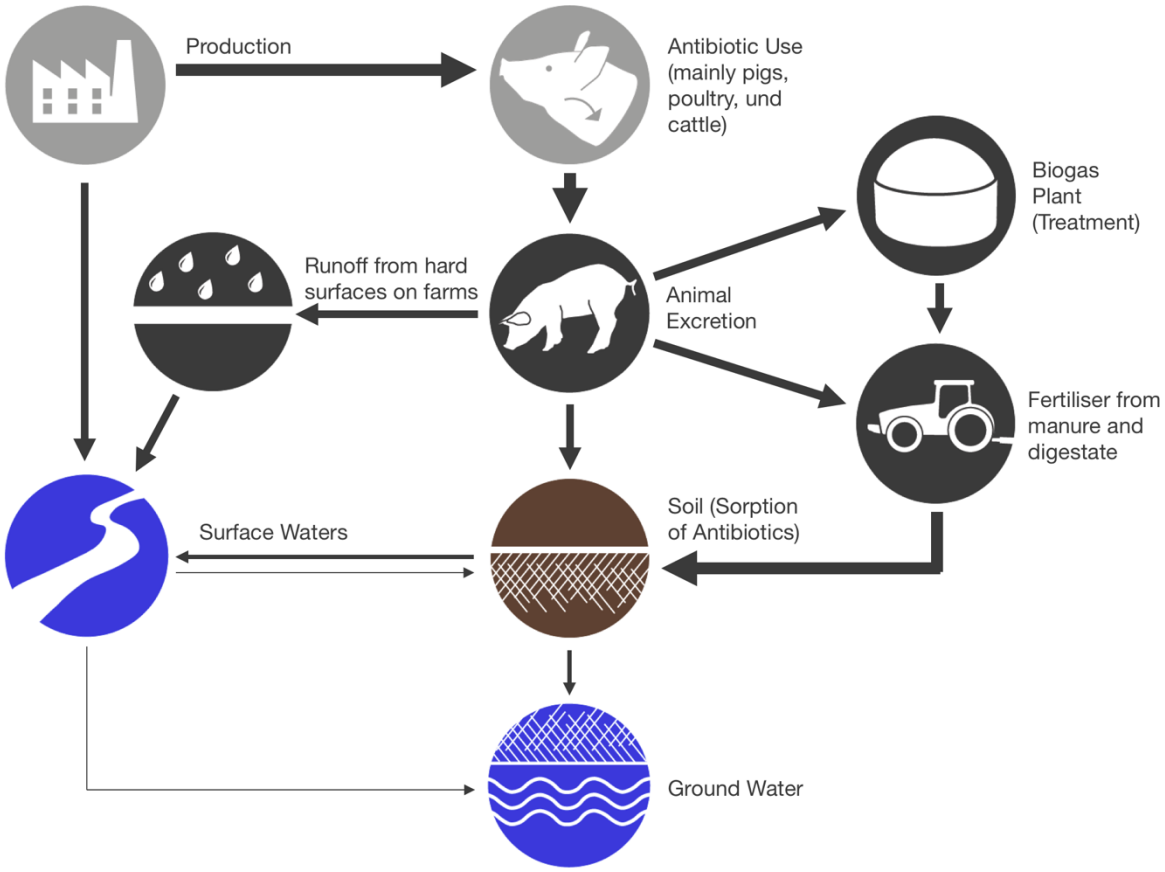

Figure 2 – ***Main pathways to the environment.*** *Based on: A.B.A. Boxall, "Contamination From the Agricultural Use of Growth Promoters and Medicines", Editor(s): Dominick A. Dellasala, Michael I. Goldstein, Encyclopedia of the Anthropocene, Elsevier, 2018, Pages 257-262 as well as Report UBA Ahting et al. 2018 "Recommendations for reducing micropollutants in waters" and Schönfeld, J. Konradi S. Berkner S. Westphal Settele K. UMID 2/2017 Artikel Antimikrobielle Resistenzen in der Umwelt – Gibt es Neues zum bekannten Phänomen? Links to online PDF's of the latter two sources can be found in the appendix.*

What affects the behaviour of antibiotics and their elimination and mobilization? (Excerpts from presentations of Dr. Astrid Spielmeyer[1] and Prof. Sören Thiele-Bruhn[2])

Often the disappearance of antibiotic compounds is seen as "degradation", but the sorption or metabolism/transformation of the compounds are usually the reason why they are no longer detected. Sorption does not necessarily mean that the compounds cannot be released again. Metabolites and transformation products can also have the same antibiotic properties as the parent compound or, in some cases, can transform back to the parent compound. It appears that the antibiotics can be bound in soil and create a long-term problem.

[1] Justus Liebig University Gießen, Institute of Food Chemistry and Food Biotechnology
[2] University of Trier, Soil Science Department

Some of the factors, which can affect sorption include:

- Treatment
  - Substrate mixtures (maize silage, for example, raised the potential for sorption)
  - Temperature (mesophilic, thermophilic conditions)
  - pH
  - Duration
- Manure itself – its particle size and composition – has an effect on the sorption properties of the soil that it is applied to
- Dry mass and dissolved organic matter concentrations (due to the origin of the manure)
  - Livestock Nutrition
  - Stable Management
  - Manure Storage
- Other compounds found in the soil, which "compete" for sorption, such as phosphates
- Soil properties
- Physical chemical properties of the pharmaceuticals

Measurement and analysis methods (Excerpts from presentations of Dr. Sebastian Zühlke[3], Dr. Marcel Nikolausz[4] and Frank Schütz[5])

Several of the speakers had extensive experience in the measurement of pharmaceuticals and other micro pollutants in manure, digestate and soil. They were able to give advice on proper measurement methods and possible explanations for variations in the results.

Highlights from the presentations:

- No results are better than false results. The risk of misinterpretation is not to underrate.
- Liquid chromatography–mass spectrometry (LC-MS) allows sensitive and selective detection of antibiotics in environmental samples.
- Only extractable compounds can be measured with LC-MS and other methods of measurement have shown that other amounts can be bound in the solid phase and are therefore not measurable with LC-MS. It is possible that these bound compounds could be released later.
- Suitable internal reference standards or standard addition method are needed for correct quantitation
- Important metabolites and their possible transformation into active compounds must be considered
- It would be practical for monitoring authorities to have a method that can detect a broader range of compounds, although it would clearly not be able to be carried out with the same amount of certainty, since the optimal measurement conditions vary for different substances. MFPA has been testing a method for thirteen antibiotic residues, which was developed by HU et al.[6]
- Stable isotope concepts can also be used to determine the presence of the compounds. They are typically more expensive, but are not distorted by effects, such as sorption.

[3] Technical University of Dortmund, Chemistry Department, Head of Laboratory
[4] Helmholtz Centre for Environmental Research - UFZ
[5] MFPA Weimar
[6] HU, Xian-Gang; Yi, Luo; ZHOU, Qi-Xing; XU, Lin (2008): Determination of Thirteen Antibiotics Residues in Manure by Solid Phase Extraction and High Performance Liquid Chromatography. In: Chinese Journal of Analytical Chemistry 36 (9), S. 1162–1166.

The stable isotope concepts were explained with the example of a different chemical (Benzene) and with anaerobic treatment. Using this method it was convincingly possible to demonstrate that carbon from the compound was broken down and used is methane / carbon dioxide production. This is a very interesting approach if applications were to be identified for antibiotic compounds.

Biotransformation of antibiotics – metabolites and transformation products (Excerpts from the presentation of Dr. *Stefan Achermann[7])*

One of the areas that requires further investigation is the transformation of the antibiotic compounds. They can only be traced if they are known. Stefan Achermann demonstrated the biotransformation process based on sulfonamide antibiotics, which the Swiss Federal Institute of Aquatic Science and Research (Eawag) has been researching in wastewater treatment plants. Since sulphonamide antibiotics are used in human and veterinary medicine, it is also relevant for the discussion on animal husbandry.

Figure 3 shows the complexity for just one compound. There are several different pathways for transformation and still many aspects, such as environmental and operational conditions, that can affect the pathway. Eawag has also done research on developing a pathway prediction system, which would be extremely helpful in the fate of antibiotics in the environment, but still requires a great amount of work considering the wide range of compounds and conditions.

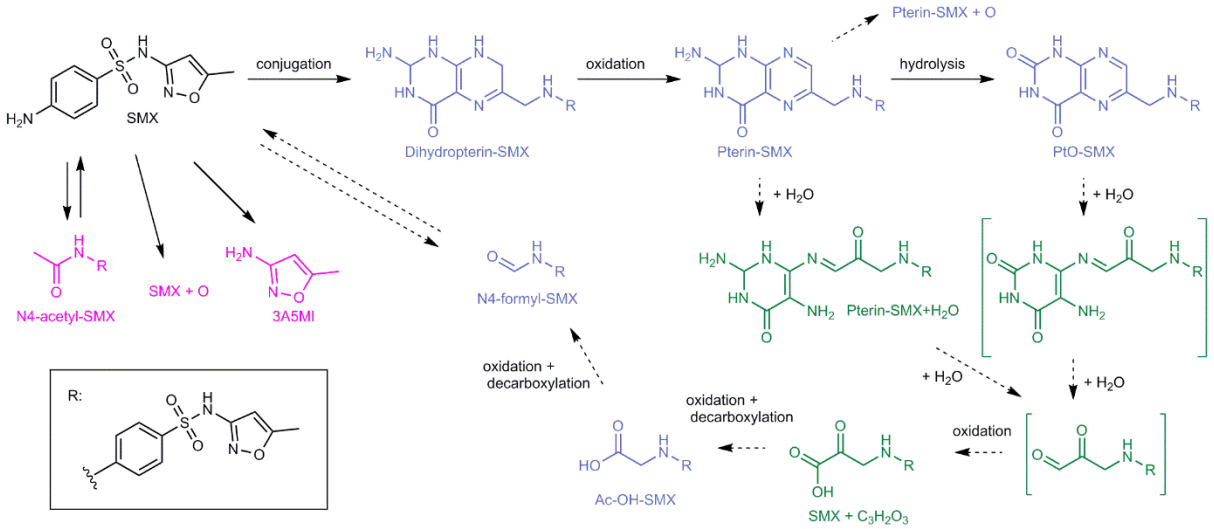

Figure 3 – ***Biotransformation of Sulfamethoxazole.*** *Reprinted with permission from Biotransformation of Sulfonamide Antibiotics in Activated Sludge: The Formation of Pterin-Conjugates Leads to Sustained Risk; Stefan Achermann, Valeria Bianco, Cresten B. Mansfeldt, Bernadette Vogler, Boris A. Kolvenbach, Philippe F.X. Corvini, and Kathrin Fenner; Environmental Science & Technology 2018 52 (11), 6265-6274. Copyright (2018) American Chemical Society.*

[7] Eawag: Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, Switzerland

Discussion / Brainstorming session

The following questions were the focus of the group discussions:

1. What treatment possibilities for manure to reduce antibiotics are promising?
2. What research and applied research projects are necessary to get deeper insight into antibiotics degradation processes, effects of degradation products in the environment?
3. Do we need (realistic) limit values for antibiotics in manure and environmental habitats?

What treatment possibilities for manure to reduce antibiotics are promising?

The group discussion on this topic was one of the most interesting, but due to the large amount of information still required it is not yet possible to make recommendations. It is often unknown whether the antibiotics are in the solid phase or liquid phase. This aspect is relevant for developing potential treatment methods to answer the question: Do we have to deal with the liquid phase or with the solids?

What research and applied research projects are necessary to get deeper insight into antibiotics degradation processes, effects of degradation products in the environment?

- Antibiotic Resistance
- Treatment possibilities and potential risks
    - Effects on resistance by spreading as fertiliser
    - Horizontal gene transfer, multi-resistancy due to mixtures from different origins
    - inhibition due to antibiotics affecting the bacteria needed for biological treatment
- Pre-treatment with fungi, oxidative enzymes
- Enrichment / isolation of antibiotic-degrading microbes
- Assessment of true biodegradability, not just elimination through adsorption and dilution

Do we need (realistic) limit values for antibiotics in manure and environmental habitats?

The short answer to this question was “yes”. The long answer is more complicated. It might be difficult to clearly define the lowest antibiotic concentration in the environment that still promotes resistant bacteria. In addition, antibiotics also directly affect aquatic and soil organisms at specific concentrations. Dr. Sabine Konradi from the German Environment Agency (Umweltbundesamt – UBA), Section Pharmaceuticals, informed that UBA recently published a background paper on “Antibiotics and Antibiotic Resistances in the Environment” (UBA 10/2018), presenting the research needs and outlining seven areas of actions. For precautionary reasons, the UBA recommends the inclusion of antibiotics into the water framework directive and the introduction of a threshold value for antibiotics for groundwater and soil. Another suggestion is to link the compulsory documentation of antibiotic use in livestock with the documentation of antibiotic agents consequently present in manure and pass this information on throughout manure transportation, input in biogas plants until application on fields. In this way mixtures of manures containing different spectra of antibiotics, for example manure from swine and poultry, could be avoided to reduce the risk of multi-resistant bacteria formation. A databank of information of antibiotic agents applied with manure to agricultural soil would allow targeted monitoring of specific antibiotics and resistance genes in agricultural soils and ground water over long periods of time. A list of references and links to articles from UBA can be found in the appendix as a good starting point for an overview of the subject matter. They also include project reports on establishment and ring tests of a standard test protocol on biotransformation in manure.

Figure 4 – Discussion in groups on questions selected by the participants

ACKNOWLEDGMENTS

The organizing group of Institute for Biogas, Waste Management and Energy along with MFPA Weimar would like to thank all of the speakers and participants for their contributions and ideas, our ABIOTEC partners as well as the TAB (Thüringen Aufbaubank) and the ELER program for the financial support.
We would also like to thank the IBBA supporting organizations, who were critical in establishing contacts to many of the scientists involved in the workshop and letting others know about the event.
More information on the IBBA, which also organizes events on other biogas related topics, can be found at www.ibbaworkshop.eu.

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Appendix – Literature for further reading

Project reports on degradation in manure

Uba Texte 80/2016 (Draft standardized test protocol see annex 1)
Harmonization of environmental exposure assessment for veterinary pharmaceuticals and biocides: ring test for validation of a draft test protocol for studies on transformation in manure. Thomas Junker, Jörg Römbke, Dieter Hennecke, Monika Herrchen, Rolf Alexander Düring, Sören Thiele-Bruhn, Maria Meinerling, Silke Fiebig, Ed Topp, Wolfgang Völkel
Link: https://www.umweltbundesamt.de/publikationen/harmonization-of-environmental-exposure-assessment-1

Uba Texte 79/2016
Harmonization of environmental exposure assessment for veterinary pharmaceuticals and biocides: Literature review of studies on occurrence and transformation of veterinary pharmaceuticals and biocides in manure.
Rolf-Alexander Düring, Manuel Wohde, Thomas Junker, Dieter Hennecke, Monika Herrchen, Sören Thiele-Bruhn
https://www.umweltbundesamt.de/publikationen/harmonization-of-environmental-exposure-assessment-0

UBA Texte | 78/2016
Harmonization of environmental exposure assessment for veterinary pharmaceuticals and biocides: Influence of different experimental set-ups on observed mineralization.
Monika Herrchen, Dieter Hennecke, Thomas Junker, Rolf Alexander Düring, Sören Thiele-Bruhn
https://www.umweltbundesamt.de/publikationen/harmonization-of-environmental-exposure-assessment

UBA Background papers:

Antibiotics and antibiotic resistances in the environment, UBA, 10/2018
German: https://www.umweltbundesamt.de/publikationen/antibiotika-antibiotikaresistenzen-in-der-umwelt
English: https://www.umweltbundesamt.de/publikationen/antibiotics-antibiotic-resistances-in-the

AMR Workshop Brussles 07.11.2018 “Act Now – Antibiotics and Antibiotic Resistance in the Environment”
English: https://www.ecologic.eu/16161, German: https://www.ecologic.eu/de/16104
Presentation UBA Position: Options to minimize antibiotics and antibiotic resistances in the environment
https://www.ecologic.eu/sites/files/event/2018/5._klasen_j_-_uba_amr_workshop_-_brussels_7_nov_2018_0.pdf

Schönfeld, J. Konradi S. Berkner S. Westphal Settele K. UMID 2/2017 Artikel Antimikrobielle Resistenzen in der Umwelt – Gibt es Neues zum bekannten Phänomen?
https://www.umweltbundesamt.de/sites/default/files/medien/3240/publikationen/umid_02-2017_uba_antibiotika_0.pdf

Recommendations for reducing micropollutants in waters, UBA 2018
https://www.umweltbundesamt.de/en/publikationen/recommendations-for-reducing-micropollutants-in

UBA Informationsportal Tierarzneimittel in der Umwelt (Lehrmaterial, Zielgruppenorientierte Information)
https://www.umweltbundesamt.de/tierarzneimittel

UBA Texte Band 115/2018 Kommunikations-strategien zur Verminderung von Tierarzneimitteleinträgen aus der Landwirtschaft in die Umwelt
https://www.umweltbundesamt.de/sites/default/files/medien/1410/publikationen/2018-12-19_texte_115-2018_tam-kommunikation-abschlussbericht.pdf

The project "Communication Concept for Mitigating Veterinary Pharmaceutical Inputs from Agriculture into the Environment" aimed to prepare the current knowledge on the subject of the environmental impact of veterinary medicines (VETs) and measures for the environmentally conscious use and handling of VETs in a target group-oriented manner for people from agriculture and veterinary medicine.