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Ultimate biodegradability and ecotoxicity of orally administered antidiabetic drugs

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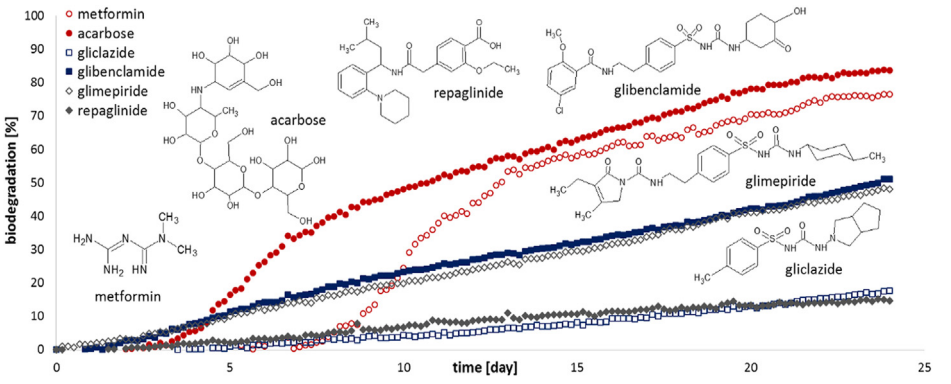
HIGHLIGHTS

- Ultimate biodegradation of antidiabetic drugs was examined.
- Metformin and acarbose showed high degradability.
- Glibenclamide and glimepiride were moderately degradable.
- Low extent of degradability of gliclazide and repaglinide was shown.
- Low ecotoxicity or no effect up to solubility limit was found in *Daphnia magna* test.

GRAPHICAL ABSTRACT



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ABSTRACT

Hypoglycaemic pharmaceuticals are recently more and more frequently detected in the environment. In our previous study, we have shown that even though many of them undergo significant primary degradation some are transformed to stable products or undergo such transformation that a large part of the structure is still preserved. One of the main routes of elimination from wastewaters or surface waters is biodegradation and a lack thereof leads to accumulation in the environment. Within this work we tested the ultimate biodegradability of six oral antidiabetics: metformin and its main metabolite guanylurea, acarbose, glibenclamide, gliclazide, glimepiride and repaglinide. We also compared the experimental results obtained in this and accompanying work with models designed to predict biodegradability and showed that these models are only moderately successful. Additionally, we examined these compounds in acute *Daphnia magna* test to check if they might pose an ecotoxicological threat. Combining the results of biodegradability and toxicity tests allows a preliminary assessment of their potential environmental impact.

1. Introduction

In the first part of this study we have examined biotransformation of several pharmaceuticals, often prescribed in treatment of

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type-2 diabetes mellitus [1]. We have shown that although some of them do undergo biological transformation the quantitative and more importantly qualitative extent of that transformation is sometimes limited.

Primary and ultimate degradability examined in this and previous study supply important information regarding susceptibility to biotic breakdown but have very different implications [1]. Primary degradation test is designed to check if microbial inoculum is able to alter the structure of compound. Since the test is usually based on measuring concentration of parent compound it might be, and is often the case, that only a minor alteration of structure occurs (e.g. hydroxylation). If the study is not backed up by the analysis of transformation products one can only guess the qualitative extent of degradation. In such cases, an ultimate biodegradation test, designed to assess whether or not a compound can be completely utilised by microorganisms leaving simple products, delivers more information.

To obtain broader knowledge on biotic transformation of chosen antidiabetics (Table 1) we hereby also scrutinized the ultimate biodegradation levels. Furthermore, we tested the ecotoxicity towards *Daphnia magna* as a way of screening for compounds that might raise concerns. *Daphnia magna* was chosen as a model organism as it is a key species used in assessment of environmental impact of chemicals and because many invertebrates (including *Daphnia* species) were shown to possess insulin signalling pathway similar to humans [2]. We also compared the experimental data to biodegradability/ecotoxicity parameters predicted using QSAR (Quantitative Structure Activity Relationships) as in many cases there are large discrepancies between experimentally obtained and predicted values. Low levels of both primary and ultimate biodegradability and/or significant ecotoxicity indicate that the test compound is potentially dangerous to the environment. A testing scheme is shown in SI file (Fig. S1) and more details can be found in first part of this study

Metformin is both the most often prescribed and most often detected in the environment antidiabetic drug [8–14]. We have shown that it undergoes full primary degradation within 15 days and is in most cases transformed to guanylurea [1]. No biodegradation in closed bottle test was observed. However, in manometric measurement, where a higher cell density is used, one of replicates showed approximately 48% of degradation (other two did not record any). Interestingly in the latter test 57.5% of metformin was mineralized when sodium acetate was added to the test medium suggesting that metformin might be co-metabolised. In Zahn-Wellens test about 50% removal was observed by measuring dissolved organic carbon when a much higher concentration of metformin was used (172.5 mg L^{-1}). Despite relatively high degradation rate metformin was classified as not readily biodegradable. Additionally, in tests, in which biodegradation took place, guanylurea was detected as a sole, dead-end metabolite [9,13].

Metformin showed rather moderate ecotoxicity with EC_{50} of 64 mgL^{-1}, 110 mgL^{-1} and above 320 mg L^{-1} for *Daphnia magna*, *Lemna minor* and *Desmodesmus subspicatus* respectively [15].

Acarbose is metabolised by gastrointestinal flora to a large extent and only a fraction of the parent compound is excreted [8]. No information regarding ultimate biodegradability of acarbose is available in the literature; we have however shown previously that the parent compound is transformed very quickly leaving no stable products [1]. Acarbose was also shown to have low toxicity towards daphnids and fish (EC_{50} > 1 gL^{-1}) therefore not raising significant environmental concerns [16].

No data regarding ultimate degradation of glibenclamide is available so far but we have previously shown that it can be transformed by WWTP organisms, in addition it undergoes 40–60% primary degradation in soils under aerobic conditions and only 10% under anaerobic with hydroxyl- and carboxyl-metabolites being formed [17]. Cunningham et al. reported low ecotoxicity (EC_{50} > 100 mg L^{-1}) in test with daphnids, algae and fish after ROCHE Pharmaceuticals Sustainability Database [16]. Such concentration is well above aqueous solubility of GLB which puts the result in question.

Data regarding glimepiride, gliclazide and repaglinide are even scarcer. No information regarding ecotoxicity and ultimate degradability is available. We have previously shown that glimepiride can be fully transformed to lower molecular weight products but gliclazide remains unchanged under similar conditions [1].

2. Materials and methods

2.1. Chemicals

Antidiabetic drugs: Repaglinide (REP) CAS No.135062-02-1, glibenclamide/glyburide (GLB) CAS No.10238-21-8, gliclazide (GLZ) CAS No.21187-98-4, acarbose (ACB) CAS No.56180-94-0, metformin (MET) CAS No.657-24-9, and a metformin transformation product – guanylurea (GU) CAS No.207300-86-5 were obtained from Sigma Aldrich (St. Louis, USA). Glimepiride (GMP) CAS No.93479-97-1 was obtained from Tokyo Chemical Industry (Tokyo, Japan), benzoic acid used as positive control was purchased from Acros Organics (Geel, Belgium) and allylthiourea use as nitrification inhibitor was obtained from Merck KGA (Darmstadt, Germany).

2.2. Ultimate biodegradability

We used a manometric respirometry method according to OECD 301 F which measures the decrease of pressure in test vessels caused by consumption of oxygen used by bacteria to degrade the test chemical [18]. Test mixture of final volume of 432 mL contained: mineral medium (8.5 mg L^{-1} KH_2PO_4, 21.75 mg L^{-1} K_2HPO_4, 22.13 mg L^{-1} $Na_2HPO_4{\cdot}2H_2O$, 1.7 mg L^{-1} NH_4Cl, 27.5 mg L^{-1} $CaCl_2$, 22.5 mg L^{-1} $MgSO_4{\cdot}7H_2O$ and 0.25 mg L^{-1} $FeCl_3$), microbial inoculum, nitrification inhibitor (allylthiourea 5 mg L^{-1}) and 20 mg L^{-1} of test substance. Microbial inoculum was derived from activated sludge from an aeration tank of the municipal WWTP in Delmenhorst, Germany. Prior to the experiment the flocks were allowed to settle and were discarded. The remaining supernatant, containing 0.4 g L^{-1} dry mass of sludge, was aerated for another 5–7 days and finally used as inoculum after addition of medium. The test substances were weighed separately for each sample and placed as a solid in test bottle (Oxitop™, WTW). In order to obtain reliable measurement of biodegradation in some cases the amount of test compound added to the test bottles exceeded its water solubility, meaning that the suspension in dynamic equilibrium was tested as permitted by the guideline. Additionally due to limited sensitivity of the technique (lowest measurable range is 40 mg O_2 L^{-1}) the concentrations of test substance are significantly higher than expected environmental concentrations. After adding the test compounds, the bottles were closed and stirred for 2 h to allow for temperature equilibration and dissolution of the test compound. Each sample was run in duplicate and was accompanied by blank samples, to account for endogenous cellular breathing, and positive controls containing benzoic acid in the same concentration as the test substance (20 mg L^{-1}). The temperature during the test was set at 20 °C and controlled. Decrease in pressure inside the bottle caused by oxygen consumption was measured, recorded and recalculated into biological oxygen demand (BOD). The% degradation was calculated from the BOD value and theoretical oxygen demand according to [18]. BIOWIN v.4.10, US EPA EPI Suite was used for