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Ultimate biodegradability and ecotoxicity of orally administered antidiabetic drugs

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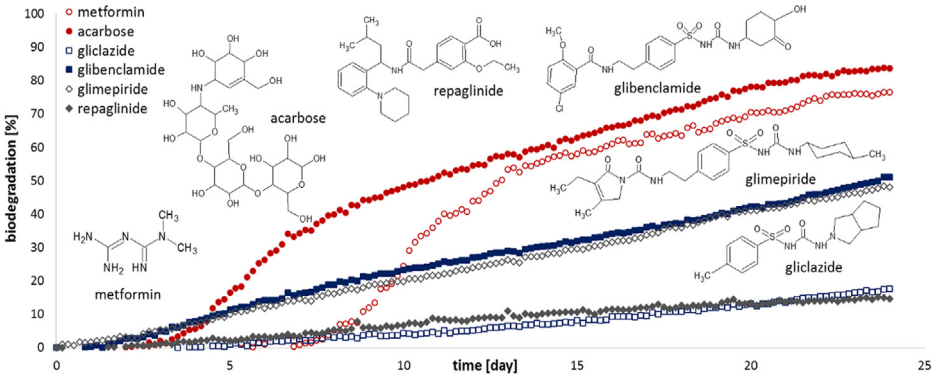
graphical abstract



highlights

- Ultimate biodegradation of antidia-betic drugs was examined.
- Metformin and acarbose showed high degradability.
- Glibenclamide and glimepiride were moderately degradable.
- Low extent of degradability of gli-clazide and repaglinide was shown.
- Low ecotoxicity or no effect up to sol-ubility limit was found in Daphnia magna test.

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.

Keywords: Biodegradation, Ecotoxicity, Pharmaceuticals
Antidiabetic drugs, Sulphonamides

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

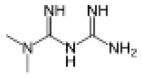
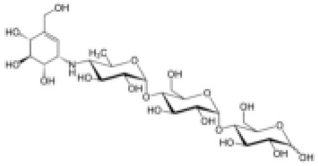
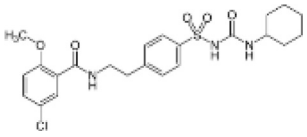
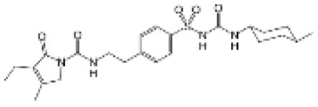
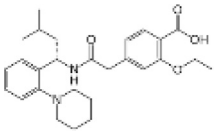
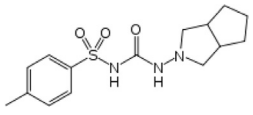
2.1. Chemicals

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

Table 1
Some environmentally relevant properties of the investigated pharmaceuticals.

Name (abbreviation) MW $logK_{ow}$[a]	Chemical structure	Consumption in US [t $year^{-1}$][b]	Human metabolism and pharmacological activity of metabolites[c]
Metformin (MET) MW = 129.16 $gmol^{-1}$ $logK_{ow}$ = -2.64		12 913 313	not metabolised, excreted as active parent compound
Acarbose (ACB) MW = 645.60 $gmol^{-1}$ $logK_{ow}$ = -8.08		4 095	metabolised extensively by gastrointestinal flora[d]
Glibenclamide (GLB) MW = 494.00 $gmol^{-1}$ $logK_{ow}$ = 4.79		10 533	extensively metabolised with limited activity of metabolites
Glimepiride (GMP) MW = 490.62 $gmol^{-1}$ $logK_{ow}$ = 4.70		5 877	extensively metabolised, some metabolites are active
Repaglinide (REP) MW = 452.59 $gmol^{-1}$ $logK_{ow}$ = 6.19		719	extensively metabolised to inactive compounds
Gliclazide (GLZ) MW = 323.41 $gmol^{-1}$ $logK_{ow}$ = 2.12		no data	extensively metabolised to inactive compounds

[a] K_{ow} value predicted using EPI Suite™ KOAWIN v.1.68.
[b] Value obtained by multiplying number of patients prescribed the drug in 2012 according to [3] by maximum recommended daily dose [mg kg^{-1} of body weight per day] assuming average adult weight of 60 kg [4,5].
[c] Source [6,7].
[d] Acarbose might be metabolised by human gastrointestinal track microflora to a high extent [6]. Abbreviations: MW – molecular weight, K_{ow} – octanol-water partition coefficient.

predicting probability of biodegradation [19]. Details of the models used are given in supplementary material.

2.3. *Acute* Daphnia magna *immobilisation test*

The 48 h acute immobilization test with *Daphnia magna* was performed using the commercially available Daphtoxkit F (MicroBioTest Inc., Gent, Belgium) according to ISO 6341 standard. Compounds were tested in different concentration regimes: GLB and GMP in concentration of 0.05–5 mg L^{-1} (maximum soluble concentration without cosolvent addition), GU and GLZ at 5–150 mg L^{-1} as their solubility in test medium was high enough. In case, a full dose response curve was not obtained within this range no higher doses were tested and the EC_{50} value was reported as higher than highest tested concentration.

Daphnia neonates were hatched from dormant ephippia at 20 °C under constant illumination. For each replicate five pre-fed animals, less than 90 h old, were placed in 10 mL of mineral medium controls) or solution of test substances in mineral medium. Four replicates on each concentration level were used. The number of immobilized or dead organisms was checked after 24 and 48 h. The relative toxicity of the samples was expressed as a fraction of not affected organisms compared to the controls. All substances were tested in two independent experiments (five concentrations, four replicates each).

3. Results and discussion

3.1. *Ultimate degradation*

The biodegradation curves are presented in Fig. 1A–F. The replicates within one test are marked to indicate the variability and the average values are plotted in Fig. 1G. Four to six degradation curves for each compound were obtained in two to three independent experiments. Our positive control, benzoic acid, reached 60% degradation within five days in each case, confirming the validity of the test. When calculating the averaged biodegradability only these results which exceeded 5% degradation were taken into account and only then when a majority of replicates showed a significant biodegradation. This was the case for metformin and glimepiride. In case of repaglinide no such clear trend was observed therefore all results were taken into account.

The average degradation of MET in manometric respirometry test reached 76% (Fig. 1G). Three replicates reached 100% of degradation and fulfilled the ready biodegradability criterion. Another three replicates showed 51%, 30% and 0% mineralisation (Fig. 1A). This is much higher than reported before in the same test which

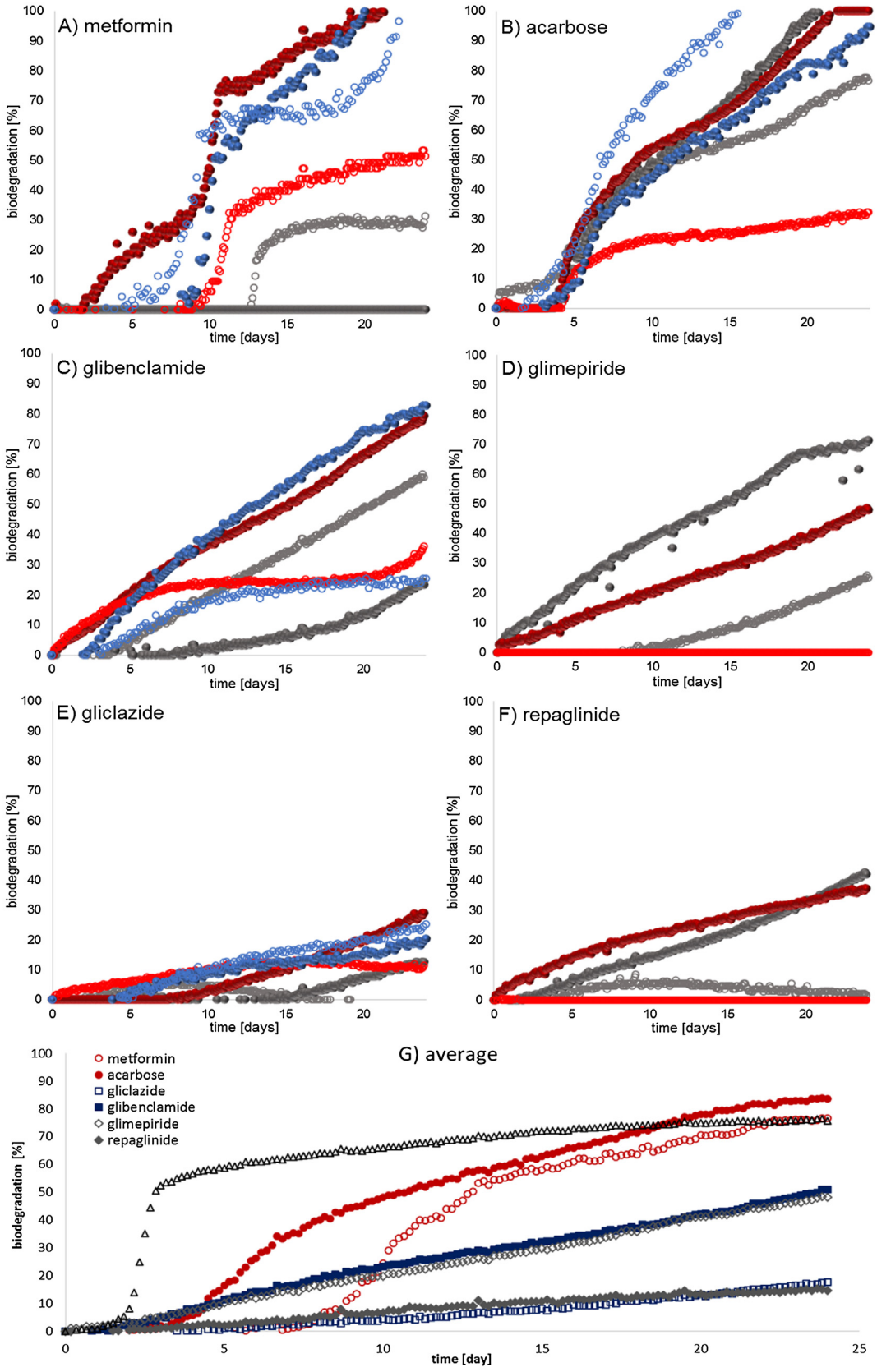


Fig. 1. Ultimate biodegradation of antidiabetic drugs: MET (A), ACB (B), GLB (C), GMP (D), REP (E), GLZ (F) and averaged results of ultimate biodegradation of all drugs (G) and positive control (benzoic acid). Open and closed symbols in Figure A-F in the same colour are the replicates within the same test.

might partially be due to lower concentration of MET used here ($20\,mg\,L^{-1}$) as compared to $50\,mg\,L^{-1}$ used in [9] – even though no toxic influence on inoculum was observed in the latter. It is also probable that the differences are caused by diversity in microbial community. It seems therefore that the degradation of MET might proceed faster (or occur at all) when specific degraders are present.

In the first part of this study we have confirmed previously reported degradation of MET to guanylurea (GU) [1]. Double dealkylation leading to formation of GU under aerobic conditions requires three oxygen molecules corresponding to 100% degradation in manometric test system (hypothetical structures are shown in Supplementary Information, Fig. 2S). At this point any further degradation, if occurring at all, would not cause oxygen depletion and therefore proceed undetected. Another theoretically possible pathway is sequential removal of two urea molecules with dimethylamine formation. Urea removal is an enzymatic hydrolysis reaction and would not cause any oxygen depletion in the manometric system giving dimethylamine as a product. Theoretically the breakdown of dimethylamine consumes three oxygen molecules and accounts for 100% degradation in this test.

ACB was degraded to a large extent with final degradation of 100% in three samples, and then 94%, 77% and 31% (Fig. 1B) giving the average of 84% (Fig. 1G). Four of the replicates fulfilled the ready biodegradability criterion. In primary degradation test performed before almost immediate total disappearance of ACB peak and gradual appearance of several peaks at shorter retention times, corresponding to smaller molecular weight metabolites were also observed [1]. A biodegradation route of ACB most probably proceeded by breaking one of *O*-glycosidic bonds and either releasing entire maltose molecule at once or two glucose molecules one after another. Separation and degradation of two molecules of glucose or one molecule of maltose corresponds to around 47% degradation (BOD based) and most probably occurred in all samples except one where it seems to be incomplete (only 32% degradation). Breaking down of sugar-like acarviosin is more difficult, since due to the presence of unsaturated valienamine and *N*-glycosidic bond it shows inhibitory activity towards glucosidases (which also is its main mechanism of therapeutic action) [20]. It seems however, that in our case acarviosin must have been (at least partially) broken down. Most probably as soon as the *N*-glycosidic bond is broken and acarviosin loses its enzyme inhibiting activity the degradation can proceeded fully.

In the ultimate biodegradation test GLB reached 83%, 80%, 60%, 36% and two times 25% of mineralization (Fig. 1C). Averaging the samples amounted to 52% mineralization showing that GLB is not readily but significantly biodegradable (Fig. 1G). The analysis of primary biodegradation samples performed previously showed full transformation within 10 days. Radjenović et al. observed glibenclamide-hydroxide as main transformation product of GLB [21]. Such a transformation would correspond to merely 2% degradation in our test system and therefore could not explain the observed results. Since the degradation seems to start at the cyclohexyl ring it is only logical that it will continue there. Complete degradation of that moiety amounts to approximately 32% degradation in manometric system, and breaking of the sulphonamide group accounts for additional 2%. The amount of degradation close to these values was observed in three of the replicates with lowest biodegradation scores. In the two of the samples reaching highest levels of mineralisation (80–83%) almost full degradation can be expected as some of the carbon present in the molecule can be directly build into biomass therefore causing no oxygen consumption.

In case of GMP the degradation reached 71%, 48%, 25% and 0% degradation (48% on average) and was too low to fulfil ready biodegradability criteria. In first part of this study we proposed a metabolic pathway which proceeds through removal of methylcyclohexyl ring and deamination leaving carbamic acid that is further methylated to final metabolite [1]. Such a transformation would be recorded as approximately 36% degradation in manometric respirometry test which is slightly lower than the average experimental value we obtained in current test suggesting that in some replicates degradation reached higher extent than that.

Degradation of GLZ in manometric respirometry test reached 29%, 25%, 23% and 11% in two replicates (18% on average). No significant levels of degradation were observed in the last sample (Fig. 1E). In primary degradation tests we observed only minor decrease in GLZ concentration [1]. Even though breakdown of the sulfonamide bond is generally possible as was shown even previously for glimepiride or glibenclamide we detect rather modest degree of degradation of GLZ in manometric test [1]. Nevertheless, taking into account that conventional WWTP uses activated sludge of much higher biomass content there is a possibility that in such conditions higher degradation or sorption can occur. More tests, especially under less stringent conditions would be necessary to exclude persistency of GLZ.

Study of biodegradability of repaglinide is reported here for the first time. Two samples showed 42% and 37% and two other hardly any degree of degradation (Fig. 1F). Because in this case there was no clear tendency in distribution of results, we could not dismiss the negative results as outliers. All of them were therefore averaged giving 20% degradation (Fig. 1G). This is a somewhat discouraging result and REP is expected to be degraded rather slowly in the environment. Nonetheless, it seems that the general metabolic capacity for its degradation exists. REP has rather low market share compared to other antidiabetics tested here and is metabolised extensively in human body meaning that expected release will be comparatively low.

Even though some sulfonylurea derivatives exhibit almost no biodegradability in manometric respirometry tests, in simulated activated sludge system containing higher biomass levels they were degraded within few days. Subsequent addition of test compounds to the same test vessel resulted in rapid degradation proving that microbial adaptation can significantly enhance elimination of these xenobiotics. Interestingly enough cross-adaptation was also observed as adapted activated sludge organisms were able to rapidly degrade also different sulphonamides than those to which they were initially adapted [22]. Similar phenomena might occur in the environment.

3.2. Implications of the ultimate degradation tests

The compounds achieving 60% degradation in manometric test system within 10 days are classified as readily biodegradable. According to OECD guideline 301 they are expected to break down rapidly and completely in the environment under aerobic conditions. As was shown on the example of metformin 100% degradation in manometric test system does not always mean complete degradation to CO_2, H_2O or NH_3, far from it actually. Without preforming primary degradation, analysis of metabolites, dissolved organic carbon measurement or at least careful examination of the structure one could think that metformin was indeed completely mineralised. Out of six OECD 301 tests all these based on CO_2 evolution or O_2 consumption (301 B, C, D, E, F) would deliver similar – falsely positive – results and only those based on DOC removal (if based on high temperature catalytic oxidation techniques) would be able to pick up the incomplete degradation of MET to GU. Therefore, some kind of analytical confirmation of degree of degradation in the final sample is necessary. Particularly beneficial are these techniques which not only show how much of molecule was degraded (e.g. total organic carbon analysis) but also what were the transformation products.

As shown in Fig. 1 there is sometimes significant variability between replicates caused most probably by variability in microbial inoculum. The test conditions were carefully controlled and inoculum came always from the same source. Nevertheless, it was sampled on three different occasions between April and July. The microbial community of wastewater treatment plant can vary considerably depending on the composition of influent, environmental conditions etc. Therefore, in each test we were most certainly using inoculum of different microbial composition. Considerable differences can exist even in the same batch of sludge (dependent replicates within one test) because the composition of microbial community changes also during the test. Such differences are rather common since the composition of microbial inoculum is usually not well defined which is a general shortcoming of biodegradation testing [9]. It seems a reasonable solution to actually examine the microbial composition before and after the test to try to identify specific degraders and check for their presence in the samples that fail to show degaradation. This requires advanced biochemical techniques (e.g. like in-situ RNA extraction and sequencing). Some variability might have been caused by the fact that the substance was added directly into each bottle instead of preparing solutions. This was done to deal with poor solubility of some of the compounds and was therfore unavoidable

3.3. *Biodegradation – comparison with QSAR models*

We used QSAR models to predict timeframe of ultimate and primary degradation (Table 2). MET was predicted to be not readily biodegradable nevertheless the degradation timeframes are rather short and generally corroborated by our results. Interestingly, almost identical timeframes are predicted for GU which is in strong opposition to both our and previous results [1,9].

ACB is the only out of the antidiabetics tested here which is expected to be readily biodegradable based on QSAR, and was shown to be so experimentally in four out of six replicates. Our results show that the average degradation timeframes would be slightly longer than predicted but confirm the general tendency that ACB is rather easily degradable.

GLB, on the other hand, is the only tested compound that was predicted to be recalcitrant. This is in contrast to our experimental results which showed averaged ultimate 52% degradation within a little more than three weeks. The main structural feature responsible for a low QSAR score is presence of chlorine atom and high molecular weight. High degree of halogenation severely limits degradability of chemicals [23]. Nevertheless the molecules containing one chlorine atom can be metabolised or co-metabolised under aerobic conditions [24,25]. This together with complete primary degradation reported previously and appearance of metabolites suggest that there is a potential for degradation [21].

QSAR indicate that GMP and REP are not readily biodegradable and that primary and ultimate degradation might take weeks and months respectively. Our experimental results for GMP tend to be more promising. We observed a low degree of mineralization for REP suggesting that indeed this compound will be degraded slowly. The BIOWIN returns 'not readily biodegradable' for GLZ with the primary and ultimate biodegradation timeframes being days-weeks and weeks-months correspondingly. Our experimental results indicate rather longer timeframes and generally poor degradability. Especially worrying is almost complete lack of primary degradation suggesting that microbial metabolic capacities allowing to initiate the degradation might be limited. In general QSAR predicted biodegradability of ACB, MET and GMP relatively accurately whereas the values of REP and GLZ seem to be somehow overestimated, especially for the latter one. The model seems to fail for GU and GLB in opposite directions. GU predicted to undergo primary degradation within weeks did not show any susceptibility

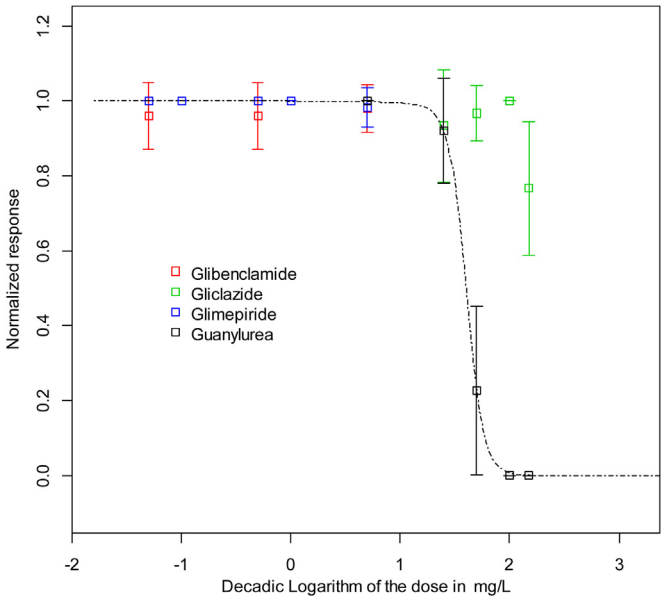


Fig. 2. Dose-response for GLB, GLZ, GMP and GU in acute immobilisation test with Daphnia magna.

to it, generating a false positive result [1]. GLB on the other hand which was classified as recalcitrant by the model showed rather significant degradability (a false negative).

3.4. *Ecotoxicity towards* Daphnia magna

The ecotoxicity of antidiabetic drugs and guanylurea was examined in acute *Daphnia magna* immobilisation test. The EC_{50} of MET and ACB was reported to be 64 mg L^{-1} and above 1 g L^{-1} respectively [15,26]. The results for other antidiabetics are shown in Fig. 2.

Only in case of GU a full dose response curve was obtained with the EC_{50} value of 40 mg L^{-1}. This shows that both parent compound (MET) and its metabolite (GU) have comparable, relatively low toxicities. The concentrations of MET and GU detected in surface waters were up to 643 ng L^{-1} and 391 ng L^{-1} respectively [13]. The expected environmental concentrations therefore remain three to five orders of magnitude below EC_{50} values for *Daphnia magna*. This suggests that at the moment no immediate danger exists, at least based on this test. Nevertheless, resistance of GU to degradation suggest that a build-up of this substance in the environment might occur with time and the concentration of GU should be monitored in the environment. For GLZ only minor effect was observed in 150 mg L^{-1} solutions, GLB and GMP did not cause any effects up to the concentration of 5 mg L^{-1}. The three pharmaceuticals show low toxicity towards *Daphnia magna*: GLZ due to inherent low toxicity, GLB and GMP due to low water solubility and therefore low exposure, at least of pelagic organism. Nevertheless, these results should be treated as preliminary assessment and more data on toxicity employing organisms at different taxonomical levels but also biodegradability and bioaccumulation is needed.

The experimental results were again compared with the models predicting baseline toxicity and excess toxicity for *Daphnia magna* (Table 3). Out of all examined drugs the ACB was the least toxic with no effect up to 1 g L^{-1} [26]. It is difficult to assess the accuracy of the first model for ACB as no exact experimental EC_{50} value can be obtained and the predicted value exceeds water solubility of ACB. It is nevertheless apparent that ACB shows generally low toxicity. The baseline model underestimated toxicity of MET by four and of GU by three orders of magnitude – which would be expected as it is not meant to be used for ionisable compounds (both MET

Table 2
Summary of results of experimentally measured primary (from [1]) and ultimate degradation and QSBR predicted primary and ultimate biodegradation – classification and score (in the brackets) are given.

compound	experimental degradation		predicted degradation[c]	
	primary[a]	ultimate	primary	ultimate
MET	100% (14 days)	76%	days-weeks (3.7)	weeks (2.9)
GU	0% (27 days)	n.a.	days-weeks (3.7)	weeks (3.0)
ACB	100% (3 day)	84%	hours-days (4.6)	days (3.8)
GMP	100% (15 days)	48%	weeks (3.1)	months (2.0)
GLB	100% (19 days)	52%	weeks (3.2)	recalcitrant (1.7)
REP	n. a.[b]	20%	weeks (3.1)	months (1.8)
GLZ	15% (26 days)	18%	days-weeks (3.3)	weeks-months (2.4)

[a] The extent of removal and time frame is given in the brackets.
[b] n.a. – not available, the degradation was not measured.
[c] BIOWIN classification criteria 5–hours, 4–days, 3–weeks, 2–months, 1> months.

Table 3
Comparison of experimental and ECOSAR predicted EC_{50} values (mg L^{-1}) towards Daphnia magna assuming baseline toxicity and excess toxicity. For compounds belonging to more than one group expected to show excess toxicity the group with lowest EC_{50} value was chosen.

antidiabetic	measured EC_{50}	predicted EC_{50}	
		baseline toxicity	excess toxicity
ACB	>1000.00 [26]	$134.00 \cdot 10^9$	24.39[a]
MET	64.00 [15]	$57.00 \cdot 10^4$	1927.34[b]
GU	40.00	$27.00 \cdot 10^3$	209.39[b]
GLB	>5.00	0.90	0.45[c]
GLZ	>150.00	119.00	12.30[d]
GMP	>5.00	1.10	0.03[e]

Chemical classes:
[a] Ally/vinyl alcohol.
[b] Aliphatic amines.
[c] Amides.
[d] Hydrazines.
[e] Carbonyl ureas.

and GU carry positive charge at circumneutral pH). Owning to the aliphatic amine groups present in the structure both MET and GU are expected to show excess toxicity. Predicted baseline and excess toxicities differ by two orders of magnitude showing that applying class specific model in that case increases the accuracy – the EC_{50} values are overestimates by a factor of thirty and five for MET and GU respectively. The discrepancies between baseline and excess toxicity are lower for other three hypoglycaemics tested but the predicted toxicity seems to be overestimated by both models. For GLB the baseline and excess toxicity predictions differ by the factor of only two, for GLZ by one and for GMP by two orders of magnitude.

The ECOSAR package was developed as a screening tool to be used in absence of data for a first estimation and detecting potentially toxic chemicals. According to European Chemical Agency the compound is identified as toxic if, among others, the EC_{50} in acute test with *Daphnia magna* is lower than 1 mg L^{-1}. If the ECOSAR package was to be used to spot such chemicals among our test set the models would signal the GLB and GMP as potentially toxic to daphnids which turned out to be a false positive. More importantly the model did not show any false negatives – so the compounds that are toxic but model failed to find them.

4. Conclusions

We have investigated biodegradability and ecotoxicity of type-2 oral antidiabetics. Out of all compounds tested ACB and MET are degraded fast and to high extent. The latter one seems to often be transformed to a dead-end product. This is especially worrying as neither MET nor its metabolite GU were flagged by the models as persistent showing that testing cannot always be replaced by the QSARs. The fact that MET was detected in virtually every water sample examined and that concentration levels could by no means be described as negligible points out that a more efficient method of wastewater treatment is necessary and that the presence of both MET and GU should be monitored in natural waters [13].

Two out of tested compounds, gliclazide and repaglinide, were degraded in less than 20% and might therefore be treated as potentially persistent even if the QSAR model does not suggests so. Fortunately preliminary ecotoxicological tests for target compounds described within our work do not indicate immediate threats. Nevertheless, the fact that some of the test compounds are degraded only partially or to minor extent and frequently detected in surface waters indicates a need for more stringent regulation.

We have observed, sometimes significant, variability between replicates in manometric respirometry test even though the quality criteria of the test were fulfilled. This shows that, as in case of ecotoxicity, biodegradability should also be tested in several independent test runs to account for variability of tests organisms (microbial inoculum). Alternatively, analysis of taxonomic composition of inoculum could indicate if there were any major differences in the community or could help to identify organisms that are most probably involved in the transformation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2017.03.030.

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