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Possible adverse effects of tetracyclines on the human health and the environment

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Abstract. Medicines have an important role in the treatment and prevention of disease in both humans and animals. But it is because of the very nature of medicines that they may also have unintended effects on animals and microorganisms in the environment. Although the side effects on human and animal health are usually investigated in thorough safety and toxicology studies, the potential environmental impacts of the manufacture and use of medicines are less well understood and have only recently become a topic of research interest. This is further complicated by the fact that some pharmaceuticals can cast effects on bacteria and animals well below the concentrations that are usually used in safety and efficacy tests. In addition, breakdown products and the combination of different biologically active compounds may have unanticipated effects on the environment. Although it may be safe to assume that these substances do not substantially harm humans, we have only recently begun to research whether and how they affect a wide range of organisms in the environment and what this means for environmental health. The aim of this work is to predict the possible adverse effects of some tetracyclines on the human health and the environment.

Persistent, bioaccumulative, acute and chronic toxic were predicted for ten tetracyclines by baseline models and a software of (Q)SAR Application Toolbox. Possible metabolic activation (observed and predicted) of some tetracyclines was applied by a software of (Q)SAR Application Toolbox. Results show that some of them are persistent, do not bioaccumulate in the food chain and are with moderate to low toxicity. The tetracyclines were metabolically activated in the liver and their protein and DNA binding was estimated. Observed metabolic pathways weren't observed. Predicted metabolites have different mechanisms of protein and DNA binding.

Keywords: tetracyclines, hepatotoxicity, persistence, bioaccumulation, acute and chronic toxicity


Introduction

Different classes of pharmaceuticals, such as antibiotics, hormones, anaesthetics and anti-inflammatories, are in the aquatic environment. Although they are present at trace levels, their continuous introduction into the environment is characterized as a “pseudo-persistence”, which may result in toxic effects (Bautitz and Nogueira, 2007). Among these pharmaceuticals, antibiotics are of special concern due to their extensive use in human and veterinary medicine. The presence of trace amounts of antibiotics in the environment can induce the development of antibiotic-resistant pathogens, which may cause serious problems for human health (Hirsch et al., 1999).

Today’s livestock farming requires the usage of many pharmaceuticals, in order to keep the animals sound and increase the production rate. Products are used to stimulate growth, to induce ovulation, but more important in prevention or treatment of parasites and bacterial diseases (Boxall et al., 2003). These farm animals are often bred on a large scale and as a consequence animal medicine or other drugs are applied in large quantities. These amounts are catching up with those of typical agrochemicals (Jones et al., 2001; Benbrook, 2002). Only in some situations the dosage is specifically adapted to the needs of one or a small group of animals. In most cases the entire livestock is treated even if the majority does not show any symptoms. Unadjusted dosages lead to incomplete metabolisation and not metabolised and degradation products are excreted by the animal (Boxall et al., 2003). The excessive use of pharmaceuticals has led to an increase in concentrations of these potentially harmful compounds in natural soils and aquatic systems (Hamscher et al., 2002; Kolpin et al., 2002). Active components can form a serious risk for both public health and natural ecosystems (Boxall et al., 2003; Kümmerer, 2008; Sibley and Hanson, 2011).

Antibiotics and other pharmaceuticals bring forth biological effects, even at low concentrations. They are specifically constructed for this purpose, making them potentially more harmful than other chemical compounds (Williams, 2005). According to Boatman (1998) more than 2500 tons of the antibiotic tetracyclines are used every year in veterinary therapy, of which tetracycline, chlorotetracycline, and oxytetracycline are applied the most. This group of antibiotics are broad-spectrum agents, active against a wide range of microorganisms. Because of its abundance in the environment, researchers have been aware of the potential problem and tried to assess the environmental risks (Vangheel, 2012).

The aim of this work is to predict the integrity of possible adverse effects of some tetracyclines on the human health and the environment.

Material and methods

Persistent, bioaccumulative, acute and chronic toxic were predicted for ten tetracyclines by baseline models and a software of PBT Profiler. Possible metabolic activation (observed and predicted) of some tetracyclines was applied by a software of (Q)SAR Application Toolbox. The tetracyclines were metabolically activated in the liver and their protein and DNA binding was estimated.

* e-mail: yanuriana@abv.bg
<table>
<thead>
<tr>
<th>№</th>
<th>CAS number</th>
<th>Name of compound</th>
<th>Structure of compound</th>
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<td>1</td>
<td>57-62-5</td>
<td>7-Chlorotetracycline</td>
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<tr>
<td>2</td>
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<td>5-Hydroxytetracycline</td>
<td><img src="structure2.png" alt="Structure" /></td>
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<td>3</td>
<td>60-54-8</td>
<td>Tetracycline</td>
<td><img src="structure3.png" alt="Structure" /></td>
</tr>
<tr>
<td>4</td>
<td>127-33-3</td>
<td>6-Demethyl-7-chlorotetracycline</td>
<td><img src="structure4.png" alt="Structure" /></td>
</tr>
<tr>
<td>5</td>
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<td>N-(Pyrrolidinomethyl) tetracycline</td>
<td><img src="structure5.png" alt="Structure" /></td>
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<tr>
<td>6</td>
<td>992-21-2</td>
<td>N-Lysinomethyltetracycline</td>
<td><img src="structure6.png" alt="Structure" /></td>
</tr>
<tr>
<td>7</td>
<td>1181-54-0</td>
<td>N-2-Hydroxymethylchlorotetracycline</td>
<td><img src="structure7.png" alt="Structure" /></td>
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<td>8</td>
<td>914-00-1</td>
<td>6-Methylene-5-oxytetracycline</td>
<td><img src="structure8.png" alt="Structure" /></td>
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<tr>
<td>9</td>
<td>564-25-0</td>
<td>alpha-6-Deoxy-5-hydroxytetracycline</td>
<td><img src="structure9.png" alt="Structure" /></td>
</tr>
<tr>
<td>10</td>
<td>10118-90-8</td>
<td>7-Dimethylamino-6-demethyl-6-deoxytetracycline</td>
<td><img src="structure10.png" alt="Structure" /></td>
</tr>
</tbody>
</table>
Compounds. Some tetracyclines (Website for data of rat and mouse) were investigated which are presented in Table 1.

Acute Terrestrial Toxicity Data. The experimental data for rat and mouse (oral LD₉₀ values) were collected from the literature (Website for data of rat and mouse).

\( \text{Log } P \): Data for the logarithm of the 1-octanol-water partition coefficient (log P) were obtained from the KOWWIN software (US EPA, KOWWIN). Where possible measured log P values were verified and used in preference to calculated values.

Baseline models. In this study several models were used for non-polar compounds to terrestrial species to determine the acute toxicity of tetracyclines (Table 3).

Baseline model (saturated alcohols and ketones) of rat (oral) (Lipnick, 1991):

\[
\log(1/LD_{50}) = 0.805 \log P - 0.971 \log(0.0807 \times 10^{4s}+1) + 0.984
\]

where \( n \) is the number of observations; \( R^2 \) is the square of the correlation coefficient adjusted for degrees of freedom; \( s \) is the standard error on the estimate; \( F \) is Fisher’s statistics. \( \lg \) is the octanol-water partition coefficient and \( LD_{50} \) is the lethal dose required to kill 50 percent of a population of test animals (e.g. mouse).

Baseline model (saturated ketones) of Mouse (oral) (Tani et al., 1986):

\[
\log(1/LD_{50}) = 0.557 \log P - 0.908 \log(0.049 \times 10^{4s}+1) + 1.201
\]

Excess toxicity: The property – excess toxicity – was used to define the toxicity of chemicals (reactive or non-reactive) (Lipnick, 1991). The extent of excess toxicity was determined as the toxic ratio (TR), which was calculated by the following equation:

\[
TR = \frac{\text{(predicted baseline toxicity)}}{\text{(observed toxicity)}}
\]

Criteria used by the PBT Profiler. The PBT Profiler is a screening-level tool that provides estimates of the persistence, bioaccumulation, and chronic fish toxicity potential of chemical compounds. It is designed to be used when data are not available. In order to help interested parties make informed decision on a chemical’s PBT characteristics, the PBT profiler automatically identifies chemicals that may be persistent in the environment and bioaccumulate in the food chain. These chemicals are identified using thresholds published by the Environmental Protection Agency (EPA) (Criteria used by the PBT Profiler).

Persistence criteria. The PBT Profiler combines the persistence criteria for water, soil, and sediment and highlights chemicals with an estimated half-life ≥ 2 months and < 6 months as persistent and those with an estimated half-life ≥ 6 months as very persistent. The half-life in air is not used in the PBT Profiler’s Persistence summary (chemicals with an estimated half-life > 2 days are considered persistent). The PBT Profiler uses 30 days in a month for its comparisons.

Bioaccumulation criteria. The PBT Profiler combines the bioaccumulation criteria and highlights chemicals with a BCF ≥ 1000 and < 5000 as bioaccumulative and those with a BCF ≥ 5000 as very bioaccumulative.

Toxicity criteria. To highlight a chemical that may be chronically toxic to fish, the PBT profiler uses the following criteria: Fish ChV (Chronic Value) > 10 mg/l (low concern), Fish ChV = 0.1 - 10 mg/l (moderate concern) and Fish ChV < 0.1 mg/l (high concern).

OECD (Q)SAR Application Toolbox. (Quantitative) Structure-Activity Relationships [(Q)SARs] are methods for estimating properties of a chemical from its molecular structure and have the potential to provide information on the hazards of chemicals, while reducing time, monetary costs and animal testing currently needed. To facilitate practical application of (Q)SAR approaches in regulatory contexts by governments and industry and to improve their regulatory acceptance, the OECD (Q)SAR project has developed various outcomes such as the principles for the validation of (Q)SAR models, guidance documents as well as the QSAR Toolbox (OECD (Q)SARs Application Toolbox).

Metabolic pathways documented for 200 organic chemicals in different mammals are stored in a database format that allows easy computer-aided access to the metabolism information. The collection includes chemicals of different classes, with variety of functionalities such aliphatic hydrocarbons, alicyclic rings, furans, halogenated hydrocarbons, aromatic hydrocarbons and haloaromatics, amines, nitro-derivatives, and multifunctional compounds. In vivo and in vitro (predominantly, with liver microsomes as experimental systems) studies were used to analyse the metabolic fate of chemicals. Different sources, including monographs, scientific articles and public websites were used to compile the database (OECD (Q)SARs Application Toolbox; Mekenyan et al., 2004).

<table>
<thead>
<tr>
<th>Name of compound</th>
<th>Persistence</th>
<th>Bioaccumulation</th>
<th>Fish ChV, mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Percent in Each Medium</td>
<td>BCF</td>
<td></td>
</tr>
<tr>
<td>Chlortetracycline</td>
<td>21%; 79%; 0%; 0%</td>
<td>3.2</td>
<td>3.9</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>23%; 77%; 0%; 0%</td>
<td>3.2</td>
<td>40</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>27%; 73%; 0%; 0%</td>
<td>3.2</td>
<td>7.3</td>
</tr>
<tr>
<td>Demeclocycline</td>
<td>22%; 78%; 0%; 0%</td>
<td>3.2</td>
<td>6.2</td>
</tr>
<tr>
<td>Rolitetracycline</td>
<td>9%; 91%; 0%; 0%</td>
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<td>4.8</td>
</tr>
<tr>
<td>Lymecycline</td>
<td>18%; 82%; 0%; 0%</td>
<td>3.2</td>
<td>780</td>
</tr>
<tr>
<td>Clomocycline</td>
<td>27%; 73%; 0%; 0%</td>
<td>3.2</td>
<td>8.8</td>
</tr>
<tr>
<td>Methacycline</td>
<td>22%; 77%; 0%; 0%</td>
<td>3.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>22%; 77%; 0%; 0%</td>
<td>3.2</td>
<td>7.6</td>
</tr>
<tr>
<td>Minocycline</td>
<td>41%; 59%; 0%; 0%</td>
<td>3.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>
Results and discussion

Chemicals that are persistent, bioaccumulative, and toxic have the potential to concentrate to levels that may cause significant adverse impact on human health and the environment. The results of estimation of some tetracyclines for persistence, bioaccumulation and toxicity are presented in Table 2. The PBT Profiler is a screening-level tool that provides estimates of the persistence, bioaccumulation, and chronic fish toxicity potential of chemical compounds. It is designed to be used when no data are available. In order to help interested parties make informed decision on a chemical’s PBT characteristics, the PBT profiler automatically identifies chemicals that may persist in the environment and bioaccumulate in the food chain. These chemicals are identified using thresholds published by the EPA (Criteria used by the PBT Profiler). Analysis of data in Table 2 reveals that all tetracyclines are persistent and toxic (FishChV). The PBT Profiler estimates that they are not expected to bioaccumulate in the food chain because it does not exceed the BCF criteria. The compounds are with moderate to low toxicity.

All organic chemicals have the potential to cause narcosis. Their ability to do so is mainly governed by their concentration and their ability to cause more serious toxic effects, which would mask any narcotic effect the chemical may cause (van Wezel and Opperhuizen, 1995). In general, chemicals which have a more specific mode of action, especially hydrophilic ones, produce greater toxicity than that expected from baseline non-polar narcosis. These chemicals often contain specific structural fragments responsible for their mechanism of action (Aptula and Roberts, 2006).

A number of reliable baseline equations are available for different organisms (terrestrial, rat and mouse) and endpoint (LD₅₀). Baseline models (eqs 1-2) for different species (terrestrial) were applied to tetracyclines (Table 3). On the basis of calculated and experimental values for acute toxicity, the toxicity ratio (TR) as the ratio of the calculated baseline toxicity over the experimentally determined value was calculated. A TR-value less than one could indicate rapid hydrolysis and/or biotransformation of the parent compound by the organism to non-toxic metabolites (Aptula and Roberts, 2006).

There are no experimental data of tetracyclines for the terrestrial species (rat) with endpoint (LD₅₀) – chlorotetracycline, rolitetracycline, lymecycline, clomocycline, methacycline and minocycline and for the terrestrial species (mouse) with endpoint (LD₅₀) – demeclocycline, lymecycline, methacycline. Results of acute toxic prediction for rat (LD₅₀) in Table 2 reveal that some tetracyclines (oxytetracycline, tetracycline and demeclocycline) are TR less than one but doxycycline is with TR greater than one, i.e. it is toxic. The predictions for mouse (LD₅₀) reveal that tetracyclines are toxic (TR greater than one). Only minocycline is on the border.

The results of the probable metabolic activation in liver (observed and predicted) of some tetracyclines are presented in Table 4. Electrophilic metabolites may not only react with nucleophilic sites in DNA but may also bind to proteins, RNA, and to endogenous substances of lower molecular weight such as glutathione (Miller, 1998). The complexity of the reaction of electrophilic metabolites with the various nucleophilic sites within cells and the reasons why different electrophilic reagents react at different sites have been interpreted on the basis of the concepts of hard and soft electrophiles/nucleophiles (hard and soft acids/bases) (Pearson and Songstad, 1967; Prescott, 1980; Soni et al., 2001).

All tetracyclines haven’t observed metabolic pathways but they have predicted metabolites with different behaviour to DNA and protein binding. Some of the generated metabolites are active (they can react with proteins and DNA) but others are non-active. The active metabolites of tetracyclines have different mechanism of protein binding – Michael-type nucleophilic addition. Nucleophilic cycloaddition to diketones, Schiff base formation, Nitroso protein binding but only tetracycline (7-dimethylamino-6-demethyl-6-deoxytetracycline) has DNA binding – Aromatic amines and Aromatic N-Hydroxylamines.

Conclusion

Implementation and enforcement of stringent regulations regarding usage of antibiotics is urgently needed. Using theoretical methods is essential in studying the possible adverse effects antibiotics could cause when used with farm animals. They have effects on the organs of farm animals (liver), the environment (persistence, bioaccumulation and toxicity) and the ultimate consumer – man.

Acute toxicity is one of the endpoints used in environmental risk assessment to determine the safe use and disposal of organic chemicals. The endpoints are a result of different routes of exposure in various species. The effect of a chemical is dependent on the species, route of exposure, and dose. The PBT Profiler is an online risk-screening tool that predicts a chemical’s potential to persist in the environment, bioconcentrate in animals, and be toxic, properties

<table>
<thead>
<tr>
<th>Table 3. Experimental and predicted values of acute toxicity of tetracyclines to terrestrial species (rat and mouse)</th>
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<tbody>
<tr>
<td>Name of compound</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>Chlorotetracycline</td>
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<td>Oxytetracycline</td>
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<td>Tetracycline</td>
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<td>Demeclocycline</td>
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<td>Clomocycline</td>
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<tr>
<td>Methacycline</td>
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<tr>
<td>Doxycycline</td>
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<tr>
<td>Minocycline</td>
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which cause concern for human health and the environment. Using this tool for the investigated tetracyclines it was established that according to the criteria used by the PBT Profiler some of them are persistent, do not bioaccumulate in the food chain and are with moderate to low toxicity.

Drug-induced hepatotoxicity will remain a problem that carries both clinical and regulatory significance as long as new drugs continue to enter the market. Unfortunately, recognizing toxicity of specific drugs is limited by the relatively rare overall incidence of hepatotoxicity as well as underreporting.

Acknowledgement

This work was funded by the University Prof. Dr. Assen Zlatarov – Burgas (project No.: 257/2012).

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Criteria used by the PBT Profiler: http://www.pbtprofiler.net/criteria.asp


Miller JA, 1998. The metabolism of xenobiotics to reactive electrophiles in chemical carcinogenesis and mutagenesis: a collaboration with Elizabeth Cavert Miller and our associates. Drug Metabolism Reviews, 30, 645-674.


OECD (Q)SARs Application Toolbox: http://www.oecd.org/chemicalsafety/nrtios/90876885.pdf


US EPA, KOWWIN; software available at: http://www.epa.gov/oppt/exposure/pubs/episuite.htm


Website for data of rat and mouse: http://chem.sis.nlm.nih.gov/chemidplus/

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Results are presented in understandable tables and figures, accompanied by the statistical parameters needed for the evaluation. Data from tables and figures should not be repeated in the text. Tables should be as simple and as few as possible. Each table should have its own explanatory title and to be typed on a separate page. They should be outside the main body of the text and an indication should be given where it should be inserted.

Figures should be sharp with good contrast and rendition. Graphic materials should be preferred. Photographs to be appropriate for printing. Illustrations are supplied in colour as an exception after special agreement with the editorial board and possible payment of extra costs. The figures are to be each in a single file and their location should be given within the text.

Discussion: The objective of this section is to indicate the scientific significance of the study. By comparing the results and conclusions of other scientists the contribution of the study for expanding or modifying existing knowledge is pointed out clearly and convincingly to the reader.

Conclusion: The most important consequences for the science and practice resulting from the conducted research should be summarized in a few sentences. The conclusions shouldn't be numbered and no new paragraphs be used. Contributions are the core of conclusions.

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Studies performed on experimental animals should be carried out according to internationally recognized guidelines for animal welfare. That should be clearly described in the respective section “Material and methods”.

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Papers shall be submitted at the editorial office typed on standard typing pages (A4, 30 lines per page, 62 characters per line). The editors recommend up to 15 pages for full research paper (including abstract references, tables, figures and other appendices).

The manuscript should be structured as follows: Title, Names of authors and affiliation address, Abstract, List of keywords, Introduction, Material and methods, Results, Discussion, Conclusion, Acknowledgements (if any), References, Tables, Figures.

The title needs to be as concise and informative about the nature of research. It should be written with small letter /bold, 14/ without any abbreviations.

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