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Identification of phototransformation products of thalidomide and mixture toxicity assessment: An experimental and quantitative structural activity relationships (QSAR) approach

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ABSTRACT

The fate of thalidomide (TD) was investigated after irradiation with a medium-pressure Hg-lamp. The primary elimination of TD was monitored and structures of phototransformation products (PTPs) were assessed by LC–UV–FL–MS/MS. Environmentally relevant properties of TD and its PTPs as well as hydrolysis products (HTPs) were predicted using *in silico* QSAR models. Mutagenicity of TD and its PTPs was investigated in the Ames microplate format (MPF) aqua assay (Xenometrix, AG). Furthermore, a modified luminescent bacteria test (kinetic luminescent bacteria test (kinetic LBT)), using the luminescent bacteria species *Vibrio fischeri*, was applied for the initial screening of environmental toxicity. Additionally, toxicity of phthalimide, one of the identified PTPs, was investigated separately in the kinetic LBT.

The UV irradiation eliminated TD itself without complete mineralization and led to the formation of several PTPs. TD and its PTPs did not exhibit mutagenic response in the *Salmonella typhimurium* strains TA 98, and TA 100 with and without metabolic activation. In contrast, QSAR analysis of PTPs and HTPs provided evidence for mutagenicity, genotoxicity and carcinogenicity using additional endpoints *in silico* software. QSAR analysis of different ecotoxicological endpoints, such as acute toxicity towards *V. fischeri*, provided positive alerts for several identified PTPs and HTPs. This was partially confirmed by the results of the kinetic LBT, in which a steady increase of acute and chronic toxicity during the UV-treatment procedure was observed for the photolytic mixtures at the highest tested concentration. Moreover, the number of PTPs within the reaction mixture that might be responsible for the toxification of TD during UV-treatment was successfully narrowed down by correlating the formation kinetics of PTPs with QSAR predictions and experimental toxicity data. Beyond that, further analysis of the commercially available PTP

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phthalimide indicated that transformation of TD into phthalimide was not the cause for the toxification of TD during UV-treatment.

These results provide a path for toxicological assessment of complex chemical mixtures and in detail show the toxic potential of TD and its PTPs as well as its HTPs. This deserves further attention as UV irradiation might not always be a green technology, because it might pose a toxicological risk for the environment in general and specifically for water compartments.

1. Introduction

When pharmaceuticals are released into the environment, they can be transformed through many abiotic and biotic processes that can contribute to their degradation and elimination or lead to the formation of transformation products (TPs) (Fatta-Kassinos et al., 2011; Khaleel et al., 2013). Therefore, the removal of pharmaceuticals and their TPs provides a new challenge to treatment systems for drinking water, wastewater and water reuse. Ultraviolet (UV) light treatment is an established method for water disinfection and sterilization (Canonica et al., 2008), It is also in discussion as a technology for wastewater purification (Liberti and Notarnicola, 1999; Meneses et al., 2010). However, photodegradation can lead to phototransformation products (PTPs) which can have more toxic effects than the parent compound investigated for different toxicological endpoints (Vasquez et al., 2013; Wang and Lin, 2012). Therefore, it is important to gather more information about environmental properties of pharmaceuticals and their TPs and to consider this information in environmental risk assessment.

In the early 1960s, Thalidomide (TD) was withdrawn from the market due to its teratogenic effects when given in early pregnancy. In 1998, its use is being revived since the FDA approved TD for the treatment of erythema nodusum leprosum associated with leprosy (Sweetman, 2009). Recently, TD is expected to be a promising drug in the treatment of a number of inflammatory and cancers diseases (Bosch et al., 2008; Sweetman, 2009). Consequently, a potential increased influx of TD into the aquatic environment has to be expected. According to our best knowledge, no study until now has detected TD in the aquatic environment. For sure as a human pharmaceutical the toxic nature of TD has been well investigated, but studies of the toxic effects of TPs are limited in general and even more for TD. In 1994, McBride proposed that TD also may be a human germ cell mutagen based on clinical observations (McBride and Read, 1994). However, Ashby et al. had provided evidence that TD neither exhibited mutagenic responses in different *Salmonella typhimurium* strains (with and without metabolic activation), nor induced chromosome aberration or micronucleus formation *in vivo* and *in vitro* (Ashby et al., 1997). The non-genotoxic properties of the compound were confirmed further by studies from Teo et al. (2000). According to the best knowledge of the authors, there is no information available in published literature regarding the toxicity of TD towards environmental bacteria. The same applies to most of the previously known hydrolytic products (HTPs) and PTPs.

TD is sensitive to hydrolytic decomposition leading to formation of twelve HTPs (Schumacher et al., 1965) (Supplementary material Table S1). The exact metabolic route and fate of thalidomide is unknown, although it appears to undergo non-enzymatic hydrolysis in plasma (Sweetman, 2009). Only the three HTPs which contain the intact phthalimide moiety showed teratogenic activity (Meise et al., 1973).

TD undergoes photolysis using xenon lamp and UV lamp without complete mineralization. New PTPs are formed during photolytic process, including phthalimide (Mahmoud et al., 2013). Phthalimide is classified as a high production volume chemical and it is a degradation intermediate formed from many products. Although phthalimide is readily biodegradable, it was detected in concentrations less than 5 μg/L in the effluent of the wastewater treatment plant of a production site in Japan (OECD, 2005). Phthalimide undergoes hydrolysis in water to ammonia and phthalic acid which is readily biodegradable and also one of the HTPs of TD (Lu et al., 2002).

Generally, experimental toxicity testing of TPs is difficult as many of them are not available commercially. Computer models based on quantitative structure activity relationship (QSAR) are important tools to solve and overcome this problem (European Commission, 2003). Once structure elucidation of any TPs is performed, these structures can be investigated in QSAR programs in order to predict the toxic potential of TPs at different toxicological endpoints and other environmental parameters (Escher et al., 2009).

The aim of this work was to characterize TD and it PTPs after photolysis and monitor their toxicity experimentally in combination with *in silico* QSAR models. The mutagenicity was investigated using the Ames Microplate format (MPF) assay. Moreover, a modified luminescent bacteria test with *Vibrio fischeri* (kinetic luminescent bacteria test, kinetic LBT) was used for an initial screening of microbial toxicity of TD and its PTPs (Menz et al., 2013). Furthermore, phthalimide, one of the identified PTPs of TD, was assessed separately in the kinetic LBT due to the contradiction between different *in silico* software regarding the predicted phthalimide toxicity against *V. Fischeri*.

2. Experimental

2.1. Chemicals

All the chemicals used in this study were of analytical grade. Acetonitrile and Methanol (HiPerSolv CHROMANORM, LC-MS grade, BDH Prolabo), and formic acid were purchased from