



Photolytic transformation products and biological stability of the hydrological tracer Uranine



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HIGHLIGHTS

- Uranine (UR) was not biodegraded in water and water-sediment system (WST).
- Only small degradation rate occurred in OECD 301 D and WST.
- Photolysis leads to incomplete mineralization of UR.
- A total of 5 stable photo-TPs were found for UR, structures were elucidated.
- Similar to the parent compound, only small biodegradation of the photo-TPs was found.

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ABSTRACT

Among many fluorescence tracers, Uranine (sodium fluorescein, UR) has most widely been used in hydrological research. Extensive use of UR for tracing experiments or commercial use might cause a potential risk of long-term environmental contamination. As any organic substance released to the environment, also UR is subjected to chemical and physical reactions that can be chemical, biological and photolysis processes. These processes transform the parent compound (PC) and have not been extensively investigated for UR. This study applies two OECDs (301 D and 301 F) tests and a screening water sediment test (WST) to investigate the biodegradability of the PC. Photolysis in water was explored by Xe lamp irradiation. Subsequently, the biodegradability of the photolysis mixtures was examined. The primary elimination of UR was monitored and structures of its transformation products (TPs) were elucidated by HPLC–FLD–MS/MS. UR was found not readily biodegradable, although small degradation rates could be observed in the OECD 301 D and WST. HPLC–FLD analysis showed high primary elimination of the tracer during photolysis. However, the low degree of mineralization found indicates that the UR was not fully degraded, instead transformed to TPs. A total of 5 photo-TPs were identified. According to MS/MS data, chemical structures could be proposed for all identified photo-TPs. Likewise the parent compound it was demonstrated that photo-TPs were largely recalcitrant to microbial degradation. Although we did not find indications for toxicity, target-oriented studies on the environmental impact of these photo-TPs are warranted. Results obtained in this study show that deeper investigations are necessary to fully understand fate and risk connected to the use of UR.

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1. Introduction

Fluorescent dyes are routinely used as hydrological tracers to monitor surface and subsurface water movement (Käss, 1994; Reichert and

Hoetzel, 1991). One of the most important applications of tracers is to assess the flow pathways and water residence times in the area of drinking water facilities. In hydrological and hydrogeological communities Uranine (sodium fluorescein, UR) is referred to as an ideal, nearly conservative tracer for groundwater studies (Leibundgut et al., 2009; Käss, 1998; Adams and Davis, 1991; Smart and Laidlaw, 1977). UR was first used to trace subsurface flow connections in a karst system in Southwest Germany (Knop, 1878). UR gained popularity because of its low detection limits and ease of analysis at low concentrations, while it is known that it is subject to photodegradation by sunlight (Smart and Laidlaw, 1977; Käss, 1998). Recently, UR was applied as a reference substance to mimic photolytic decay of a contaminant (e.g. pesticide) in surface waters (Lange et al., 2011).

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UR has also widely been used in industry and health care applications, mainly because of its bright green color, high water solubility, moderately low costs and low toxicity (Ikeya et al., 2009; Jean et al., 2007; Smart and Laidlaw, 1977). UR is a complex organic molecule belonging to the xanthene dyes with one of the most intense fluorescence and very high quantum yield value of 0.85–0.94 (Adams and Davis, 1991; Ikeya et al., 2009; Heller et al., 1974; Schmidt, 2005).

Extensive use of UR for tracing experiments or commercial use might cause a potential risk of long-term environmental contamination. As with any organic substances released into the aquatic environment, fluorescence tracers can principally undergo non-biotic and biotic degradation processes such as photolysis, hydrolysis, oxidation and reduction. While photodegradation of UR is well known in surface waters and half-life times have been quantified ($t_{1/2}$ at concentrations of 100 and 10 mg m⁻³ are 38.1 and 33.3 min, respectively), observations of biodegradation are still speculative (Käss, 2004; Kranjc, 1997). Within soils, biodegradation was reported to be negligible, as timeframes typically span only 5–48 h in staining experiments (Alaoui et al., 2011; Anderson et al., 2009; Duwig et al., 2008).

If the degradation of an organic compound is incomplete, transformation products (TPs) are formed (Fenner et al., 2013) which can be more toxic and present at higher concentrations than their parent compounds (PCs) (Mañas et al., 2009; Olsson et al., 2013). However, only few studies have addressed TPs of fluorescence dyes so far. Gombert & Carre (2011) have highlighted formation of UR TPs and other popular tracers in lab scale simulated water treatment processes (with gaseous chlorine and UV/visible light irradiation) with their HPLC analysis of the water samples. However, they only identified one TP for Tinopal and none for UR by LC–MS.

According to Ishibashi (1965), possible photo-decomposition products of UR are phthalic acid and resorcinol. The latter one appears to be no mutagenic, as found by Heddle et al. (1983). General toxicity evaluations classify UR as a safe tracer (e.g. Leibundgut & Hadi, 1997; Behrens et al., 2001) but rarely distinguish between experiments with and without light exposure. Hence it is not clear if photolytic TPs are included or not. In 48 h tests Tonogai et al. (1978) found no acute toxicity to fish (*Oryzias latipes*) for UR and its photolytic TPs. At the same time, toxicity of the halogenated dyes increased through irradiation. For 10 days Walthall & Stark (1999) exposed *Daphnia pulex* to UR at a light–dark regimen and found chronic mortality at UR concentrations in excess of 0.25 g L⁻¹. Recently, Gombert & Carre (2011) exposed rats, *Daphnia magna* and micro-algae (*Pseudokirchneriella subcapitata*) to unidentified mixture of degradation products of UR and other fluorescent tracers at initial concentration of 1 g L⁻¹ and found no acute toxicity and only moderate ecotoxicity for the tracer sodium naphthionate. Generally, concentrations used during toxicity testing are rarely reached during tracing experiments, since UR has a distinct green color already at concentrations of 1 mg L⁻¹.

Since synthetic organic dyes (e.g. monoazo, diazo, anthraquinone, triphenylmethane dyes) are prominent water pollutants, their removal from wastewater has attracted various research groups (e.g. Muhammad et al., 2012). Biodegradation by microorganisms, particularly by fungi, is an effective method (e.g. Knapp et al., 1995; Novotný et al., 2004). Most knowledge exists for azo dyes, xanthene dyes like UR are less prominent in waste water and hence underrepresented in research.

This is especially true for TP formation. During photolysis, which is omnipresent for UR, TPs may be formed following radical reactions. However, knowledge regarding their fate and properties is very limited. Furthermore, if these TPs turn out to be persistent, they will be of special interest for environmental risk assessment. Laboratory tests to identify the combined effect of photolysis and aerobic biodegradation on the formation of persistent TPs were successfully applied for two formulations of herbicide pesticides (Gutowski et al., 2014). However such, studies have not yet been performed for UR.

A combination of photolysis under simulated sunlight irradiation, two biodegradation tests (Closed Bottle test and Manometric Respiratory

test, OECDs 301 D, F) and a water sediment test was carried out to evaluate the primary elimination of UR monitored by high performance liquid chromatography with fluorescence detector (HPLC–FLD). The degree of mineralization was evaluated by means of non-purgeable organic carbon (NPOC) analysis. Photo-TPs were analyzed in terms of ready biodegradability, and their structures were elucidated and identified with liquid chromatography tandem mass spectrometry (LC–FLD–MS/MS).

In the newly developed water sediment test (WST) (Baginska et al., 2015) a complex matrix (i.e., water–sediment interface) was introduced to increase reproducibility and stability of the test system. This allows one to investigate processes like biodegradation, sorption, elimination from water phase, and abiotic degradation in one set.

2. Materials and methods

2.1. Chemicals

The analytical standard of UR (98.5–100.5% chemical purity, CAS Nr. 518-47-8) was obtained from Fluka (Sigma-Aldrich, Steinheim, Germany). HPLC grade acetonitrile (CAS Nr. 75-05-8) and ammonium acetate (CAS Nr. 631-61-8) were purchased from VWR (VWR International, GmbH, Darmstadt, Germany). Aniline (CAS Nr. 62-53-3) was purchased from the same supplier; calcium carbonate (CAS Nr. 471-34-1), quartz (CAS Nr. 14808-60-7) and clay (CAS Nr. 1318-74-4) were purchased from Carl Roth, Germany. Sodium azide (CAS Nr. 26628-22-8) was purchased from Sigma-Aldrich, Germany. Peat (from *Sphagnum Moss*) was obtained from Aurich-Wiesmoor-Torfvertriebs-GMBH, Germany. All aqueous solutions were prepared using ultrapure water 18.2 MΩ cm (Ultra Clear UV TM, Barsbüttel, Germany).

2.2. Sunlight simulated photolysis experiments in aqueous solution

UR solutions were dissolved in ultrapure water the day prior to the experiment and stored in the dark. UR was subjected to the photolysis at three initial concentrations of 10 mg L⁻¹, 20 mg L⁻¹ and 60 mg L⁻¹. 800 ml of the test solution was transferred to the photo-reactor under gentle stirring using a magnetic stirrer. Temperature was set to 20–22 °C controlled by circulating cooler (WKL230, LAUDA, Berlin). Photolysis in water was performed in an ilmasil quartz immersion tube using a xenon lamp (TXE 150, UV consulting Peschl, Mainz, Germany) as a radiation source. The lamp emits spectra similar to natural sun light 200–800 nm with the highest intensity in the visible range (200–280 nm: 1.61 e⁻² W/m², 280–315 nm: 1.16 e⁻² W/m², 315–380 nm: 3.75 e⁻² W/m², 380–780 nm: 5.58 e⁻¹ W/m²) (data provided by the manufacturer). Before every experiment the lamp was warmed up for 3 min to reach its maximum intensity. Photolysis experiments were performed for 8.0 h in order to mimic an average daily sunshine duration from sunrise to sunset. Samples were collected every hour for HPLC and LC–MS/MS analysis. Samples for NPOC determination were collected at the time increments of 0.0 h, 4.0 h and 8.0 h.

Samples before (0.0 h) and after (8.0 h) photolysis were collected and subsequently submitted to the ready biodegradability tests: Closed Bottle test (CBT), Manometric Respiratory test (MRT) and to screening WST. The final concentration of UR was adjusted by measuring NPOC of the tested substance (i.e. before photolysis) and photolysis treated samples, to provide required carbon content, and to reach adequate theoretical oxygen demand (ThOD), for each CBT, MRT and WST respectively (described further in Sections 2.3 and 2.4). In parallel to every experiment, a HPLC analysis was run to support the NPOC measurements and to determine primary elimination of the parent compound.

2.3. Closed Bottle test (OECD 301 D)

CBT was performed according to the guidelines of the Organization for Economic Co-operation and Development OECD (1992). This test is

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