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Biodegradability of the antioxidant diaryl-p-phenylene diamine using a modified inherent biodegradation method at an environmentally relevant concentration

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HIGHLIGHTS

- The antioxidant DAPD was degraded in a modified inherent biodegradability test.
- No parent compound was measured after 28 d.
- 37% Mineralisation was measured after 63 d and 29% incorporation into biomass.
- Silica gel and surfactant were used to increase bioavailability.

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ABSTRACT

The chemical product diaryl-p-phenylene diamine (DAPD), produced by The Goodyear Tire & Rubber Company as POLYSTAY 100® (CAS 68953-84-4), is employed as an antidegradant in polymers used in tires and industrial rubber products. Previous evaluations pertaining to the ecological fate of DAPD indicated a lack of biodegradative activity in aquatic media. In order to further pursue the biodegradation potential of DAPD, it was deemed necessary to enhance the sensitivity of the aquatic biodegradation assay through (a) employment of a radiotracer of the test substance, and (b) optimisation of conditions for achieving maximal solubilisation of test material in the aquatic media of the incubation vessels. Test vessels were prepared according to the OECD ready biodegradability test guidelines, with DAPD added on silica gel at concentrations of 10 or 100 μg L^{-1}, together with a surfactant to aid solubilisation. After 63 d incubation up to 37% mineralisation was measured and up to 29% of the applied radioactivity was incorporated into cell biomass. Also, after 28 d no DAPD could be measured in solution by radio-TLC and HPLC–MS. These three results demonstrate that the antioxidant DAPD undergoes microbiologically mediated biodegradation and is highly unlikely to persist in the environment.

1. Introduction

The chemical product diaryl-p-phenylene diamine (DAPD), produced by The Goodyear Tire & Rubber Company as POLYSTAY 100® (CAS 68953-84-4), is employed as an antidegradant in polymers used in tires and industrial rubber products. Historically, various aromatic amines have served this purpose, but one substance used previously in this application (2-naphthylamine) was considered an occupational hazard due to its carcinogenic activity (NTP, 2011). In contrast, DAPD has been subjected to chronic toxicity studies, and exhibited no evidence of carcinogenicity or other significant long-term health effects (Iatropoulos et al., 1997).

More than eighty percent of the manufactured product consists of three constituents with the structures below (Fig. 1). In contemporary terminology, it is considered to be a "multi constituent substance" (ECHA, 2008). In addition to these three components, the product contains <20% of higher molecular weight compounds and trace levels of starting reactants, e.g., aniline.

Previous evaluations assessing the ecological fate of DAPD in a standard biodegradation test indicated a lack of biodegradative activity in aquatic media (Ricerca, 1995). Important physical properties that plausibly influenced this inactivity in standard assays include the components' low water solubilities (<1 mg L^{-1}), high log*K*ow values (⩾3.3), and elevated log*K*oc values (>4.3) (Chemex, 2010a). These chemical structures are not compatible with abiotic hydrolysis. Known oxidation products of many aromatic amines are phenolic and quinone substances, which have higher polarity

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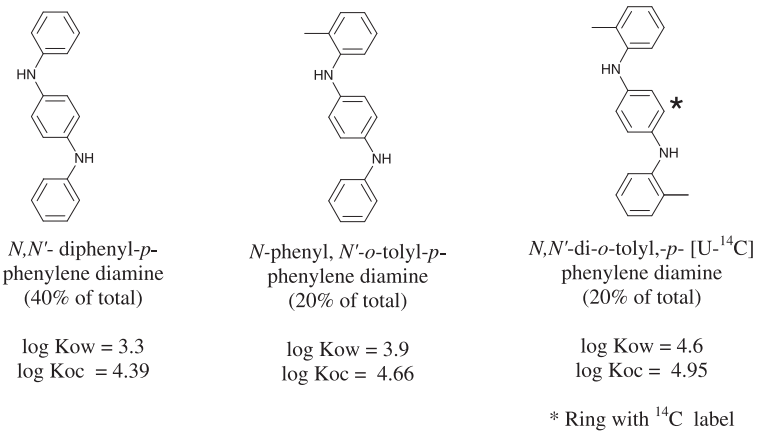

Fig. 1. Components of POLYSTAY 100®.

and lower partition coefficients than the parent compound, leading to the possibility that these compounds are more biodegradable (Kuczkowski, 2003). In a fish bioaccumulation test performed with radiolabelled R898, rapid depuration of radioactivity was observed ($t½ < 5$ d), consistent with the formation of a polar metabolite(s) (Brixham Environmental Laboratory, 2011). Additional work is required to identify metabolic products occurring in the fish. Past attempts to assess the microbial biodegradation of the chemical according to standard testing guidelines, such as the OECD 301B carbon dioxide evolution test (OECD, 1992) have not shown activity in sludge-inoculated incubation media from sewage treatment plants. The method included exposure of 30 mg test chemical per liter in biodegradation incubation flasks which greatly exceeded the water solubility of the chemical's constituents. The absence of measurable CO_2 release in this assay indicated negligible ultimate degradation under these assay conditions (Ricerca, 1995). No assessments of primary degradation i.e. removal of DAPD were made.

Toxicity testing in aquatic and terrestrial tests has shown the toxicity of DAPD is very different in the presence or absence of sediment or soil. Previous testing in aquatic toxicity tests to algae, daphnia and fish displayed high toxicities (EC50s < 1 mg L^{-1}) (Chemex, 2010b; Springborn Laboratories, 1995a,b, 1996, 1997a). However, testing of the chemical in the presence of sediment and soil totally eliminated toxic activities (NOECs = 1000 mg kg^{-1} soil and sediment) in resident species (chironomids and earthworms) (Chemex, 2010c; Mambo-Tox Ltd, 2010a, 2010b). These toxicity data suggest that the strong adsorption of DAPD (supported by log*K*oc values $\geqslant$ 4.39) to sediment in aquatic systems attenuates the chemical's presence in the water column, and the level of toxicity of the chemical in standard laboratory aquatic testing in the absence of sediment lead to results that do not represent *bona fide* ecological conditions. While chironomid and earthworm species may be more resistant to DAPD exposures and toxicity than aquatic species, the very marked potencies observed for the aquatic species suggest other factors are important in the attenuation of effects observed in soil/sediment species.

In order to further pursue the biodegradation assessment of DAPD, it was deemed necessary to enhance the sensitivity of aquatic biodegradation testing through (a) employment of a radiotracer of the test substance, and (b) optimisation of conditions for achieving maximal solubilisation of test material in the aquatic media of incubation vessels. The former was done through the *de novo* synthesis of carbon-14 labeled R-898 (di-o-tolyl-p-phenylene diamine, CAS 15017-02-4) with the central ring being the site of carbon 14 labeling. A combination of surfactant plus silica gel substrate was employed to enhance solubilisation of the radiolabelled test chemical in the aquatic media as permitted by the REACH technical guidance for degradation and persistence assessments (ECHA, 2008). The choice of R-898, which constitutes approximately 20% by mass of the antioxidant POLYSTAY 100®, as the sentinel chemical for DAPD for this assay was based upon the fact that R-898 is the component with the highest partition coefficient and lowest water solubility, and was projected to be the least likely to biodegrade due to its higher degree of methyl substitution compared to other product components. Previous laboratory assays using standard ready biodegradability methods showed DAPD was not readily biodegradable, raising the possibility that it may be persistent in the environment. However, due to the known abiotic oxidative degradation of this substance in its role as a polymer antioxidant, it was considered important to reassess degradation of DAPD including modifications and enhancements described in the REACH guidance (ECHA, 2008). The modifications chosen were (a) testing at a low concentration using a radioisotope, (b) adding the test substance on an inert support (silica to enhance surface area exposure to incubation media), and (c) use of a surfactant to elevate solubilisation. The test was also enhanced by increasing exposure time to 63 d, plus use of one treatment (5 replicates) with a higher inoculum concentration.

2. Materials and methods

2.1. Materials

Radiolabelled N,N′-di-o-tolyl,-p-[U-^{14}C]phenylene diamine (R-898; CAS 15017-02-4) was obtained from Selcia Limited, Ongar, UK and had a specific activity of 18.7 μCi mg^{-1}. The radiochemical purity of the R-898 was determined as 95.5%. Silica gel, Synperonic PE105 surfactant, and mineral salts were obtained from Sigma–Aldrich, Poole, UK. Reverse osmosis water with a conductivity of <15 μS m^{-1} was obtained from the in-house reverse osmosis system.

Radiolabelled benzoic acid was obtained from American Radiolabeled Chemicals, St. Louis, USA, and was used as a positive control substance, to demonstrate activity of the inoculum. This was combined with non-radiolabelled sodium benzoate from Sigma–Aldrich, Poole, UK before dosing, to adjust the amount of radioactivity to the required test concentration.

Activated sludge was collected from the aeration basin of a waste water treatment plant at Totnes, Devon, UK, a plant which treats sewage of predominantly domestic origin.

The mineral medium was made up according to the OECD 302C guideline (OECD, 1981) and contained the following nutrients per litre of reverse osmosis water: 25.5 mg of KH_2PO_4, 65.25 mg of