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Short Communication

Reductive dehalogenation of DDT with folate models: Formation of the DDT metabolite spectrum under biomimetic conditions

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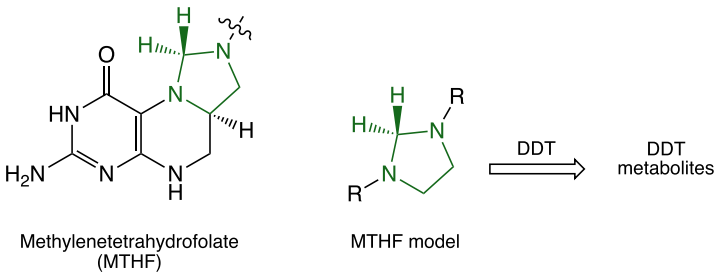
HIGHLIGHTS

- The interactions between MTHF model compounds and DDT are studied *in vitro*.
- The full metabolite spectrum of DDT is obtained under aerobic and anaerobic conditions.
- The degradation pathway of each individual DDT metabolite is mapped.
- Formation of the metabolite DBP does not require cytochrome P450.

GRAPHICAL ABSTRACT



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ABSTRACT

The insecticide DDT is an omnipresent environmental contaminant and an ongoing toxicological concern. The recent discovery that methylenetetrahydrofolate (MTHF) models are capable of reducing a range of halocarbons to hydrocarbons under biomimetic conditions has prompted us to investigate the possible role of MTHF in the metabolism of DDT. We now report that the reaction of MTHF models with DDT produces no less than five known *in vivo* metabolites of DDT, namely DDD, DDE, DDMU, DBP, and DDM. The capability of the MTHF models to produce the full spectrum of known DDT dehalogenation products is strong evidence that the mechanistically obscure metabolism of DDT may involve MTHF. The findings also suggest that DDT should be capable of disrupting folate-dependent pathways.

1. Introduction

First described in 1874 (Zeidler, 1874), DDT only gained widespread use after Paul Herrmann Müller identified it as a highly potent insecticide (Läuger et al., 1944). The use of DDT as an effective suppressant for insect-borne diseases, such as typhus and malaria, was recognized by the Nobel Prize for medicine in 1948. The ability of DDT (Bitman et al., 1968) and its metabolites to act as endocrinological disruptors towards estrogen receptors (Bitman et al., 1968; Gellert et al., 1972) and androgen receptors (Kelce et al., 1995) resulted in the ban of DDT in the United States and most other countries. Despite legal restrictions on its production and use, the great persistence of DDT and its metabolites in the environment represents an ongoing concern (Longnecker et al., 1997; Beard, 2006; Tiemann, 2008), which has made DDT one of

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Abbreviations

DDT	1,1,1-trichloro-2,2-*bis*(4-chlorophenyl)ethane
DDD	1,1-dichloro-2,2-*bis*(4-chlorophenyl)ethane
DDE	1,1-dichloro-2,2-*bis*(4-chlorophenyl)ethene
DDMU	1-chloro-2,2-*bis*(4-chlorophenyl)ethene
DDM	*bis*(4-chlorophenyl)methane
DBP	4,4′-dichlorobenzophenone

the most studied insecticides from both environmental and toxicological perspectives.

While details of the metabolic degradation of DDT depend on the organism, the reduction of carbon-chlorine bonds is common for all organisms studied to date (Beard, 2006; Smith, 1962; Kallman and Andrews, 1963). The existence of this reductive pathway was demonstrated by Kallman & Andrews through *in vivo* studies with yeasts as early as 1963. In 1964, Datta, Laug, and Klein confirmed the operation of the dehalogenation metabolism for DDT in mammals (Datta et al., 1964). Subsequent studies demonstrated the dominance of the dehalogenation pathway for a wide range of chlorinated halocarbons, in addition to DDT (Mohn and Tiedje, 1992). Remarkably, the agent involved in the reductive dehalogenation of DDT has remained obscure.

Reduced cytochromes were postulated as reducing factors by Wedemeyer in 1966 (Wedemeyer, 1966), and the subsequent finding that simple iron porphyrin models are capable of reducing DDT *in vitro* (Miskus et al., 1965; Castro, 1964; Mansuy et al., 1978) can be taken as supporting evidence. Nicotinamide adenine dinucleotide phosphate (NADPH) was implied as the metabolic reducing agent by K. A. Hassel in 1972 (Hassel, 1972), while subsequent studies favoured the involvement of flavin adenine dinucleotide (FAD) (Esaac and Matsumura, 1980; Sugihara et al., 1998; Wedemeyer, 1967). Remarkably, the ubiquitous hydride donor methylenetetrahydrofolate (MTHF) **2H** does not seem to have been implicated in the reductive metabolism of DDT, or indeed any other halocarbon.

2. Results and discussion

The starting point of our investigation was the surprising discovery that MTHF models **1H** (Fig. 1) are capable of reducing a wide variety of simple halocarbons to the respective hydrocarbons under biomimetic conditions (Denk et al., 2017). The findings suggested that DDT might likewise be reduced by the MTHF models. We now show that simple MTHF models **1H** are not only capable of reducing DDT under biomimetic conditions, but also produce the five most common DDT metabolites. Using two standard protocols, one aerobic and one anaerobic, the reduction of DDT by the MTHF models **1H** (R: *tert*-butyl, *para*-tolyl) formed the metabolites **4**–**8** identified by previous *in vivo* studies (Fig. 2).

Comparison of aerobic and anaerobic conditions revealed that reaction of DDT and **1H** gave **4**, **5**, **6**, and **7** under anaerobic conditions. Under aerobic conditions (Subba-Rao and Alexander, 1985; Langlois et al., 1970), metabolite **8** has been observed as well (Langlois et al., 1970) (Scheme 1). The formation of DBP **8** is of particular interest as it has previously been attributed to the oxidation of DDT by cytochrome P450 (Xiao et al., 2011). Our observations show that **8** can be obtained from MTHF models **1H** under aerobic conditions without the involvement of cytochrome P450.

To map the degradation pathways in detail, the reactions of the

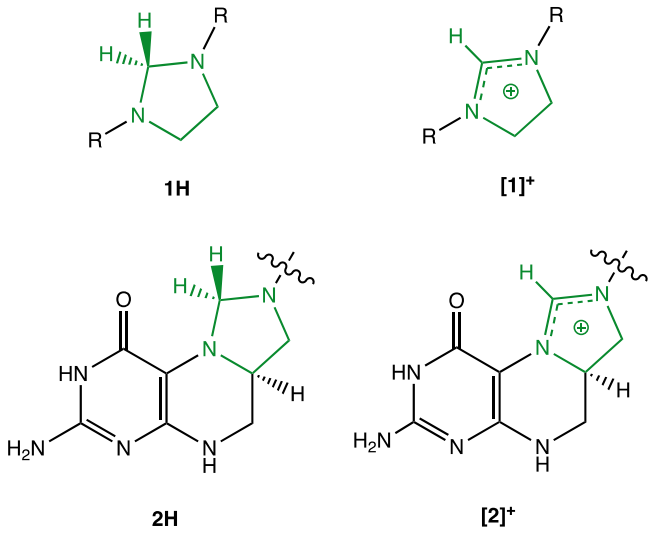

Fig. 1. Structural relationship between MTHF models **1H**, methylenetetrahydrofolate (MTHF) **2H**, and the oxidized forms $[\mathbf{1}]^+$ and $[\mathbf{2}]^+$.

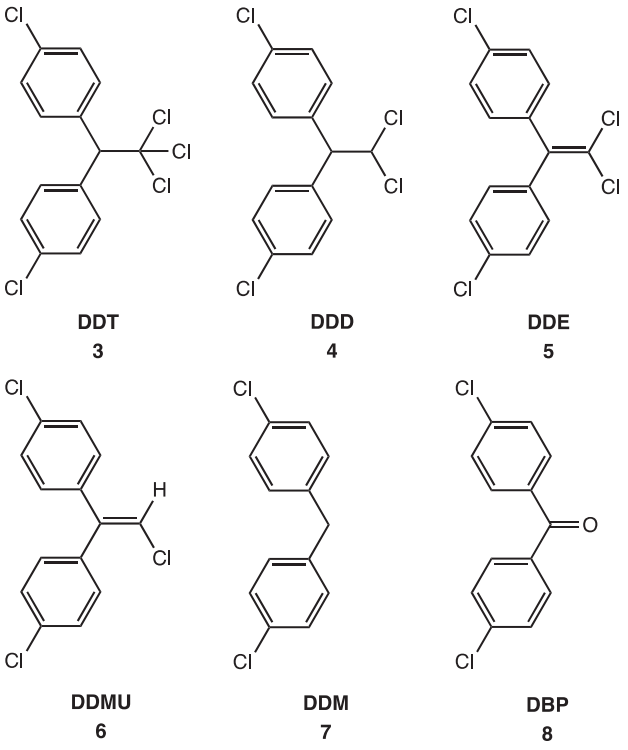

Fig. 2. DDT (1,1,1-trichloro-2,2-*bis*(4-chlorophenyl)ethane **3**, and its metabolites DDD (1,1-dichloro-2,2-*bis*(4-chlorophenyl)ethane) **4**, DDE (1,1-dichloro-2,2-*bis*(4-chlorophenyl)ethene) **5**, DDMU (1-chloro-2,2-*bis*(4-chlorophenyl)ethene) **6**, DDM (*bis*(4-chlorophenyl)methane) **7**, and DBP (4,4′-dichlorobenzophenone) **8**.

individual DDT metabolites **4**–**8** with **1H** (R: *tert*-butyl) were examined. The observed degradation pathways are summarized in Scheme 1. DDMU **6** forms from both DDD **4** and DDE **5** and is the final stage of the degradation processes under both aerobic and anaerobic conditions.

Surprisingly, DDM **7** does not form from any of the metabolites **4**, **5**, **6**, **8**, and must accordingly form directly from DDT **3**. DBP **8** is obtained under aerobic conditions from DDT and from the pure metabolites **4**–**6**. However, as its formation from DDT is much