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OVERVIEW OF THE BIOREMEDIATION AND THE DEGRADATION PATHWAYS OF DDT

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Abstract
DDT is a persistent pesticide that was banned in most countries due to its significant environmental and human health hazards. Bioremediation has the potential for in-situ treatment of DDT contaminated sediment and soils. With the right microorganisms and conditions, DDT and its primary metabolites, DDD and DDE can be degraded into 4-chlorobenzoic acid or 4,4- dichlorobenzophenone under aerobic and anaerobic conditions, respectively. The extent of degradation and time required will depend on the initial concentration, respiration mode and microorganisms present. This review provides brief overview of bioremediation and discusses some of the key degradation pathways of DDT.
Key Words: bioremediation, DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane, degradation, metabolism

Introduction

DDT (1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane) is an organochlorine pesticide also called dichloro-diphenyl trichloroethane. It was first synthesized in 1874 by Othmer Zeidler, a German doctoral student (Proskauer, 1992). DDT usage did not become widespread until after World War II for the control of malaria and agricultural pests. Its use in many industrialized countries was banned by the early 1980'2 due to its recalcitrance and many environmental and health hazards (Lackmann et al., 2004). A few examples of the environmental impacts include thinning of bird eggshells, altering reproductive development in reptiles and being an endocrine disruptor (Barreto-Castro et al., 2010; Nadeau et al., 2008; Purnomo et al., 2010; Thomas et al., 2011). The human health effects from DDT span causing leukemia, being an endocrine disrupter, causing early pregnancy loss to being a suspected carcinogen (Aislabie et al., 1997; Bidlan and Mannmani 2002; Chikuni et al., 2002; Liu et al., 2008; Thomas et al., 2011; Van Zwieten et al., 2003).

Although banned more than 30 years ago, DDT contamination is still widely prevalent. This is in part due to the fact that DDT has a reported half-life of 4-35 years (Corona-Cruz et al., 1999; Muendo et al., 2012; Toan et al., 2009; Wang et al., 2007), with the specific half-life being dependent on the type of soil. Only areas of tropical climates have reported DDT half-lives less than one year (Van den Berg, 2009). In addition, three countries still produce DDT commercially. India, China and North Korea produce DDT for the control of malaria in Africa as well as a raw material in dicofol (Morisawa et al., 2002; Turgut et al., 2009; van den Berg et al., 2009). Bioremediation is being studied by numerous scientists world wide as a viable remediation method for DDT. A recent study by Sudharshan et al. (2012) provides an overview of the different bioremediation schemes of DDT and other key persistent organic pollutants. This paper will provide a brief introduction on the requirements for bioremediation and examples of the key metabolic pathways for DDT contaminated soils.

Basic Requirements for Bioremediation

Bioremediation is the process of altering a contaminant using a living organism, such as bacteria, fungi and plants. Mineralization is the complete conversion of an organic compound such as DDT, into biomass, carbon dioxide, salts and water. Complete mineralization can occur given the correct environmental parameters, bacteria and sufficient time. Biodegradation/bioremediation is used to describe any stage of the contaminant breakdown prior to complete mineralization. The primary requirement for any successful bioremediation treatment is the presence of an adequate microbial population. Soils with a viable microbial density will have at least 10^4 colony forming units (CFU)/g of soil. It is important to note that when dealing with recalcitrant compounds such as DDT, simply verifying the total microbial count will not be sufficient. Instead, having at least 10^4 CFU/g of active degraders will be required. An active degrader refers to the ability of the microbe to use the target compound as the primary carbon source (i.e., use DDT). The extent of bioremediation that can be achieved will also depend on complexity of the target compound and the presence of key factors to key the microbe(s) alive.

If only nutrients and/or a terminal electron acceptor (TEA) are added, the process is referred to as biostimulation. Bioaugmentation refers to treatments that also add a foreign microbial source. It is important to note that when dealing with compounds such as DDT one cannot simply track the disappearance of the parent compound as intermediates may be toxic. DDD (1,1-dichloro-2,2-(4-chlorophenyl)ethane) and DDE (1,1-dichloro-2,2-(4-chlorophenyl)ethylene), the key degradation byproducts of DDT, are highly toxic and more recalcitrant than DDT (Gautam and Suresh, 2009).

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Regardless of the approach, several key parameters have to be in place for bioremediation to be successful. For normal microbial functioning the appropriate TEA, pH, temperature, nutrients and moisture must be present as well as the absence of toxic material(s). Often the nutrients and/or foreign microbial source are added in a water solution thereby maintaining the proper moisture level. The toxic material may be a different contaminant, an intermediate by product or even another microbial species. The specific growth requirements can also be used to delineate the type of microorganisms. For instance, microbes that require oxygen as their TEA (i.e., respiration mode) are classified as aerobic while those that do not require oxygen as their TEA are anaerobic. Those species that can switch their respiration between aerobic and anaerobic depending on the TEA available are called facultative species. Additional, more in-depth details on the approaches to bioremediation can be found in Cookson (1995), Cutright (2005) and Eweis et al. (1998).

The bioavailability of the contaminant will also impact the extent of bioremediation (Baczynski et al., 2012). Bioavailability is dependent on the contaminant characteristics (solubility, K_{ow}, hydrophobicity, etc.) and soil type (extent and type of organic matter and clay), which may tightly bind DDT. For instance, clays with a higher organic content were found to sorb more DDT than clays with a lower organic faction (Dai et al., 2008).

A majority of the available literature simply report non-identified microbial or fungal species isolated from DDT contaminated soil. Often the identification of fungi is limited to classification of either white rot or brown rot fungi. Each of these fungi classifications have over three hundred different species. A partial listing of known DDT degraders by bacteria and fungi are shown in Table 1 and 2, respectively. Recent research has found that certain plants can assist with the degradation of DDT (Huang et al., 2007; Mo et al., 2008;Whitefield-Aslund et al., 2010). Even though phytoremediation (i.e., use of plants to facilitate treatment) is a subset of bioremediation, only the pathways associated with microbial (bacteria or fungi) degradation will be presented.

Degradation Pathways of DDT

The most frequently proposed reactions during DDT degradation are: reductive dechlorination, dehydrohalogenation, dioxygenation, hydroxylation,

Table 1. Partial list of bacteria that involved in at least one step of DDT degradation

Species	Respiration Mode	Reference
Alcaligenes sp. DG5	Aerobic	Gao et al., 2011
Alcaligenes sp.	Anaerobic	Beunink and Rehm 1988
	Aerobic	Xie et al., 2011
Alcaligenes denitrificans ITRC-4	Facultative	Ahuja and Kumar, 2003
Bacillus cereus	Aerobic	Mwangi et al., 2010
Clostridium sp.	Anaerobic	Bao et al. 2012
Eubacterium limosum	Anearobic	Sudharshan et al., 2012
Flavimonas oryzihabitans	Aerobic	Barragan-Huerta et al., 2007
Methanogenic granular sludge	Anaerobic	Baczynski et al., 2010
Mixed sediment consortium	Anaerobic	Chiu et al., 2004
Pseudomonas putida	Aerobic	Barragan-Huerta et al., 2007; Gautam and Suresh, 2009
Pseudoxanthomonas sp wax DT-1P	Aerobic	Wang et al., 2010
Pseudoxanthomonas jiangsuensis	Aerobic	Wang et al., 2011
Serratia mercascens DT-1P	Aerobic	Bidlan et al., 2002
Shewanella decolorationis S12	Anaerobic	Li et al., 2010
Sphingobacterium sp D6	Aerobic	Fang et al., 2010

Table 2. Partial list of fungi that involved in at least one step of DDT degradation

Species	Respiration Mode	Reference
Anaerobic sludge	Anaerobic	You et al., 1996
Brown rot fungi	Aerobic	Purnomo et al., 2011
Daedalea dickinsii	Aerobic	Purnomo et al., 2010
Fomitopsis pinicola	Aerobic	Purnomo et al., 2010
Fusarium solani	Aerobic	Mitra et al., 2001
Phanerochaete chrysosporium	Facultative	Corona-Cruz et al., 1999
Phanerochaete chrysosporium	Aerobic	Bumpas et al., 1993; Kaplan 1992; Thomas and Gohil, 2011
*Phlebia brevispora*TMIC34596	Aerobic	Xiao et al., 2011
Phlebia lindtneri GB-1027	Aerobic	Xiao et al., 2011
Pleurotus ostreatus	Aerobic	Purnomo et al., 2010
Trametes versicolor	Aerobic	Sari et al., 2012
White rot fungi	Aerobic	Zhao et al., 2010
Wood rot fungi	Aerobic	Thomas and Gohil, 2011

hydrogenation and meta-ring cleavage (attack between the 2,3 carbons on the ring structure). The first primary intermediate of DDT from the aforementioned reactions is either DDD or DDE; the occurrence will depend on the respiration mode and microbe being used. Most studies cite that DDD is the most common anaerobic metabolite while DDE is associated with aerobic conditions (Hay and Focht, 2000; Liu et al., 2008). However, as will be shown below the metabolic pathway and degradation mechanism is bacteria/fungi specific.

It is critical to keep in mind that not all laboratory studies will be able to be duplicated in the field. For instance, several researchers have found DDT will degrade more rapidly to DDD anaerobically. In a laboratory setting it will be easier to control the environment to facilitate the required respiration mode. It will be difficult, if not impossible, to maintain an anaerobic environment in the field. If the desired bacteria/fungi requires a strict anaerobic environment, in situ applications will most likely occur only after DDT has migrated to lower soil horizons or sediments (Muendo et al., 2012) or in the occurrence of high contaminant levels. Natural aerobic environments are associated with the vadose (~top three feet), as well as low levels of contamination.

Aerobic Degradation

In a historically contaminated soil, in-situ aerobic treatment would occur in the vadose zone. Nadeau et al. (1998) reported the first aerobic degradation of DDT, a modified version is shown in Figure 2. Here DDT metabolism was initiated by directly attacking the ring structure, via oxygenation to form 2,3-dihydrodiol DDT. After forming 2,3-dihydroxy DDT, meta-cleavage occurred with successive steps leading to the formation of 4-chlorobenzoic acid (4-CBA). The incorporation of two oxygen molecules requires the presence of dioxygenase enzymes. Thus the bacteria that can initiate DDT degradation by attacking the ring structure are hypothesized to produce dioxygenases. In some instances the production of specific enzymes can be influence by a secondary carbon source. Aerobic degradation with bacteria strain KK, later identified as *Alcaligenes sp*., was effective at degrading 65% DDT present. The degradation rate was greatly influenced by presence of 0.5% glucose (Xie et al., 2011). Although glucose inhibited DDT degradation by *Serratia marcaescens* DT-1P, degradation was significantly enhanced when a mixture of yeast, peptone and tryptic soy broth was present (Bidlan and Manonmani, 2002).

If dechlorination had been the first step of attack,

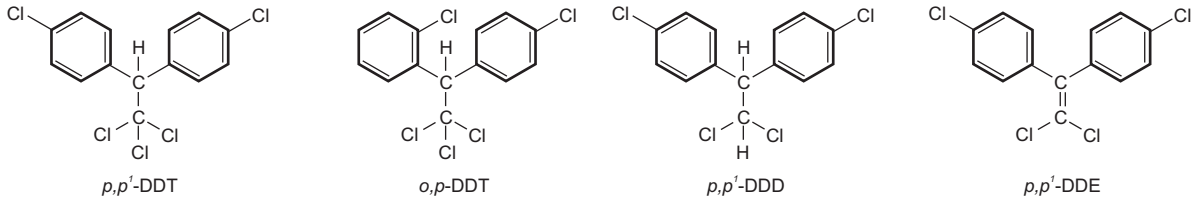

Figure .1 Structure of DDT and its metabolites. (Aislabie et al., 1997; Thomas and Gohil, 2011).

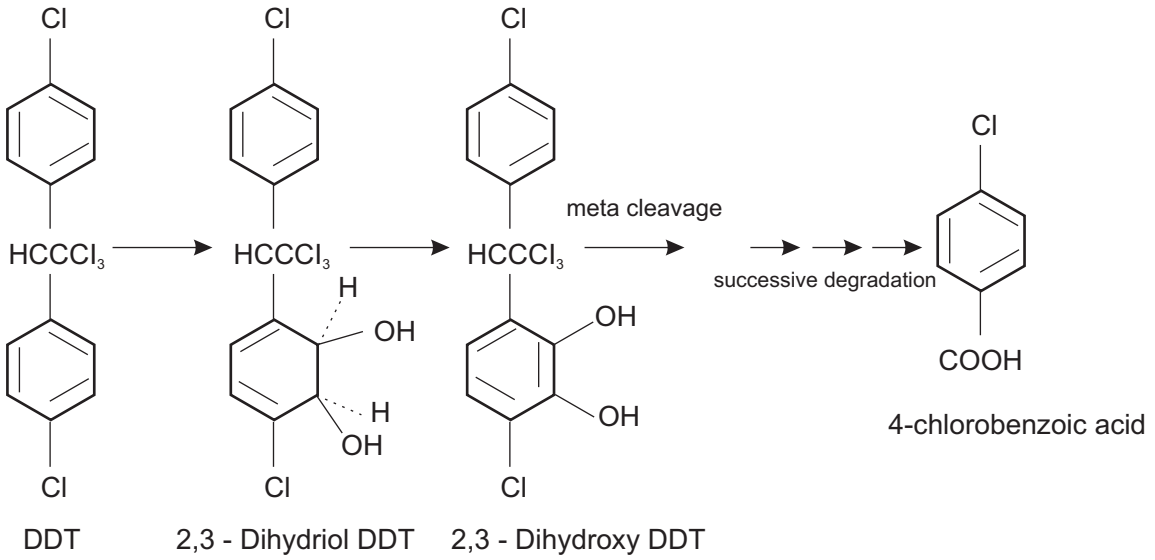

Figure 2. Modified aerobic degradation pathway of DDT as described by Nadeau et al. (1998)

it would have occurred at the alkyl chain. In this pathway, the removal of the chlorine leaves the alkyl chain unstable leading to the double bond in p,p'-DDE (Xie et al., 2011). In some instances, the double bond is more stable and thereby more difficult to degrade leaving p,p'-DDE an end product (Nguyen et al., 2011). The subsequent degradation of DDE (as with other intermediates) will require a consortium of bacteria (Mwangi et al., 2010; Wang et al., 2010). For instance, Hay and Focht (2000) reported that DDE could only be degraded when *Pseudomonas acidovorans* M3GY was added after another microbe had initiated DDT degradation. Research by Megharaj et al. (1998) also found that DDE could be degraded aerobically, but at a much slower rate.

As shown in Table 2, certain fungal strains can also degraded DDT under aerobic conditions. A modified pathway by Xiao et al (2011) via a combined culture of *P. lindtneri* and *P. brevispora* that could degrade 15.5 µmol/L DDT in 21 days is shown in Figure 3. This is a novel pathway in that the first metabolite under aerobic conditions is DDD. This is followed by several non-identified intermediates prior to the occurrence of DDA (2,2-bis(4-chlorophenyl)acetic acid) shown in Figure 3. After several successive steps, DBP (4,4-dichlorobenzophenone) would be the next key intermediate. At this point, DBP can be further degraded via two different routes, both of which lead to 4-dichlorobenzoic acid (4-DCB). Most of the other fungal pathways reported are based on the initial work by Bumpus et al. (1993) where both DDD and DDE can be formed. In that pathway DDD undergoes hydroxylation to form dicofol before being further metabolized to 4-DCB. As such, 4-DCB is often viewed as the 'end-product' of DDT. Although studies have documented the successful degradation of DBP, they were initiated with DBP and not DDT (McCullar et al., 2002).

Anaerobic Degradation

Rapid DDT degradation is typically associated anaerobic conditions via reductive dechlorination (Xiao et al., 2011). This mode of attack is usually restricted to the alkyl chain. For instance, 74% of 5.8 mM DDT underwent reductive dechlorination to DDD under anaerobic conditions by *Clostridium sp.* BXM (Bao et al., 2012). An unidentified anaerobic sediment culture was able to degrade 10µg/L DDT into DDD within 15 days (Chiu et al., 2004). Baczynski et al. (2010) reported that 19 mg/kg DDT was converted into DDD within the first two weeks. Although both DDD and DDE can be formed under anaerobic conditions (depending on the microbe used), DDE is not desirable as it can be more resistant to subsequent treatment (Barragn-Huerta et al., 2007; Van Zwieten et al., 2003).

A representative anaerobic pathway with DDD as the primary initial metabolite is shown in Figure 4 (You et al., 1996). This pathway is one of the most complete in that it identifies seven key intermediates before the formation of DBP (4,4-dichlorobenzophenone). DBP is often considered the 'end product' under anaerobic conditions (Baczynski et al., 2010). Fang et al. (2010) reported a similar pathway for their study with *Sphingobactrium sp*., which could degrade 12.9% of the DDT present in 90 days. A recent study by Yu et al. (2011) documented the formation of these metabolites in-situ for contaminated sediments under anaerobic conditions via both dehydrochlorination and reductive dechlorination of the alkyl chain. They also found that if DDE was formed, it would degrade into p,p'-DDMU (1-chloro-2,2-bis-(p-? chlorophenyl)ethylene) followed by successive degradation into p,p'-DDNU (2,2-bis(p-chlorophenyl)ethylene) (Yu et al., 2011).

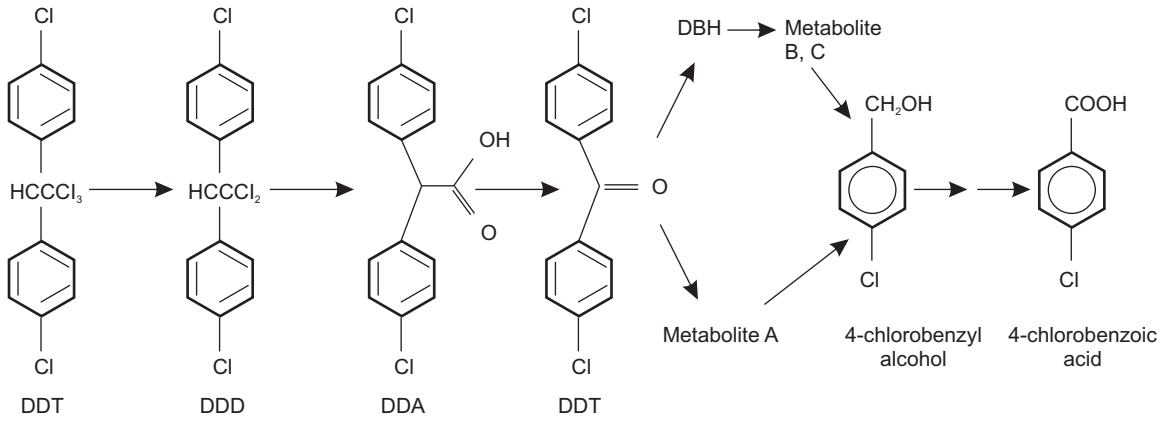

Figure 3. Novel aerobic pathway for DDT by fungi as proposed by Xiao et al. (2011)

Conclusions

These findings outlined above indicate that bioremediation can be a viable method to treat DDT contaminated sites. Degradation of DDT has been demonstrated under both aerobic and anaerobic conditions by a variety of different organisms. The primary 'end product' reported for most pathways has been 4-chlorobenzoic acid (4-CBA). Under anaerobic conditions, the end product is often dichlorobenzophenone (DBP). Although complete mineralization may be possible for DDT and its metabolites, it has not been reported to date. This is in part due to the fact that most studies often focused on the efficiency of one or two microbes. Complete mineralization, especially in efficient time frames, will require a consortium of different microbes. Additional research with aerobic consortiums is recommended in order to facilitate future in-situ treatments of the vadose soils.

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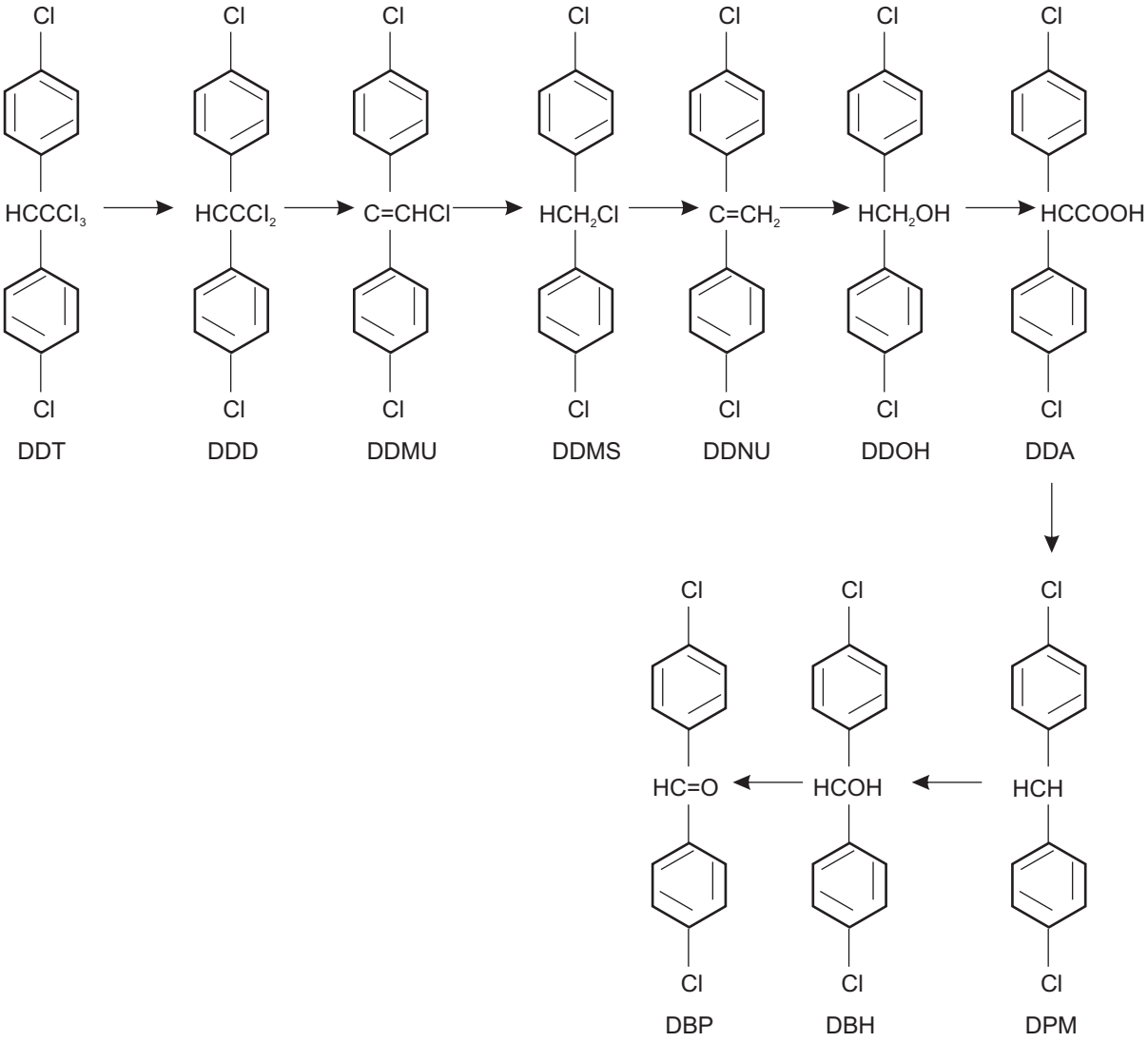

Figure 4. Metabolic pathway of DDT under anaerobic conditions as modified by research of You et al. (1996). DDMU is 1-chloro-2,2-bis(p-chlorophenyl)ethylene; DDMS is 1-chloro-2,2-bis(p-chlorophenyl)ethane; DDNU unsym-bis(p-chlorophenyl)ethylene; DDOH is 2,2-bis(p-chlorophenyl)ethanol; DDA is dichlorodiphenylacetate; DPM is dichlorophenylmethane; DBH is dichlorobenzylhydrol and DBP is dichlorobenzophenone.

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