DDT Metabolite
Bis(Chlorophenyl)acetic Acid: The Neglected Environmental Contaminant

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Up to the present date, DDA (2,2-bis(chlorophenyl)acetic acid) resulting from the degradation of DDT (2,2-bis(chlorophenyl)-1,1,1-trichloroethane) residues in the environment has been neglected as an environmental contaminant of the aquatic system. More than 60 years after the invention of DDT as an insecticide and more than 25 years after its use was banned in most of the developed countries, DDA was found as major contaminant up to the microgram per liter level in the surface water of the Teltowkanal in Berlin, Germany. DDA is formed together with some other intermediates of DDT such as DDD (2,2-bis(chlorophenyl)-1-chloroethane), DDE (2,2-bis(chlorophenyl)-1,1-dichloroethylene), DDMU (2,2-bis(chlorophenyl)-1,1-dichloroethane), DDOH (2,2-bis(chlorophenyl) ethanol), DDM (2,2-bis(chlorophenyl)-1-chloroethane), and DDDMU (2,2-bis(chlorophenyl)acetonitrile), and DBP (dichlorobenzophenone), which were identified at lower concentrations in surface water of the canal. Our results add some new aspects to the ongoing controversy over the fate of DDT in the natural environment and confirm some laboratory experiments about the natural remediation of DDT residues. Moreover, the polar degradation product DDA is also a potential problem for drinking water production because it is the only DDT derivative that also leaches into the groundwater wells of a neighboring drinking water plant at concentrations exceeding the European maximum tolerance levels for pesticide residues in drinking water.

Introduction
At the end of the 1930s, Paul Müller discovered the insecticidal properties of 2,2-bis(chlorophenyl)-1,1,1-trichloroethane (DDT) (1). In the following decades, DDT was used worldwide as highly active insecticide in agriculture, for pest control in forestry and for vector control in human hygiene against diseases such as malaria and typhus. In the 1960s, there was some evidence that DDT and its metabolites DDE (2,2-bis(chlorophenyl)-1,1-dichloroethylene) and DDD (2,2-bis(chlorophenyl)-1,1-dichloroethane) are highly persistent in the environment and accumulate in higher animals (2, 3). Other studies have shown that DDT derivatives are toxic and responsible for the thinning of egg shells of birds (3).

In 1972, DDT was banned in the United States except for emergency control of vector-borne diseases. Most other industrialized countries, including West Germany, also banned the use of DDT in the early 1970s. In the former German Democratic Republic (GDR), however, DDT was still produced and used until the end of the 1980s, and some developing countries still use DDT (4–7), especially for vector-borne disease control (8). Voldner and Li (4) estimated the cumulative global usage of DDT between 1950 and 1993 at 2.6 million tons with 990 000 t from 1970 to 1993.

DDT residues are distributed globally by atmospheric transport (9). They are also found in arctic mammals (10). Further studies began to unravel the endocrine effects of DDT residues in wildlife. The studies suggest that some isomers of DDT derivatives act as endocrine-disrupting chemicals causing emasculation, abnormal sexual development, and impaired reproduction in wildlife (11–15). Additionally, studies by Kelce et al. (16) have shown that p,p'-DDE is a potent androgen receptor antagonist. Pereira et al. (17) concluded that the environmental effects of DDT and its derivatives would begin to manifest themselves several decades after the introduction of these compounds.

DDT residues are extremely persistent in the environment and degradation strongly depends on the environmental conditions. In investigations of DDT in the Lauritzen Canal and in Richmond Harbor in San Francisco Bay, Pereira et al. (17) found that under anaerobic conditions DDT is reductively dechlorinated primarily to DDD, whereas under aerobic conditions it is mainly dehydrochlorinated to DDE and to a lesser degree to dichlorobenzophenone (DBP). However, in zones with high DDT residue concentrations, aerobic degradation was found to be diminished or inhibited (17). Pereira et al. (17) also described environmental findings of DDMU (2,2-bis(chlorophenyl)-1-chloroethylene), another important degradation product of DDT, and its derivatives. In 1998, Quensen et al. (18) in laboratory experiments also found DDMU as an important degradation product mainly deriving from DDE residues that were for a long time regarded as terminal residues in the environment. These findings started a new controversy over whether persistent DDT residues can be naturally degraded by microbial action and whether the laboratory results can be transferred to the natural conditions found at DDT-contaminated Superfund sites (19).

Despite all these discussions, it is difficult to believe that until now DDA, the polar metabolite of DDT, DDD, DDE, and DDMU, has not been recognized in any of the many investigations of DDT residues in the aquatic environment. Many positive findings of DDT residues in seawater, surface water, groundwater, or drinking water have been reported worldwide; some of these being recent publications (20–29). However, DDA has never been included in these investigations, although DDA was one of the first DDT metabolites to be known. In 1945, it was isolated and identified by White and Sweeney (30) in the urine of rabbits exposed to DDT. Ware et al. (31) called DDA “perhaps the universal DDT metabolite in microorganisms, plants, insects, and higher animals”. DDT is also converted readily into DDA by purely chemical action or by photodecomposition (31). In 1978, Marei et al. (32) detected DDA as one important metabolite on incubation of DDT with sewage sludge in laboratory experiments. They also postulated the occurrence of DDA in the aquatic environment; nevertheless, we could not find any reliable data in the literature about the occurrence of DDA.

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were analyzed by tandem mass spectrometry (GC and MS) for confirmation of the identity of the DDT derivatives, samples were collected with standard equipment from the middle of the Teltowkanal in Berlin. Furthermore, the results confirm many of the postulations made by Marei et al. (32) and add some new aspects to the debate concerning the fate of DDT residues in the environment.

**Experimental Section**

**Analytical Methods.** DDA was analyzed according to an analytical method originally developed for the analysis of polar pesticides and drug residues (35–38). This method applies solid-phase extraction (SPE) using an end-capped reversed-phase octadecyl material (RP-C18), derivatization with pentafluorobenzyl bromide (PFBBr), and analysis with capillary gas chromatography–mass spectrometry (GC–MS) in selected ion monitoring (SIM) mode. The nonpolar DDT derivatives were extracted by SPE using an end-capped RP-C18 material (38). The elutes from SPE were analyzed applying GC with electron capture detection (ECD) and for confirmation purposes GC–MS with SIM (38). Additionally, for the confirmation of the identity of the DDT derivatives, samples were analyzed by tandem mass spectrometry (GC–MS/MS).

**Materials.** All solvents were highly purified products from Merck (Darmstadt, Germany). Pentafluorobenzyl bromide was obtained from Aldrich (Steinheim, Germany); triethylamine was from Merck (Darmstadt, Germany). Sample vials, screw caps, and septa were purchased from Zinsser (Frankfurt, Germany); 200-μL inserts for the sample vials from CS-Chromatographie Service (Langerwehe, Germany). DDT, DDD, DDE, DDMU, DDOH, DDA, DBP, and the internal standard 2-(4-chlorophenoxy)propanoic acid were of analytical purity and purchased from Riedel de Haen (Seelze, Germany), or from Aldrich (Deisenhofen, Germany). The surrogate standard 2-(4-chlorophenoxy)butyric acid was synthesized according to a procedure described elsewhere (39). Solid-phase extraction (SPE) was carried out with a vacuum manifold (spe 12G) using cartridges of polypropylene with a volume of 6 mL and end-capped RP-C18 or Bakerbond Polar Plus RP-C18 material all from Mallinckrodt Baker (Griesheim, Germany).

**Sample Collection.** As shown in Figure 1, 10 representative sampling locations were selected for the screening of the surface water at the DDT contaminated Superfund site in the Teltowkanal in Berlin, Germany. The samples were collected with standard equipment from the middle of the waters at a depth of 2 m. All samples were stored at 4°C until they were extracted and analyzed for DDT residues on the next day.

**Sample Preparation for Nonpolar DDT Derivatives.** A water sample of 0.5 L was adjusted to a pH of 8.5 for SPE. Each SPE cartridge was filled with 1 g of an end-capped RP-C18 adsorbent. Conditioning was performed successively with 10 mL of acetone, 10 mL of methanol, and finally 10 mL of distilled, deionized water. The solvents were drawn through the cartridge by means of a gentle vacuum, and the cartridge was not permitted to run dry during the whole conditioning procedure. The water sample was then percolated through the cartridge at a maximum flow rate of approximately 8 mL/min by applying a low vacuum.

After the cartridge was dried for 2–3 h by flushing with nitrogen, the analytes were eluted from the cartridge with dichloromethane and toluene. The eluate was dried under a gentle stream of nitrogen. The residue from the sample preparation was dissolved in 100 μL of toluene and injected directly into the gas chromatograph.

**Sample Preparation for o,p′- and p,p′-DDA.** A water sample of 0.5 L was mixed with 100 μL of a solution of 2-(4-chlorophenoxy)butyric acid in methanol (1 mg/L) as surrogate standard to give a concentration of 200 ng/L. The sample was adjusted to a pH < 2 before SPE. Each SPE cartridge was filled with 1 g of a non-end-capped RP-C18 adsorbent. Conditioning was performed successively with 10 mL of acetone, 10 mL of methanol, and finally 10 mL of distilled, deionized water (pH < 2). The solvents were drawn through the cartridge by means of a gentle vacuum, and the cartridge was not permitted to run dry during the whole conditioning procedure. The water sample was then per-
TABLE 1. Results from the Surface Water Monitoring of DDT Residues in the Teltowkanal in Berlin, Germany

<table>
<thead>
<tr>
<th>sampling location no.</th>
<th>surface water</th>
<th>o,p'-DDT (ng/L)</th>
<th>p,p'-DDT (ng/L)</th>
<th>o,p'-DDE (ng/L)</th>
<th>p,p'-DDE (ng/L)</th>
<th>o,p'-DDD (ng/L)</th>
<th>p,p'-DDD (ng/L)</th>
<th>o,p'-DDA* (ng/L)</th>
<th>p,p'-DDA (ng/L)</th>
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<td>nd</td>
<td>nd</td>
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* The results represent the average of a 3-fold determination of each sample. ** o,p'-DDA was not commercially available; therefore, the p,p'-DDA standard was used for its quantitation. 500 mL of the water samples has been processed in sample preparation. The recovery for all compounds was better than 70%. The limits of detection were below 1 ng/L for all compounds. The limits of quantitation were always below 5 ng/L. *** nd, not detected.

**Results and Discussion**

**DDT Contaminated Superfund Site in the Teltowkanal in Berlin.** The Teltowkanal in the south of Berlin was built between 1901 and 1906. The canal was used as drainage for rainwater and industrial wastewater from districts formerly located outside of Berlin. Additionally, it was used as a shipping canal for industrial supply and as a short cut for the shipping routes between the rivers Oder and Elbe. The canal has a length of approximately 35 km and connects the rivers Dahme and Havel. At the beginning of the Teltowkanal close to the confluence of the river Dahme with the canal, a large chemical production plant is located (Figure 1). In this production plant in former East Berlin more than 60 000 t of DDT were produced and formulated between 1943 and 1986 (41). It is an open secret that production residues were released into the soil and into the neighboring water course, the Teltowkanal. It is, however, not clear when and how much DDT was discharged into the canal.

**DDT Residues in the Teltowkanal Sediment.** Random canal sediment samples were collected and analyzed for DDT residues (34). Total DDT concentrations up to more than 1 mg/kg (dry weight) were found in these samples. Interestingly, DDT (o,p'- and p,p'-DDT) itself was only detected at maximum concentrations of 0.05 mg/kg. It has also totally been converted to DDD, which was found at concentrations up to more than 1 mg/kg. DDE was produced to a lesser extent and detected at maximum concentrations of 0.2 mg/kg in the sediment samples. DDA was not detected at significant concentrations in the sediment. The DDD residues dominating in the sediments of the canal clearly indicate that DDT was converted under anaerobic conditions, as shown by Pereira et al. (17) in San Francisco Bay.

**Occurrence of DDA and Other DDT Derivatives in the Surface Water.** Surface water samples were collected in July 1997 from the river Dahme and the Teltowkanal up to the bank filtration area of a drinking water plant close to the canal (Figure 1). The purpose of these investigations was to identify DDT residues, to investigate their distribution in the canal, and to determine the individual ratios of the DDT derivatives present in the aqueous phase. Some results of these investigations are compiled in Table 1.

DDA was found at maximum concentrations up to 1000 ng/L and is clearly the dominant DDT derivative in the aqueous phase of the canal. The individual ratios of the o,p'- and p,p'-isomers reflect the ratios of o,p'- and p,p'-DDT in corresponding time windows. The tuning of both mass spectrometers was performed weekly using the autotuning macro. Precolumns and/or insert liners were exchanged after 50 injections at the latest.
FIGURE 4. Proposed degradation pathway of DDT residues in the Teltowkanal. Names of DDT derivatives are printed in bold when they were found in our investigations. Names of derivatives that were found as key metabolites in the aqueous phase are underlined.

DDT, which dissolves even better in the aqueous phase than DDT or DDE. DDD is converted to DDA, which seems to be a very persistent key metabolite in the aquatic system. Figure 4 shows a possible degradation pathway of DDT to DDA in the Teltowkanal. This pathway is derived mainly from the known anaerobic reductive metabolism of DDT observed in microorganisms in laboratory experiments (42, 43). The DDT metabolites that have been detected at considerable concentrations in the surface water of the Teltowkanal are underlined. p,p′-DDMU, which is only found at trace-level concentrations, may be formed from DDD or mainly from DDE as proposed by Quensen et al. (18). The very low concentrations of p,p′-DDMU and DDE as compared to those of DDD, DDA, or DDMS are intermediate metabolites in the degradation of DDD to DDA. DDD may also be dechlorinated directly to DDMS without the intermediate formation of DDDM (42, 43).

DBP may be formed together with DDE under aerobic conditions as reported by Pereira et al. (17). DBP is however also described as a photodegradation product of DDA (31). Remarkably, only the p,p′-isomer but no o,p′-DBP was detected in the surface water, which should occur when DPB is built from DDA by photodegradation.

DDCN, which is also found in the Teltowkanal, was reported by Albone et al. (44) to be formed under anaerobic conditions in sewage sludges. It was also found by Jensen et al. (45) in investigations of lake sediments. It is however not clear whether DDCN is formed directly from DDT or from DDA (44).

The occurrence of the DDT residues only at sampling location nos. 5–10 clearly identified the chemical production plant as the contamination source. Downstream of the canal at sampling location no. 6, the maximum concentrations...
for all DDT derivatives were measured. In 1989, water samples from this sampling site were analyzed for DDT residues (DDT, DDD, DDE, and DDMU) by Heinisch and Wenzel (46). Heinisch and Wenzel found p,p′-DDT as the major contaminant at concentrations of 140 ng/L. This value corresponds exactly to our result for p,p′-DDT at the same location 8 years later. Thus, there is some indication that there has been little or no change in the degree of DDT contamination of the aqueous phase during all these years. It seems as if there is a steady-state condition in the Teltowkanal where the aqueous phase is continuously fed by DDT residues accumulated in the canal sediment. Heinisch and Wenzel (46) should also have found DDA in their investigations, but it was unfortunately not included in the analytical program.

**DDA Residues in the Groundwater Wells of a Drinking Water Plant.** As shown in Figure 1, a drinking water plant that uses up to 62% of bank filtrate in drinking water production is located downstream of the canal. Using bank filtration, the surface water from the Teltowkanal is expected to be clarified of microorganisms and micropollutants. Nevertheless, polar organic contaminants such as drug residues (33) and DDA are found in groundwater wells from the bank filtration area. We detected o,p′- and p,p′-DDA at individual concentrations up to 0.28 and 1.7 μg/L, respectively (34). The DDA concentrations are even higher than those measured in the Teltowkanal directly in front of the bank filtration area. This might be explained by higher DDA intakes in the past and indicates the persistence of DDA residues in the groundwater aquifers. It can also be concluded that polar contaminants leach easily through the subsoil into the groundwater aquifers because polar drug residues are also found at concentrations comparable to those detected in the canal (35). In Berlin, polar contaminants are also a problem in drinking water production because there is no special purification of the resulting raw waters, e.g., activated carbon is not used. Thus, the Berlin water works closed down several of the drinking water wells to keep the DDA concentrations below the maximum tolerance levels for pesticide residues in drinking water set to 0.1 μg/L by the European Union (47).

The public authorities have recently initiated a first monitoring program to investigate the degree of groundwater contamination by DDA residues in this area. The results of these investigations should enable the assessment of groundwater contaminations caused by DDT residues that have been released into the Teltowkanal and into the soil of the chemical production plant.

**Why Has DDA Been Neglected as an Environmental Contaminant until Now?** Although, it is hard to believe, it is easy to see why DDA has not been analyzed for in all systematic investigations of DDT residues in the aquatic environment. DDT derivatives such as DDT, DDD, DDE, DDMU, and DBP can be analyzed together in multisidue methods developed for the analysis of nonpolar contaminants. However, due to its polar structure, DDA has to be derivatized to render it amenable to gas chromatographic analysis, and the use of mass spectrometry instead of electron capture detection (ECD) is a prerequisite for the analysis of DDA. If one of these prerequisites is lacking, DDA will not even be found by chance.

**Further Considerations.** As reported recently by Pareira et al. (17) and Quensen et al. (18), DDT residues can be naturally remediataed to DDMU and as shown here further remediated to DDA. DDA is supposed to be much less toxic than DDT, DDE, or DDD. DDA seems, however, to be very persistent in the environment, and as shown here, it leaches easily through the subsoil into the groundwater aquifers. DDA thus has become a problem in the Teltowkanal area where groundwater recharge is used in drinking water production. DDT residues have finally been converted to a less toxic form, but they obviously do not disappear completely by mineralization. In our example of the Teltowkanal, the problem of the DDT residues has been shifted by the natural degradation of DDT from the sediments to the surface water and finally into the groundwater aquifers, making DDT residues problematic for drinking water production when regarding the maximum tolerances for pesticides in drinking water set by the EU (47). This problem will remain in the future because of DDA’s persistence in the contaminated groundwater aquifers.

If the situation of the Teltowkanal is comparable to other DDT-contaminated Superfund sites, DDA should also be found there. In our investigations DDA accounts for more than 60% of the total DDT residues found in the aqueous phase of the Teltowkanal. The concentrations of DDA were on average five times higher than those measured for DDD. DDD is analyzed as one standard parameter in water analysis. It seems probable that DDA might have been present at considerable concentrations in those samples that have been reported to contain residues of DDD. In conclusion, DDA can be seen as an important parameter that should be analyzed whenever surface water, groundwater, and drinking water samples are investigated concerning DDT residues.

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**Literature Cited**
