DDT Polymorphism and the Lethality of Crystal Forms

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Dedicated to Professor Roald Hoffmann on the occasion of his 80th birthday

Abstract: DDT (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane), a contact insecticide with a rich and controversial history since its activity was discovered in 1939, has long been thought to be monomorphic. Herein we report the discovery and characterization of a second polymorph, designated Form II, which can be isolated as single crystals, but converts very slowly at room temperature to the form reported previously, now designated as Form I. Computations based on an evolutionary algorithm for crystal structure prediction revealed that Forms I and II are among the four lowest energy crystal structures of fifty calculated. A preliminary study of the contact insecticidal activity toward fruit flies (Drosophila melanogaster) indicates that Form II is more active, suggesting opportunities for more effective solid-state formulations that would allow reduced amounts of DDT, thereby minimizing environmental impact.

DDT, one of the most consequential compounds of the last century,[1] is a tactile killer (Scheme 1). Unsuspecting insects walk upon DDT crystals and absorb the poison through their hydrophobic footpads, the first steps in their demise.[2] The most effective solid-state formulation of DDT was pursued during the so-called “Green Revolution” of the 20th century,[3–7] which sought to increase crop production through technology as well as selective breeding and encompassed the heyday of synthetic pesticides (ca. 1946–1972). Research on the physicochemical properties of DDT fell sharply following the ban on agricultural use in the United States in 1972[8] because of systemic environmental concerns raised by Rachel Carson in her book Silent Spring (1962).[9] An essay in this issue of Angewandte Chemie focuses on how DDT science has been misrepresented by those with extra-scientific interests.[10]

We began a study of the solid-state chemistry of DDT because stock micrographs of crystals posted online revealed optical signatures of helicoidal twisting.[11] In fact, DDT is one of many molecular crystals that twist around the growth direction.[12–14] DDT crystal habits of varied lethality have been described,[15–18] but all were unindexed and presumed to be manifestations of the same crystal phase.[19,20] DDT has always been considered monomorphic, crystallizing in the polar space group Pca21, Z = 4, Z′ = 1 (Cambridge Structural Database Refcode: CPTCET10).[21,22] Herein, we report the discovery of a second DDT polymorph that appears under most crystallization conditions investigated in our laboratory. This prompts the question as to whether differences exist between the DDT polymorphs at the crystallographic level that may be an essential aspect of their activity as contact insecticides.

Growth of DDT crystals by evaporation from ethyl acetate, chloroform, nitromethane, tetrahydrofuran, dichloromethane, triethylamine, hexane, acetone, toluene, benzene, methyl propionate, or 2,2,4-trimethylpentane solutions on glass surfaces afforded crystalline films, some forming needles and others forming two regions with distinct morphologies (Figure S1). X-ray microdiffraction revealed that one of these could be assigned to Form I whereas the second suggested an unknown polymorph, hereby designated as Form II (Figure 1A, Figure S2). The two forms also could be distinguished by micro-Raman spectroscopy (Figures S3 and S4).

The crystallization behavior of DDT is curious, as evidenced by early observations of spherulites[23] from the melt (a.k.a. fusion prep) that were characterized initially by the presence of strained crystals, which subsequently relaxed by boundary migration across intersecting crystals.[24] In our hands, cooling of molten DDT generated a supercooled melt that can be stable for hours at room temperature (Figure 1B). Growth at 0°C from the supercooled melt when confined...
between glass slides produced fields of Form II with chaotic polycrystalline textures, punctuated by small Form I spherulites (Figure 1C, Videos S1 and S2). Spherulites also are evident in Figure 1D, but these display arhythmic, radial birefringence that is pronounced in crystals grown at $T = 78^\circ C$. Form II transformed to Form I upon heating to $80^\circ C$, with new needles of Form I aligned according to the crystallographic orientation of contacting Form I fibrils in the neighboring spherulites (Figure 1E,F). This transformation also was observed by atomic force microscopy (Video S3). At room temperature the transformation was very slow.

The supercooled melt is persistent, with Form I nucleating slowly. Nucleation can be provoked by agitation, consistent with prior observations, including patents that describe rolling and agitation to improve the friability of DDT when solidified from the melt. For example, one patent teaches that “DDT should be cooled in thin film form under such conditions that it is worked with great vigor during the cooling operation.” This behavior is consistent with differential scanning calorimetry (Figure S5), which reveals an endothermic peak from melting at $T_m = 108^\circ C$, but the absence of a crystallization event upon supercooling to temperatures even as low as $-25^\circ C$. When the supercooled melt was heated, an endothermic event assignable to a glass transition was observed $T_g = -10^\circ C$. This was followed by an exothermic event in the range $T_c = 25–42^\circ C$, depending on scan rate, that can be assigned to an amorphous-to-crystalline transition, primarily Form I based on companion measurements with Raman microscopy, X-ray diffraction, and optical microscopy. Another minor exotherm observed consistently at $T \approx 67^\circ C$ was assigned to a transformation from Form I to Form II, consistent with Raman spectroscopy (Figure S6).

Notably, the enthalpy of melting is typically larger than the enthalpy calculated from the area under the crystallization peak at $T_c$. This suggests partial crystallization at $T_c$, followed by continuous crystallization upon further heating, consistent with visual observations and a gradual heat flow up to the melting point.

The observation of an apparent second polymorph prompted us to compare the solid-state structures of both forms (Figure S7, Table S1). Needles of Form I, with the long axis coincident with the [001] direction, were grown by cooling supersaturated ethanol solutions from $70^\circ C$ to ambient temperature. The crystal structure of Form I was redetermined at a lower temperature (100 K) than that previously reported (Figure 2a). The new and previously reported structures were essentially identical, but hydrogen atoms were included in our redetermination ($Pca_2_1$, $Z = 4$).

Figure 1.
A) A crystalline film formed by evaporation of an ethyl acetate solution of DDT, revealing two distinct regions assigned to Forms I and II. B) Photograph of the DDT supercooled melt at 25°C. C) Growth from the melt at 0°C produces fields of Form II punctuated by smooth Form I spherulites. D) Twisted DDT Form II grown from the melt at −78°C viewed between crossed polarizers. E, F) Transformation from chaotic textures of Form II (E) to needles of Form I (F) after heating at 80°C.

Figure 2.
Crystal structures of Form I (a) and Form II (b) viewed along the $a$ axes. The structure of Form I (available in Cambridge Structural Database Refcode CPTCET10) was redetermined for this investigation while adding hydrogen atoms to the refined model. See Table S1.

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Form II predominated when grown from ethyl acetate or methyl propionate by evaporation of DDT solutions at 25 °C on a glass slide. Occasionally, individual crystals large enough for X-ray analysis could be retrieved from the surface. The single-crystal structure of Form II was refined in the enantiomorphous space group $P2_12_12_1$, $Z = 8$, $Z' = 2$, $a = 9.6675(8)$ Å, $b = 15.7441(13)$ Å, $c = 19.2261(17)$ Å, $V = 2926.3(4)$ Å$^3$ (Figure 2b).

The mode of action of DDT as an insecticide involves contact between insect feet and DDT crystal surfaces, with subsequent adsorption into the hemolymph fluid[29] either directly upon contact or through ingestion[29] when the insects clean their DDT-contaminated feet. Once absorbed, DDT acts as a neurotoxin, first producing hyperactivity, then paralysis, and eventually death.[29] The discovery of Form II prompted us to compare its lethality with that of Form I.

The lethality of DDT Forms I and II were compared by exposing fruit flies (Drosophila melanogaster) to each form, recording the onset of hyperactivity, and tracking the time required for their expiration. In one set of trials, performed in triplicate for each crystal form as well as a control, the flies were exposed to Forms I and II on glass cover slips prepared by evaporation of ethanol and methyl propionate solutions, respectively, placed inside polystyrene petri dishes. The fruit flies were temporarily incapacitated by CO$_2$ exposure, then transferred to the petri dishes (three dishes for each crystal form, approximately 10 flies per dish). The dishes were covered and the motion of the fruit flies was recorded (Videos S4 and S5). The fruit flies typically began exhibiting hyperactivity after approximately 45 minutes, consistent with DDT’s neurotoxicity. The onset of hyperactivity and the first death occurred earlier for flies exposed to Form II. The average fly survival time was 166 min and 128 min for Forms I and II, respectively (Figure S9). A second set of trials, performed with the same protocol but with loose powders of Forms I and II afforded average survival times of 144 and 101 minutes, respectively (Figure 3). Whereas variables such as crystal size, orientation, and morphology demand further study, in the aggregate these results demonstrate that Form II is more lethal to fruit flies than Form I. The intrinsic difference in the lethality of Forms I and II actually is greater than that indicated in Figure S9 as it is difficult to prepare Form II exclusively. Powder X-ray diffraction (PXRD) indicated that only about 50% of the polycrystalline samples from methyl propanoate were Form II.

In order to assess the relative stabilities of Forms I and II, and potentially undiscovered forms as well, prospective crystal structures were explored using systematic crystal structure prediction (CSP) based on an evolutionary algorithm, as implemented in the USPEX code[30–33] an approach that uses only molecular geometry as input. The number of molecules per asymmetric unit ($Z$) and choices of space groups, specified by the user, define the extent of the crystal structure search. Alternatively, one can perform the crystal structure search by fixing the unit cell if the cell parameters are available from experiment. GULP[34] and DFTB+ [35] codes were used for structure relaxations within USPEX (see the Supporting Information for more details).

The initial computational search for crystal structures with $Z = 1$ at ambient pressure was constrained to the 30 most common space groups, successfully returning Form I as well as other low-energy structures (Table S2). The geometries of the 50 lowest energy structures were then re-optimized using VASP code[36] at the optB88 level[37] which ranked Form I as the second-most stable structure. The final optimized structure of Form I returned a root-mean-squared deviation for 20 molecules chosen from the supercell of RMSD$_{20} = 0.186$ Å relative to a single-crystal structure collected here at 100 K (which was essentially identical to the previously reported room-temperature structure but better refined as hydrogen atoms were added to the model). Another search performed with $Z = 2$ and the five most common space groups identified Form II with a RMSD$_{20}$ value of 0.215 Å relative to the experimental single-crystal solution determined here. The 50 lowest energy structures were selected from both CSP runs and their geometries optimized at the level of optB88. Among a total of 100 structures, the 50 lowest energy structures were chosen after removal of duplicates. The energies of these structures span 16.6 kJ mol$^{-1}$, as illustrated in Figure 4. Forms I and II rank second and fourth lowest, the four lowest-energy forms (Figure S8) spanning only 4 kJ mol$^{-1}$ only slightly greater than the value of $kT$ at room temperature (2.5 kJ mol$^{-1}$). The lattice energy of Form I is slightly lower in energy than Form II (by 2.88 kJ mol$^{-1}$), in agreement with the observation that Form II transforms to Form I, and never the reverse. The lowest and third-lowest energy structures calculated, both $P2_1/c$ (A and B in Figure 4), were denser than Forms I and II. Although these rankings in this narrow energy range may be an artifact of the optB88 functional, they suggest the possibility of other polymorphs. During this investigation a benzene solvate of DDT, (DDT·0.5(benzene)), also was discovered (Table S3). This compounds converts readily to Form I upon standing under ambient conditions.

The polymorphism of DDT may have been overlooked because of declining interest in a compound subject to increasing regulation because of its environmental impact. Nonetheless, DDT polymorphism is significant because the

![Figure 3](https://example.com/figure3.png)

**Figure 3.** Comparison of the lethality of Forms I and II for a Drosophila melanogaster model.
Observation of concomitant phases\cite{38} provokes questions about the uptake of DDT by insects. It is reasonable to suggest that uptake of DDT, which involves contact between insect feet and crystal surfaces, with subsequent adsorption, would be polymorph dependent. The different insecticidal activities of DDT Forms I and II, and even the different activities of symmetry-independent crystal faces, warrants investigation. One report explains that whereas toxicity increased with increasing needle length, breadth was less important, but a suspension of needle-shaped crystals was as toxic as one containing considerably larger plate-shaped crystals\cite{13} suggesting differences in activity among various crystal faces. Symmetry-independent crystal faces would be expected to have different attachment energies between surface molecules and underlying crystal planes, which may affect DDT uptake. Furthermore, insect feet are known to secrete fluid that regulates adhesion to contacting surfaces\cite{10,11}. This fluid may solubilize DDT, suggesting crystal forms with smaller lattice energies would be more effective.

Expiring insects will hardly care whether they were poisoned by Form I or II, but solid-state chemists have the skills and tools needed to reinvestigate contact insecticides, in general, with respect to polymorphism and crystal morphology. In places where DDT is still used to combat malaria, the possibility that one crystalline form or morphology may be more active than another provides an opportunity to optimize solid-state formulations to reduce the amount of compound applied, so as to achieve the necessary protection against disease while minimizing environmental impact. Moreover, a reduced amount of DDT during application may curtail the development of resistance, which has been key factor in its diminishing use\cite{12}. DDT is one of the so-called “dirty dozen”\cite{12} contact insecticides. As a class, these compounds are so far monomorphic. It seems likely that with some effort additional phases not previously reported can and will be discovered.

\section*{Acknowledgements}
B.K. and M.D.W. are grateful for support from the U.S. National Science Foundation (DMR-1105000, DMR-1608374), as well as the Materials Research Science and Engineering Center (MRSEC) program of the National Science Foundation under Award No. DMR-1420073. Q.Z. is grateful for support from the National Nuclear Security Administration under the Stewardship Science Academic Alliances program through DOE Cooperative Agreement DE-NA0001982. J.Y. acknowledges funding support from the China Scholarship Council. The authors thank Professor Claude Desplan and Cleopatra Tsanis (NYU biology) for the generous donation of Drosophila melanogaster flies.

\section*{Conflict of interest}
The authors declare no conflict of interest.

\section*{Keywords:} crystallography · DDT · insecticides · polymorphism

\section*{How to cite:} Angew. Chem. Int. Ed. 2017, 56, 10165–10169

Angew. Chem. 2017, 129, 10299–10303

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