X-ray Crystallographic studies of fungicide Chlorothalonil

Dr Jyotsna Chauhan* Gargi bhattacharya

Assistant professor in UIT Rajeev Gandhi Technical University Airport Bypass Road ,Gandh iNagar Bhopal,M.P India

Running Title: X-ray Crystallographic studies of fungicide

****Address for correspondence***
Jyotsnachauhan2006@gmail.com

Abstract: Chlorothalonil (2,4,5,6-tetrachloroisophthalonitrile) is a polychlorinated aromatic mainly used as a broad spectrum, contact fungicide. The activity of fungicides is intimately related to its chemical structure. Knowledge about the chemical structure of a

chemical is useful for the synthesis of new compounds with more specific actions and fewer adverse reactions, to increase/decrease the duration of action of the original drug or to get a more potent compound, to restrict the action to a specific system of the body and to reduce the adverse reactions, toxicity and other disadvantages associated. We can understand the basic chemical groups responsible for drug action[1].Recently it has been observed that some of the fungicides are loosing their effects. if their structures are known. So analogous compounds(as a substitute.) can be designed .A rational approach to test these fungicides is to know the three dimensional structure of these compounds and macromolecular receptor sites as well as their molecular complex .The structures of these compounds can be obtained by X-ray diffraction method in crystalline form and they will invariably be similar to their structure in solutions. The composition of crystal **Cholrothalonil** is confirmed by comparing the infra-red spectra of two components. The unit cell parameters are a = 6.3354(5) Å b = 6.2233(4) Å ,c = 27.753(2) Å, $\alpha = 90^{o}$, $\beta = 95.397(2)^{00}$, $\gamma = 90^{o}$ **The Crystal system is Monoclinic, and space group** P21/c

Keywords: X-ray crystallography, fungicides, Triazole structure

A systemic fungicide is defined as systemic fungi toxic compound that controls a fungus pathogen remote from the point of application. These active compounds are absorbed by the roots of plant and get acropetally trans located within it through the xylem to the leaves traveling peripherically accumulates at the edges and tips., thus providing protection as well as eradicating already established infection. In the search for a more effective approach to plant disease control, the emphasis shifted to the exploitation of the biological properties of new fungicides, This new approach can be defined as being on a profound knowledge, at the physiology, biochemical and molecular levels, of the modes of action of exiting fungicides and the mechanisms of resistance to them in fungi. Such knowledge provides a ration guideline, not only in the development of new compounds, but also in strategies for judicious and sustained use of established fungicides (Fuchs, etal., 1983). The approach to disease control, thus, embraces the techniques and strategies to avoid or delay the development of field resistance to fungicides, based on investigation into the mechanism of resistance. Chlorothalonil-containing products are sold under the names Bravo, Echo, and Daconil. It was first registered for use in the US in 1966. In 1997, the most recent year for which data are available, it was the third most used fungicide in

the US, behind only sulfur and copper, with some 12 millions used in agriculture alone in a year. Chlorothalonil reduces fungal intracellular glutathione molecules to alternate forms which cannot participate in essential enzymatic reactions, ultimately leading to cell death.

Experimental: - The structures of these compounds can be obtained by X-ray diffraction method in crystalline form and they will invariably be similar to their structure in solutions. First grow the crystals of existing fungicides available and synthesize their derivatives in lab. The determination of structural perturbation in fungicide derivatives and comparison of the result of their molecular association with other receptor sites by X-Ray crystallography techniques will be done. In parallel with these structural studies, spectroscopic studies carried out on them. The goal is then to tie together the structural and spectroscopic studies to have more comprehensive account of the precise shape of these molecules, the non-covalent interaction which are likely to be involved in and the changes introduced in molecular geometry and electronic structure of these compounds as a result of their molecular association with other compounds. Thus we study the structure of variety of such compounds and correlate their structure with biological activity, so that more safer and effective fungicides at reasonable price can be developed.. Crystallization of **Cholrothalonil** was done by slow evaporation from a solution of methanol at 296°K temp.The crystals obtained were white and rectangular in shape. The density of crystal 1.334 Mg/m^3 is measured by floatation method the mixture of benzene and Bromoform .The unit cell parameters were determined by automatic computerized 4-circled Enraf-Nonius CAD-4 Diffractometer. The preliminary information about crystal is given in Table.1.

Data collection and Structure Solution: **Data collection and Structure Solution**

X-ray crystallographic data were collected at 296K with $Mo_{K\alpha}$ radiation (λ = 0.71073 Å) using a Bruker Nonius SMART CCD diffractometer equipped with graphite monochromator. The SMART software was used for data collection and also for indexing the reflections and determining the unit cell parameters; the collected data were integrated using SAINT software. The structures were solved by direct methods and refined by full-matrix least-squares calculations using SHELXTL software. All the non-H atoms were refined in the anisotropic approximation against F^2 of all reflections. The H-atoms, placed at their calculated positions and refined in the isotropic approximation; those attached to heteroatom (N and O) were located in the difference Fourier maps, and refined with isotropic displacement coefficients.

The three dimensional intensity data were collected on a computerized automatic 4-circle CAD-4 Enraf-Nonius diffractometer using graphite filtered $Mo_{K\alpha}$ (Å) radiation's at SAIF Madras.. Temperature of crystal during data collection was 296°K. All the data were corrected for Lorentz and Polarization effect. Three standard reflection were measured where hkl indices varies from -8<=h<=9, - 9<=k<=9, -35<=l<=36. The total number of reflections were 12288 out of which unique reflection were 3140. Each intensity measurement involved in a scan over the reflection peak, a back ground measurement at each end of the scan range and measurement of the peak height. The structure was solved using SHELXS- program for crystal structure solution.

REFINEMENT: The positional co-ordinates, which were obtained from SHELXS 97 and isotropic temperature factors, were subjected to refinement by SHELXL refinement program. After so many cycles of refinement the R factors dropped to 0.0458. Further refinement of the structure was carried out with individuals an isotropic temperature factors of the exponential form.**-2P_1^ 2[h^2a*^2U_{11}+----------+2hKa*bxU_{12}** reduced R factor to 0.0354. The hydrogen

atoms were fixed at this stage by geometrical considerations and were not refined.Refinement of the structure was terminated after two more cycles when all the deviations in parameters became much smaller than the corresponding estimated standard derivations. The final R value was 0.0354 for all 12288 reflections collected.

***RESULTS AND DISCUSSION*:** Atomic coordinates (x 10^4) and equivalent Isotropic displacement parameters (A^2 x 10^3) for Chlorothalonil- is shown in Table 2. Bond lengths [A] Bond angles [deg] for Chlorothalonil- is shown in Table 3 and table 4.Anisotropic displacement parameters (A^2 x 10^3) for Chlorothalonil is shown in Table 5 The anisotropic displacement factor exponent takes the form:--2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]. Torsion angles [deg] is shown in Table 6.The ORTEPdiagram is shown in fig.1. I-R is shown in fig 2.In Chlorothalonil-The structure obtained is almost accurate, In Benzene ring distance between C(1)-C(2) is 1.391(2), C(2)-C(3) is 1.389(3), C(3)-C(4) is 1.398(2) , C(4)-C(5) is 1.391(2), C(5)-C(6) is 1.392(2) and C(1)-C(6) is 1.393(2) and the theoretical Bond Lengths between C=C and C-C are 1.34 and 1.54 respectively showing the regular behavior of benzene ring.The averaged C-Cl and C≡N bond length is 1.7108(16) and1.135(2) respectively, theoretically it comes to be1.77 and 1.16 respectively. These bond lengths are very-very close to theoretical values. By the torsion angles data we can see that the arrangement of atoms are symmetric and we can say that there is no or very small disagreement between the arrangements of atoms in the molecule of Chlorothalonil.

The crystal structure consists of parallel sheets stacked along *a-axis*. Chlorothalonil reduces fungal intracellular glutathione molecules to alternate forms which cannot participate in essential enzymatic reactions, ultimately leading to cell death.

Conclusion:Thus we study the structure of variety of such compounds and correlate their structure with biological activity, so that more safer and effective fungicides at reasonable price can be developed.

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Table 1 Preliminary Crystal Structure of Cholrothalonil

Empirical formula	C8 Cl4 N2
Formula weight	265.90
Temperature	293(2) K

Wavelength	0.71073 A
Crystal system	Monoclinic
Space group	P21/c
Volume	971.62(13) A^3
Z / Calculated density	4 / 1.818 Mg/m^3
Absorption coefficient / F(000)	1.171mm^{-1}/ 520
Crystal size	0.30 x 0.25 x 0.15mm
Unit cell dimensions	a = 6.3354(5)A^o, b = 6.2233(4)A^o,c = 27.753(2)A^o $\alpha = 90^o$, $\beta = 95.397(2)^o$, $\gamma = 90^o$
θ range for data collection	3.23 – 31.22 deg.
Limiting indices	-8<=h<=9, - 9<=k<=9, -35<=l<=36
Reflections collected	12288
Unique	3140 [R(int) = 0.0264]
Completeness to theta = 31.22	99.8 %
Absorption correction	Semi-empirical from equivalents
Max. / min. transmission	0.8440 and 0.7203
Refinement method	Full-matrix least-squares on F^2
Data / Restraints / Parameters	3140 / 0 / 127
Goodness-of-fit on F^2	1.030
Final R indices [I>2sigma(I)]	R1 = 0.0345, wR2 = 0.0833
R indices (all data)	R1 = 0.0506, wR2 = 0.0926
Largest diff. peak and hole	0.327 and - 0.379e.A^{-3}

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for **Cholrothalonil** U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.

atom	x	y	z	U(eq)
C(1)	6545(2)	4210(3)	990(1)	33(1)
C(2)	5917(3)	2990(3)	1418(1)	33(1)
C(3)	4158(3)	3593(3)	1677(1)	34(1)
C(4)	3029(2)	5439(3)	1505(1)	34(1)
C(5)	3632(3)	6671(3)	1076(1)	35(1)
C(6)	5403(3)	6043(3)	822(1)	34(1)
C(7)	8389(3)	3583(3)	729(1)	42(1)
C(8)	3526(3)	2353(3)	2124(1)	43(1)
N(1)	9865(3)	3070(3)	536(1)	60(1)
N(2)	3012(3)	1370(3)	2474(1)	63(1)
Cl(1)	7352(1)	776(1)	1637(1)	50(1)
Cl(2)	893(1)	6152(1)	1840(1)	51(1)
Cl(3)	2226(1)	8902(1)	864(1)	52(1)
Cl(4)	6249(1)	7534(1)	303(1)	51(1)

Table 3. Bond lengths [A] for Chlorothalonil.

C(1)-C(2)	1.391(2)
C(1)-C(6)	1.393(2)
C(1)-C(7)	1.441(2)
C(2)-C(3)	1.389(2)
C(2)-Cl(1)	1.7110(16)
C(3)-C(4)	1.398(2)
C(3)-C(8)	1.436(2)
C(4)-C(5)	1.391(2)
C(4)-Cl(2)	1.7108(16)
C(5)-C(6)	1.392(2)
C(5)-Cl(3)	1.7061(16)
C(6)-Cl(4)	1.7112(16)
C(7)-N(1)	1.135(2)
C(8)-N(2)	1.134(2)

TABLES 4 BOND ANGLES angles [deg] Chlorothalonil

C(2)-C(1)-C(6)	119.71(14)
C(2)-C(1)-C(7)	119.69(15)
C(6)-C(1)-C(7)	120.59(15)
C(3)-C(2)-C(1)	120.24(14)
C(3)-C(2)-Cl(1)	119.76(12)
C(1)-C(2)-Cl(1)	119.97(12)
C(2)-C(3)-C(4)	119.41(14)
C(2)-C(3)-C(8)	120.29(15)
C(4)-C(3)-C(8)	120.29(14)
C(5)-C(4)-C(3)	121.02(14)
C(5)-C(4)-Cl(2)	120.87(13)
C(3)-C(4)-Cl(2)	118.11(12)
C(4)-C(5)-C(6)	118.72(14)
C(4)-C(5)-Cl(3)	120.70(12)
C(6)-C(5)-Cl(3)	120.58(13)
C(5)-C(6)-C(1)	120.89(14)
C(5)-C(6)-Cl(4)	120.63(12)
C(1)-C(6)-Cl(4)	118.48(12)
N(1)-C(7)-C(1)	178.3(2)
N(2)-C(8)-C(3)	179.4(2)

Symmetry transformations used to generate equivalent atoms:

Table 5 Anisotropic displacement parameters (A^2 x 10^3) for Chlorothalonil.

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The anisotropic displacement factor exponent takes the form:
-2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

atom	U11	U22	U33	U23	U13	U12
C(1)	32(1)	37(1)	32(1)	-2(1)	6(1)	4(1)
C(2)	33(1)	32(1)	35(1)	-1(1)	2(1)	5(1)
C(3)	33(1)	36(1)	32(1)	-1(1)	6(1)	-2(1)
C(4)	29(1)	40(1)	34(1)	-6(1)	5(1)	3(1)
C(5)	34(1)	34(1)	37(1)	-1(1)	1(1)	6(1)
C(6)	36(1)	36(1)	32(1)	2(1)	4(1)	1(1)

C(7)	39(1)	46(1)	41(1)	4(1)	9(1)	8(1)
C(8)	40(1)	49(1)	42(1)	4(1)	10(1)	2(1)
N(1)	57(1)	69(1)	59(1)	9(1)	25(1)	21(1)
N(2)	58(1)	75(1)	59(1)	21(1)	21(1)	3(1)
Cl(1)	53(1)	45(1)	53(1)	11(1)	8(1)	17(1)
Cl(2)	41(1)	63(1)	50(1)	-5(1)	16(1)	13(1)
Cl(3)	51(1)	46(1)	59(1)	7(1)	5(1)	18(1)
Cl(4)	57(1)	52(1)	46(1)	15(1)	15(1)	3(1)

Table 6 Torsion angles [deg] for Chlorothalonil.

C(6)-C(1)-C(2)-C(3)	0.0(2)
C(7)-C(1)-C(2)-C(3)	179.18(16)
C(6)-C(1)-C(2)-Cl(1)	-178.28(12)
C(7)-C(1)-C(2)-Cl(1)	0.8(2)
C(1)-C(2)-C(3)-C(4)	-0.1(2)
Cl(1)-C(2)-C(3)-C(4)	178.18(12)
C(1)-C(2)-C(3)-C(8)	-179.05(16)
Cl(1)-C(2)-C(3)-C(8)	-0.7(2)
C(2)-C(3)-C(4)-C(5)	0.4(2)
C(8)-C(3)-C(4)-C(5)	179.34(16)
C(2)-C(3)-C(4)-Cl(2)	-179.15(12)
C(8)-C(3)-C(4)-Cl(2)	-0.2(2)
C(3)-C(4)-C(5)-C(6)	-0.6(2)
Cl(2)-C(4)-C(5)-C(6)	178.96(12)
C(3)-C(4)-C(5)-Cl(3)	179.16(12)
Cl(2)-C(4)-C(5)-Cl(3)	-1.3(2)
C(4)-C(5)-C(6)-C(1)	0.5(2)
Cl(3)-C(5)-C(6)-C(1)	-179.27(13)
C(4)-C(5)-C(6)-Cl(4)	-178.51(13)
Cl(3)-C(5)-C(6)-Cl(4)	1.7(2)
C(2)-C(1)-C(6)-C(5)	-0.2(2)
C(7)-C(1)-C(6)-C(5)	-179.35(16)
C(2)-C(1)-C(6)-Cl(4)	178.81(13)
C(7)-C(1)-C(6)-Cl(4)	-0.3(2)
C(2)-C(1)-C(7)-N(1)	-34(8)
C(6)-C(1)-C(7)-N(1)	146(7)
C(2)-C(3)-C(8)-N(2)	-118(23)
C(4)-C(3)-C(8)-N(2)	63(23)

Symmetry transformations used to generate equivalent atoms: