The electronic structures of six organochlorine insecticides: \( \gamma \)-lindane (I), aldrin (II), dieldrin (III), DDD (IV), DDE (V) and DDT (VI) have been investigated by UV photoelectron spectroscopy (UPS), quantum chemical calculations and comparison with molecular modelling studies. Their electronic and molecular structures are discussed in order to rationalize their biological activity. In this work we relate the biological activity of these insecticides to their experimentally observed electronic and molecular structures.
Abstract: The electronic structures of six organochlorine insecticides: γ-lindane (I), aldrin (II), dieldrin (III), DDD (IV), DDE (V) and DDT (VI) have been investigated by UV photoelectron spectroscopy (UPS), quantum chemical calculations and comparison with molecular modelling studies. Their electronic and molecular structures are discussed in order to rationalize their biological activity. In this work we relate the biological activity of these insecticides to their experimentally observed electronic and molecular structures.

Keywords: photoelectron spectroscopy; insecticides; biological activity
1. Introduction

The compounds studied in this work (Scheme 1) are important as insecticides and organic pollutants (I-VI) [1].

Scheme 1. Organochlorine insecticides (OC) studied

They bind to ion channels which are embedded in the membranes of neurons and which influence the flow of $\text{Na}^+$ or $\text{Cl}^-$ ions. Compounds I-III affect $\text{Cl}^-$ ion transport by binding to the gamma-aminobutyric acid (GABA) receptor while IV-VI affect $\text{Na}^+$ ion transport by binding to the voltage gated sodium ion channels. In both cases the binding to various channel elements interrupts the flow of ions through the channel and disrupts the propagation of nerve impulses which induces convulsions and speedy death of an insect. Neurotoxic insecticides are the most important insecticides, because they induce quick death which prevents crop damage and spread of disease. It is important to note that neurotoxicity of these insecticides is not fully selective i.e. they affect insects and mammals, but to different extents. The reason for greater toxicity towards insects than to mammals lies in structural differences of insect binding sites compared to mammals’ as well as in different rates of metabolic activation and detoxification in insects and mammals [2]. Nevertheless, the unwanted
toxicity of OC towards humans and other vertebrates have caused them to be banned or restricted for agricultural use. However, because of their generally low rates of environmental degradation and bioaccumulation in the food chain these banned insecticides now represent important environmental pollutants. The adverse effects of these insecticides are reflected not only in increased neuroexcitability, but also in interference with the mammalian reproductive system [3]. The evidence for carcinogenic effects of insecticides is not clear at present [4]. Organochlorine insecticides studied here are ubiquitous environmental pollutants, resistant to chemical degradation and with tendency to accumulate in the food chain and environment. Furthermore, some of them show cooperative (synergistic) toxicity towards human neurons [5].

The accumulation of OC in the environment has led to the development of a plethora of quantitative-structure-property-relationship (QSPR) models and descriptors for predicting factors which govern their toxicity and environmental distribution. Various descriptors built into these models are based on lipophilic, electronic and structural properties of the respective molecules [6]. However, while these models are useful, they rely on pattern recognition driven by statistical parameters and use theoretical descriptors (HOMO-LUMO energies, hardness, effective molecular size, polarizability, electron charge distribution, vibrational modes, effective atomic charges and potentials etc.). Therefore most of these descriptors are calculated using theoretical approximations and are not experimentally observable. Such models should be complemented by detailed understanding of OC mechanism of action at the molecular level. However, detailed, mechanistic understanding is difficult to obtain because it requires the knowledge of all relevant components: the molecular target (e.g. ion channel), its active binding site and the
electronic or molecular structure of the insecticide ligand itself. This is why QSPR and QSAR models proliferate while experimental study of molecular mechanisms lags behind.

In order to further our understanding of insecticide action (without proposing another QSPR relationship) we used UV photoelectron spectroscopy (UPS) to study the electronic structure of OC which is one of the key factors governing molecular mechanisms pertaining to their biological activity. UPS is very useful for studying molecular electronic structure. It has been used previously for three organophosphorus insecticides where it was proposed that UPS can be used for qualitative identification of individual insecticides in mixtures [7]. We are interested in UPS to provide information about the energy of electrons which are localized on particular functional groups in OC molecules. These functional groups in turn determine transport and specific insecticide binding to its biological target.

Our selection of OC for this study was based on their widespread use or on their significance as persistent pollutants. Furthermore, the selected compounds are sufficiently volatile to be transferable into the gas-phase by heating (without experiencing decomposition) which is necessary for measuring photoelectron spectra.

It must be noted that the observed toxicity/biological activity of individual OC depends on many other variables besides electronic structure e.g. solubility, polarizability, environmental residence time, insecticide transport mechanisms within the individual organism. It would thus clearly be impossible to obtain simple, quantitative relationship between spectral peaks (and their associated ionization energy) and OC’s toxicity or biological activity. To reiterate therefore, our aim was more modest and comprised only the rationalization of biological activity from the viewpoint of experimentally determined electronic and molecular structure.
2. Experimental and theoretical methods

The sample compounds were purchased from Aldrich and used without further purification after checking their identity and purity by NMR spectroscopy.

The HeI/HeII photoelectron spectra (UPS) were recorded by a Vacuum Generators UV-G3 spectrometer and calibrated with small amounts of Xe or Ar gas added to the sample flow. The spectral resolution of the HeI and HeII spectra was 25 meV and 70 meV, respectively when measured as FWHM of the 3p-1 2P3/2 Ar⁺ ← Ar (1S0) line. The samples were introduced with the inlet probe heated to 850-120°C, respectively. The spectra obtained were reproducible and showed no signs of decomposition. Decomposition is usually discernible from the appearance of sharp intense peaks whose intensity varies with time and which are due to the presence of small molecules (decomposition products) in the spectrometer's ionization chamber. We did not detect any decomposition peaks related to Cl₂ (11.59 eV) or HCl (12.75 eV). Quantum chemical calculations were performed with the Gaussian 03 program [8] for the purpose of spectral assignment. We used the Green’s functions method (GF) and a 6-31G* basis set for the calculation of ionization energies [9]. The existence of shake-up states in the spectral bands does not appear to be important since according to GF calculations the pole strengths of all the assigned bands are >0.9. We have also investigated several conformers of IV and VI and found that the maximum difference in ionization energies between conformers is at most 0.2 eV (i.e. below experimental resolution) and that the orbital ordering remains unaffected by conformational changes. The molecules I-III exist predominantly in one conformer (γ-lindane) or are conformationally rigid (aldrin and dieldrin). The calculated molecular geometries, fully optimized at the B3LYP/6-31G* level were used as input into GF calculations. All the optimized structures corresponded to minima on their potential energy
surfaces as was inferred from the absence of imaginary vibrational frequencies. The calculated geometries agreed to within 0.01Å and 1.5° with the molecular structures of lindane, aldrin and dieldrin which were determined experimentally by X-ray diffraction [10].

3. Results and Discussion

*Photoelectron Spectra*

The UPS spectra are shown in Figs. 1-6. The spectral assignments are summarized in Table 1 and are based on HeI/HeII intensity variations [11], GF calculations and comparison with the assigned spectra of related molecules e.g. halobenzenes and norbornene [12-17]. Such comparison leads to assignment of all the bands below 10 eV of ionization energy as π-ionizations (see Table 1). The HeII/HeI photoionization cross-section ratios for atomic orbitals C2p, O2p, Cl3p are 0.31, 0.64 and 0.05, respectively [11]. The cross-section data suggest that bands whose relative intensity decreases significantly on going from HeI to HeII radiation correspond to ionizations from molecular orbitals with chlorine lone pair character (nCl). On the other hand, the bands whose relative intensity increases correspond to ionizations of orbitals with oxygen lone pair character (nO). The spectra allow us to determine ionization energies from MOs mainly localized on specific functional groups of individual OC molecules.

Inspection of the photoelectron spectra of I-VI shows that the relative intensity of several bands in the 10.4-12.0 eV energy range (approximately) decreases strongly on going from HeI to HeII radiation so that these bands can be readily assigned to chlorine lone pairs (nCl). The band at 10.0 eV in the spectrum of III shows relative intensity increase and can be assigned to the oxygen lone pair (nO) of the epoxide ring. The span of chlorine lone pair ionization energies in I-III group is broader than in the IV-VI group. This is due to larger number of chlorine atoms present in I-III
vs. IV-VI and also to the fact that there are three distinctive sets of equivalent chlorine atoms present in the former group compared to two equivalent sets of chlorines in the latter. Smaller number of sets of equivalents chlorine atoms leads to narrower (sharper) chlorine lone pair bands. Furthermore, the onset of nCl ionizations appears at lower energy in I-III molecules compared to IV-VI molecules. This may have repercussions for OC ligand binding to their ion channel receptors.

The other important spectral features in OC spectra comprise ionizations of π-orbitals. In aldrin (II) the two π-orbitals appear at 9.05 and 9.4 eV, while in dieldrin (III) the single π-orbital is shifted towards higher ionization energy by 0.2 eV. This shift can be attributed to inductive effect of epoxide oxygen.

Biological activity

The measurement of the electronic structure of OC was only one of our aims. We also try to relate their electronic structures to the biological activity of OC as ligands in the context of their binding to ion channel receptors [18]. Therefore a short description of the molecular basis of OC activity is deemed necessary. We shall try to answer questions like:

1. How and why do ligand-target interactions differ in the two OC classes? (We distinguish two classes of OC ligands: I-III and IV-VI.)

2. Why is the observed acute toxicity of I-III much larger than the chlorinated biphenyls IV-VI (Table 1)?

Modelling studies [18] have shown that there are two different ways of interfering with the flow of ions through the ion channel. The “indirect” mode of action proceeds via modifying conformations of protein/peptide groups in the channel wall upon binding of a ligand into the (protein/peptide’s) active pockets. Such binding, changes the conformations of constituent protein/peptide groups which in turn modifies the
structure of the channel wall and the flow of ions through it. The “direct” mode of action comprises binding or “anchoring” the ligand across the channel’s aperture and direct interference with the ion flow. These two mechanisms favor ligand molecules of different rigidities and electronic structures.

Binding of conformationally flexible chlorinated biphenyls (IV-VI) alters the positioning of linker peptides and bending of helices in such a way as to freeze the sodium ion channel in the open state with subsequent unrestricted flow of \( \text{Na}^+ \) through it. The biphenyl OC bind into active pockets in the wall of the main channel which requires them to be conformationally flexible in order to reach and fit the twisted forms of these pockets. The “indirect” mode ligand binding comprises mostly very weak hydrophobic interactions [18].

Binding of organochlorines I-III is governed by the fact that these ligands are conformationally rigid and need to be tightly bound to the GABA receptor’s ion channel aperture (in order to physically block the channel). The ligand-receptor binding interactions involving I-III comprise chlorine bonds to methyl groups of residues lining the ion channel as well as orthogonal hydrogen bonds to hydrogen bond donor groups [18c,19]. These chlorine bonds are weaker than normal hydrogen bonds, but have the advantage of having lower desolvation enthalpies compared to classical hydrogen bonds [18]. These directional chlorine bonds are known to provide (on the basis of experimental evidence) highly stabilizing directional contacts between halogen-substituted ligands and biological substrates [19].

_How do UPS data contribute to the discussion of binding for the two classes of OC ligands i.e. I-III and IV-VI?_ Examination of their photoelectron spectra shows that I-III have lower \( n_{\text{Cl}} \) ionization energy onsets and wider range of \( n_{\text{Cl}} \) energies than IV-VI. Chlorine atoms form noncovalent interactions (vide supra) with residues in the
ion channels. Lower $n_{Cl}$ ionization energy implies better electron donating or hydrogen bond accepting ability and subsequently stronger noncovalent chlorine interactions with the e.g. amide functional groups of amino acid residues in the ion channel receptor. This is consistent with the fact that I-III are more toxic (biologically potent) than IV-VI (Table 1). π electrons in II-III and especially in IV-VI have lower ionization energies than chlorine lone pairs, but their influence on ligand binding is less important for the following reasons. The steric access to π orbitals by receptor residues (amino acids) in II-III is blocked by the neighboring chlorine substituents while in IV-VI the access to π-orbitals is less constrained. Nevertheless, such ligand-receptor π-π interactions are weaker than chlorine nonbonding interactions because they are non-directional and can only take place with residues which contain aromatic groups (e.g. phenylalanine, tyrosine, tryptophan).

4. Conclusion

We have used the information on the electronic and molecular structures of several organochlorine insecticides (ligands) to rationalize the mechanism of their binding to ion channel receptors. Inspection of molecular structures of I-VI shows that I-III are more conformationally rigid than IV-VI. The latter group of molecules can exist in several conformations which can be accessed by the rotations of phenyl groups. II and III have rigid bicyclic geometry or only a single stable conformer (I). We propose that such mechanisms include (amongst others) two important factors: conformational rigidity of the OC ligand and energy of its chlorine lone pair. It is known that the existence of multiple factors which govern biological activity of each pesticide makes the establishment of quantitative structure/activity relationships based on theoretical descriptors difficult to achieve. Nevertheless, we have shown how descriptors like molecular rigidity and ionization energies may be used to rationalize the mechanisms.
of biological action.

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**Table 1.** Experimental ($E_i$/eV) and calculated (GF/eV) vertical ionization energies and orbital assignments for organochlorine insecticides$^{a,b,c,d}$
<table>
<thead>
<tr>
<th>Compound</th>
<th>Band</th>
<th>$E_i$</th>
<th>GF/eV</th>
<th>Assignment</th>
<th>LD$_{50}$ mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (γ-lindane)</td>
<td>X-E</td>
<td>10.9-11.15</td>
<td>10.66, 10.87, 10.98, 11.08, 11.12, 11.30</td>
<td>$n_{Cl}$</td>
<td>100</td>
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<td></td>
<td>F-K</td>
<td>11.9-12.5</td>
<td>11.62, 11.74, 11.80 11.88, 12.07, 12.22</td>
<td>$n_{Cl}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L</td>
<td>13.3</td>
<td>13.26</td>
<td>$\sigma$</td>
<td></td>
</tr>
<tr>
<td>II (aldrin)</td>
<td>X</td>
<td>9.05</td>
<td>8.69</td>
<td>$\pi$</td>
<td>39</td>
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<tr>
<td></td>
<td>A</td>
<td>9.4</td>
<td>9.16</td>
<td>$\pi$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>10.4</td>
<td>10.53</td>
<td>$n_{Cl}$</td>
<td></td>
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<td></td>
<td>C</td>
<td>10.9</td>
<td>10.80</td>
<td>$n_{Cl}$</td>
<td></td>
</tr>
<tr>
<td></td>
<td>D-N</td>
<td>11.4-12.35</td>
<td>10.94, 11.0, 11.18 11.28, 11.37, 11.53 11.67, 11.82, 11.85 12.14</td>
<td>$n_{Cl}$</td>
<td></td>
</tr>
<tr>
<td>III (dieldrin)</td>
<td>X</td>
<td>9.2</td>
<td>9.2</td>
<td>$\pi$</td>
<td>46</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>10.0</td>
<td>10.03</td>
<td>$n_{O}$</td>
<td></td>
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<tr>
<td></td>
<td>B-M</td>
<td>(10.4,10.9)</td>
<td>10.7, 10.92, 11.09 11.10, 11.27, 11.29 11.43, 11.60, 11.85 11.88, 11.99, 12.03</td>
<td>$n_{Cl}$</td>
<td></td>
</tr>
<tr>
<td>IV (DDD)</td>
<td>X</td>
<td>8.75</td>
<td>8.59</td>
<td>$\pi$</td>
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<td>9.2</td>
<td>9.13</td>
<td>$\pi$</td>
<td></td>
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<td></td>
<td>B-C</td>
<td>9.6</td>
<td>9.37, 9.42</td>
<td>$\pi$, $\pi$</td>
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<td>D</td>
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<td>E-K</td>
<td>11.4</td>
<td>11.1, 11.31, 11.33, 11.47, 11.51, 11.62 11.69</td>
<td>$n_{Cl}$</td>
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<td>L</td>
<td>12.1</td>
<td>12.41</td>
<td>$\sigma$</td>
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<td>8.28</td>
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<td>9.13</td>
<td>$\pi$</td>
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<td>B-D</td>
<td>9.65</td>
<td>9.33, 9.47, 10.04 $\pi$, $\pi$, $\pi_{CC}$</td>
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<tr>
<td></td>
<td>E</td>
<td>11.05</td>
<td>11.08</td>
<td>$n_{Cl}$</td>
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<tr>
<td><strong>F-G</strong></td>
<td>11.28</td>
<td>11.34, 11.35</td>
<td>n&lt;sub&gt;Cl&lt;/sub&gt;</td>
<td></td>
<td></td>
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<tr>
<td><strong>H-K</strong></td>
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<td>11.61, 11.74, 11.80, 11.98</td>
<td>n&lt;sub&gt;Cl&lt;/sub&gt;</td>
<td></td>
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<tr>
<td><strong>VI (DDT)</strong></td>
<td><strong>X</strong></td>
<td>8.75</td>
<td>8.62</td>
<td>π</td>
<td>300</td>
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<td><strong>A</strong></td>
<td>9.20</td>
<td>9.16</td>
<td>π</td>
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<tr>
<td><strong>B-C</strong></td>
<td>9.60</td>
<td>9.32, 9.38</td>
<td>π</td>
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<tr>
<td><strong>D-J</strong></td>
<td>11.40</td>
<td>11.05, 11.19, 11.29, 11.30, 11.35, 11.58, 11.63</td>
<td>n&lt;sub&gt;Cl&lt;/sub&gt;</td>
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<td><strong>K-L</strong></td>
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<td>12.0, 12.21</td>
<td>n&lt;sub&gt;Cl&lt;/sub&gt;</td>
<td></td>
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</tbody>
</table>

\^ the π<sub>CC</sub> represent orbital localized on ethane moiety of V

\^ the LD<sub>50</sub> dose (from ref. 6b) for rats

\^ the numbers in brackets represent poorly resolved shoulders

\^ the LD<sub>50</sub> = is the dose required to kill half the members of a tested population after a specified test duration.

References


Fig. 1 HeI and HeII photoelectron spectra of I (γ-lindane)
Fig. 2 Hel and Heli photoelectron spectra of II (aldrin)
Fig. 3 HeI and HeII photoelectron spectra of \textbf{III} (dieldrin)
Fig. 4 HeI and HeII photoelectron spectra of IV (1,1-dichloro-2,2-bis(4-chlorophenyl)ethane; DDD)
Fig. 5 HeI photoelectron spectrum of V (1,1-dichloro-2,2-bis(4-chlorophenyl)ethene; DDE)
Fig. 6 HeI photoelectron spectrum of VI (1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane; DDT)