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Cellular Localization of Dieldrin and Structure–Activity Relationship of Dieldrin Analogues in Dopaminergic Cells

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Abstract

The incidence of Parkinson's disease (PD) correlates with environmental exposure to pesticides, such as the organochlorine insecticide, dieldrin. Previous studies found an increased concentration of the pesticide in the striatal region of the brains of PD patients and also that dieldrin adversely affects cellular processes associated with PD. These processes include mitochondrial function and reactive oxygen species production. However, the mechanism and specific cellular targets responsible for dieldrin-mediated cellular dysfunction and the structural components of dieldrin contributing to its toxicity (toxicophore) have not been fully defined. In order to identify the toxicophore of dieldrin, a structure–activity approach was used, with the toxicity profiles of numerous analogues of dieldrin (including aldrin, endrin, and *cis*-aldrin diol) assessed in PC6-3 cells. The MTT and lactate dehydrogenase (LDH) assays were used to monitor cell viability and membrane permeability after treatment with each compound. Cellular assays monitoring ROS production and extracellular dopamine metabolite levels were also used. Structure and stereochemistry for dieldrin were found to be very important for toxicity and other end points measured. Small changes in structure for dieldrin (e.g., comparison to the stereoisomer endrin)

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Notes

The authors declare no competing financial interest.

Supporting Information

Chromatographs from GC separation of extracted dieldrin and endrin and inhibition of rabbit LDH by organochlorines. This material is available free of charge via the Internet at <http://pubs.acs.org>.

yielded significant differences in toxicity. Interestingly, the *cis*-diol metabolite of dieldrin was found to be significantly more toxic than the parent compound. Disruption of dopamine catabolism yielded elevated levels of the neurotoxin, 3,4-dihydroxyphenylacetaldehyde, for many organochlorines. Comparisons of the toxicity profiles for each dieldrin analogue indicated a structure-specific effect important for elucidating the mechanisms of dieldrin neurotoxicity.

INTRODUCTION

While the exact mechanism of neuronal loss in Parkinson's disease (PD) is unknown,¹ a majority of PD cases are thought to be due to exposure to environmental toxicants, such as insecticides, herbicides, and heavy metals.^{2–6} The correlation between pesticide exposure and PD has been corroborated by an increased concentration of pesticides found in the brains of PD patients.^{2,4–6} One pesticide of interest is dieldrin, as its level was elevated in PD patients, a result not found in the brains of patients with other neurodegenerative diseases, such as Alzheimer's disease, or in the brains of healthy, control patients.⁴ In addition, previous studies have also shown dieldrin to affect a number of cellular processes associated with PD. The consequences of these effects include increased oxidative stress, disruptions in the metabolism and trafficking of dopamine (DA), apoptosis susceptibility, and mitochondrial dysfunction.^{1,7–12} Many of the reported adverse effects caused by exposure to dieldrin, such as reactive oxygen species (ROS) production and apoptosis, lead to selective dopaminergic cell dysfunction and/or death.^{7,9,12,13} A question that remains is, what is the mechanism via which dieldrin mediates such adverse cellular effects (e.g., ROS formation and disruption of DA trafficking)? Where does dieldrin localize in a cell, and what are the targets? How does dieldrin interact with proteins, covalently or noncovalently, given that it has an epoxide?

Previous studies have assessed how structural changes to dieldrin affect its pesticidal activity in various species of insects.^{14–16} Significant alterations to insect toxicity were found even with small changes to dieldrin's structure, such as removal of chlorine atoms or a carbon bridge.^{14–16} Such data suggest that specific structural elements are critical for its insecticidal properties. Of question then is how these same structural features relate to dieldrin's dopaminergic toxicity in humans. Do the compounds with the most potent insecticidal activity also demonstrate the most detrimental effects in a model of dopaminergic cells?

To determine dieldrin's structure–activity relationship, three aspects of its structure were assessed (Figure 1: 1). The relative 3-dimensional orientation and presence of the methano bridge (Groups 1 and 2). The identity of the polar moiety (olefin, *cis*-diol, or epoxide) located opposite the chlorine atoms (Groups 2 and 3). The combined effect of the 3-dimensional orientation and polar group (Group 3). The effects of these compounds on cell viability, DA metabolism, and ROS production were investigated.

Comparison of these features and their activity will provide valuable information about the cellular targets of these compounds and will be used to gain valuable insight into the mechanistic link between dieldrin exposure and the development of PD. Ultimately, how does dieldrin exposure predispose individuals to PD? The selectivity of dieldrin toward

dopaminergic neuronal death *in vivo* is predicted to facilitate the development of PD. Therefore, it is of high importance that the mechanism of dopaminergic toxicity from dieldrin exposure is understood.

EXPERIMENTAL PROCEDURES

Caution: *The following compounds are hazardous and should be handled carefully: dieldrin, aldrin, endrin, isodrin, cis-aldrin diol, desmethylene dieldrin, and desmethylene aldrin. All are highly lipophilic and contact with skin, eyes, and clothing should be avoided.*

Chemicals

Dieldrin, aldrin, endrin, and isodrin were purchased from Chem Service (West Chester, PA). All other reagents, unless otherwise noted, were purchased from Sigma Aldrich (St. Louis, MO) without further purification. Organochlorine analogues were named based on the von Baeyer/IUPAC system for polycyclic compounds and as discussed previously.¹⁷

***cis*-Aldrin Diol (1,8,9,10,11,11-Hexachloro-4,5-(*exo*)*cis*-dihydroxy-2,3-7,6-*endo*-2,1-7,8-exotetracyclo[6.2.1.1^{3,6}.0^{2,7}]dodec-9-ene)**

cis-Aldrin diol was synthesized using a method adapted from the literature.^{18,19} Briefly, aldrin (86 mg, 0.24 mmol) was combined with dry ether (6.4 mL), pyridine (0.15 mL), and osmium tetroxide (50 mg, 0.20 mmol). The reaction was stirred for 2 h at room temperature and then allowed to stand overnight in the dark. The ether was evaporated with nitrogen, giving a brown residue that was then dissolved in dichloromethane (10 mL) and stirred vigorously overnight with a solution of water (6.5 mL), potassium hydroxide (71 mg, 1.3 mmol), and D-mannitol (630 mg, 3.46 mmol). The dichloromethane layer was removed, washed with water, and dried with sodium sulfate, giving the product in 90% yield. ¹H NMR (DMSO-*d*₆): δ 1.12 (d, 1H, CH₂), 1.60 (d, 1H, CH₂), 2.06 (m, 2H, CH), 2.60 (m, 2H, CH), 3.57 (m, 2H, CH), 4.93 (m, 2H, OH); ¹³C NMR (DMSO-*d*₆): δ 28.3, 43.0, 51.7, 73.6, 81.0, 104.7, 130.8; MS (EI) *m/z* calcd for C₁₂H₁₀Cl₆O₂ 395.9; found *m/z* 396.1.

Desmethylene Aldrin (1,8,9,10,11,11-Hexachloro-2,3-7,6-*endo*tricyclo[6.2.1.0^{2,7}]undeca-4,9-diene)

Using methods adapted from literature methods,^{14,19–21} we placed hexachlorocyclopentadiene (1.0 g, 0.004 mol, 0.6 mL) and 1,4-cyclohexadiene (1.3 g, 0.016 mol, 1.51 mL) under argon and stirred them at 110 °C for 44 h. The product was purified by column chromatography on silica gel with hexanes as mobile phase, resulting in a 25% yield. ¹H NMR (CDCl₃): δ 1.90–1.99 and 2.35–2.44 (m, 4H, CH₂), 2.98–3.09 (m, 2H, CH), 5.86 (m, 2H, HC=CH). ¹³C NMR (CDCl₃): δ 21.9, 45.8, 82.5, 103.2, 126.7, 131.6; MS (EI) *m/z* calculated for C₁₁H₈Cl₆ 349.9; found *m/z* 349.9.

Desmethylene Dieldrin (1,8,9,10,11,11-Hexachloro-4,5-*epoxy*-2,3-7,6-*endo*tricyclo[6.2.1.0^{2,7}]undec-9-ene)

Using methods adapted from literature methods,^{14,19,21,22} we added 3-chloroperbenzoic acid (*m*CPBA) (0.08 g, 0.5 mmol) in 2.0 mL of dichloromethane dropwise to desmethylene

aldrin (0.1 g, 0.3 mmol) in 2.0 mL of dichloromethane and stirred at room temperature for 2 h. The reaction mixture was extracted twice with water. The organic layer was then dried with magnesium sulfate and the product purified by column chromatography on silica gel with a mobile phase of 4:1 hexanes to acetone, resulting in a 50% yield. ^1H NMR (CDCl_3): δ 1.59–1.67 and 2.40–2.48 (m, 4H, CH_2), 2.91–2.99 (m, 2H, CH), 3.20–3.24 (m, 2H, CH–O). ^{13}C NMR (CDCl_3): δ 22.2, 42.2, 49.1, 82.3, 102.8, 132.0; MS (EI) m/z calculated for $\text{C}_{11}\text{H}_8\text{Cl}_6\text{O}$ 365.9; found m/z 365.9.

Thiol and Amine Reactivity

Thiol reactivity of dieldrin was determined using Ellman's reagent (5,5'-dithiobis(2-nitrobenzoic acid) (DTNB)) and a procedure adapted from the literature.^{23,24} *N*-Acetyl Cys (100 μM) was incubated with dieldrin (0–2.5 mM) overnight at 37 °C in DMSO. DTNB (0.5 mM) in DMSO was added along with 50 mM sodium phosphate buffer, pH 7.4 (25% v/v), and absorbance monitored at 412 nm using a Molecular Devices SpectraMax plate reader. The free thiol concentration in each well was determined by comparing the absorbance to a standard curve of DTNB reacted with varying concentrations of *N*-acetyl Cys.

The reactivity of dieldrin with primary amines was assessed using *N*-acetyl Lys. Dieldrin (0–100 μM in DMSO) was incubated with *N*-acetyl Lys (100 μM in DMSO) at 37 °C for 24 h. The concentration of dieldrin was monitored via an HPLC method adapted from Mowafy et al.²⁵ Briefly, the samples were analyzed on an Agilent 1100 Series Capillary HPLC with a Phenomenex C18 Jupiter column (5 μm , 300 Å, 150 $\times$ 1.0 mm) using isocratic conditions of water (25%) and methanol (75%), a flow rate of 50 $\mu\text{L}/\text{min}$, and the absorbance measured via photodiode array at 214 and 234 nm. Differences in peak area between dieldrin incubated with and without *N*-acetyl Lys are predicted to indicate reactivity between these two compounds, i.e., dieldrin and the dieldrin-Lys conjugate.

Cell Culture

PC6-3 cells, a generous gift from Stefan Strack (University of Iowa), were cultured in RPMI1640 medium (GIBCO, Invitrogen, Carlsbad, CA) supplemented with heat-inactivated horse serum (10%) (HyClone, Thermo Scientific, Rockford, IL), fetal bovine serum (5%) (GIBCO), penicillin (10 IU/mL), and streptomycin (10 mg/mL) and were grown in 100 mm^2 tissue culture dishes at 37 °C with 5% CO_2 . Cells (3×10^4 cells/well or 6×10^4 cells/well for DA-supplemented metabolism experiments) were seeded into six-well plates and allowed to grow for four days at 37 °C with 5% CO_2 . The cells were then differentiated with 2.5S nerve growth factor (NGF) (BD Biosciences, Bedford, MA) at 50 ng/mL for 4 days. The cells used in these experiments are a subline of PC12 cells, an established cellular model for dopaminergic neurons.^{26–29} Previous work has demonstrated that PC6-3 cells express enzymatically active tyrosine hydroxylase and metabolize dopamine, thereby demonstrating their utility as a cellular model for dopaminergic neurons.^{30–32}

Treatment of Cells with Dieldrin and Analogues

Media were removed from cells and replaced with HEPES-buffered saline containing 115 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl_2 , 0.8 mM MgSO_4 , 5.5 mM glucose, 1.0 mM NaH_2PO_4 , and 15 mM HEPES (pH 7.4). The cells were treated with the compounds

(dieldrin, aldrin, endrin, isodrin, *cis*-aldrin diol, desmethylene aldrin, or desmethylene dieldrin) at 0–900 μM in dimethylsulfoxide (DMSO, 0.6%) for 4 h. The concentrations used in this study (10–900 μM) are higher than the concentrations detected in the brains of Parkinson's patients (2.4 μM , based on reported values of μg of dieldrin per g of lipid in the striatum)^{2,4–6} but were chosen to represent the full range of cell death in this model system (20–100% loss of cell viability). This range was required to calculate the IC_{50} values, which were used for accurate comparisons between compounds and were also comparable to the concentrations used in similar studies.^{7,11,13,33–36}

Cell Fractionation and GC/MS Analysis

Upon 4 h of treatment of the PC6-3 cells with dieldrin (0–300 μM), the HEPES-buffered saline used for treatment was collected (extracellular fraction) and then lysis buffer (10 mM K_2HPO_4 with 0.1% Triton X-100 (v/v)) was added to each well. The cell lysate was collected and sonicated for 5 min using a sonicator bath. The membrane/lipid fraction was separated from the cytosol and lysed organelles (intracellular fraction) using centrifugation at 1000g for 10 min at 4 °C (Abcam Technical Bulletin, Plasma Membrane Protein Extraction Kit). The pellet (membrane/lipid fraction) was resuspended in phosphate-buffered saline, and all samples were stored at –70 °C pending analysis.

Prior to extraction, each sample was spiked with an internal standard (endrin, 1 $\mu\text{g}/\text{mL}$). The organochlorines were extracted three times using hexanes with a volume equal to the sample volume. If an emulsion formed, one part ethanol was added to the sample. The solvent was removed from the organic layer using nitrogen gas and then the sample dissolved in ethyl acetate. Dieldrin and endrin were separated using a Thermo Voyager gas chromatograph coupled with a single quadrupole mass spectrometer. Flow of the gas carrier was 20 mL/min, and the temperature was 50 °C for 2 min, increased by 10°/min to 280 °C and then held for 10 min. The mass spectrometer monitored ionization at 79 m/z and 263 m/z . Peak area quantification was completed using Xcaliber 2.0. Dieldrin peak area at 263 m/z , elution time 20.28 min, was first normalized to the internal standard (endrin at 263 m/z , elution time 19.88 min) and then compared to a standard curve. The amount of dieldrin (μg) in each of the fractions was compared to the amount of dieldrin extracted from treatment with 10 μM of dieldrin and are given as fold change of dieldrin. Values are reported as the mean $\pm$ SD, $n = 3$.

Cell Viability

The effect of each compound on cell viability was determined using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay, in which the yellow, MTT tetrazolium is converted to a purple formazan salt by cellular reductases (e.g., succinate dehydrogenase). After a 4 h treatment of the cells with each compound, the cells were incubated for 1.25 h at 37 °C with MTT (0.5 mg/mL). The HEPES-buffered saline was then removed from each well and centrifuged at 10,000g for 5 min. This pellet and the formazan salt remaining in each well were dissolved in DMSO and combined. Absorbances were then measured at 570 and 650 nm using a Molecular Devices Spectra-Max plate reader. Results are presented as % of control.

IC₅₀ Value Calculations

In order to calculate the IC₅₀ values for each compound based on their cell viability, the dose–response data was normalized between 0 and 100%, and graphed against the log[inhibitor]. A nonlinear curve fitting was applied based on log[inhibitor] vs normalized response with variable slope. The concentration at which 50% normalized activity was observed is reported as the IC₅₀ ± SEM.

Cytotoxicity Assay

The cytotoxicity of each compound was determined using Cytotoxicity Detection Kit^{PLUS} (LDH) (Roche Applied Science, Mannheim, Germany), according to the manufacturer's specifications. Briefly, a high control for each set consisted of cells incubated with 0.6% DMSO and then for the last 15 min of the experiment, lysis buffer, as provided by the manufacturer. The low control in each set was a well incubated with 0.6% DMSO and no lysis buffer. At 4 h, an aliquot of media was removed from each well and incubated with the reaction mixture provided for 20 min in the dark at RT. The reaction was then stopped and the absorbance at 492 and 690 nm measured using a Molecular Devices Spectra-Max plate reader. In order to determine the percent cytotoxicity, the value obtained for the low control was subtracted from each treated well, then divided by the difference between the high control and the low control. Dieldrin and the other analogues did not interfere with the absorbance readings for this assay.

The ability of each compound to inhibit LDH was evaluated by incubating rabbit L-LDH (0.5 U/mL) with various concentrations of each compound (0–900 μ M, 0.6% DMSO) for 30 min in HEPES-buffered saline. At this point, the reaction mixture containing the necessary enzyme substrates and cofactors was added and incubated in the dark at room temperature for 5 h. Absorbances at 492 and 690 nm were then measured and the normalized absorbance determined by subtracting the 690 nm absorbance from the absorbance at 492 nm to assess enzyme activity.

Dopamine Metabolism

The effect of each compound on the extracellular concentration of DA metabolites was monitored. Cells were treated with 0, 10, 25, 50, 100, or 300 μ M of each compound. An aliquot of the extracellular HEPES-buffered saline was removed at 0, 1, and 4 h and then mixed with perchloric acid (5% v/v) in order to precipitate the proteins and terminate any reaction. The samples were stored at –70 °C, thawed, and centrifuged at 10,000g for 5 min prior to HPLC analysis. Samples were analyzed by an Agilent 1200 Series Capillary HPLC with a Phenomenex C18 Luna column (1 × 150 mm). The dopamine metabolites 3,4-dihydroxyphenylacetaldehyde (DOPAL), 3,4-dihydroxyphenylacetic acid (DOPAC), and 3,4-dihydroxyphenylethanol (DOPET) were separated using isocratic conditions of 0.1% trifluoroacetic acid in water with 6% ACN (v/v), a flow rate of 50 μ L/min, and detection with a photodiode array detector (absorbance at 202 and 280 nm). The peak area was then converted to concentration by comparison to a standard curve calculated from metabolite standards as previously described.^{30,37–39} For the DA-supplemented metabolism experiments, cells were supplemented with 100 μ M DA 15 min prior to dieldrin treatment to initiate DA metabolism.

Flow Cytometry

Differentiated PC6-3 cells were pretreated with dihydroethidium (DHE, 10 μ M, 0.2% DMSO) and 2',7'-dichlorodihydrofluorescein diacetate (H₂DCFDA, 50 μ M, 0.2% DMSO) for 20 min at 37 °C in HEPES-buffered saline. Cells were then washed and treated with each dieldrin analogue at a nontoxic dose (<20% cell death according to MTT assay) for 1 h. The doses of each organochlorine used were dieldrin (25 μ M), aldrin (25 μ M), *cis*-aldrin diol (25 μ M), desmethylene dieldrin (10 μ M), isodrin (100 μ M), and desmethylene aldrin (10 μ M). Cells were then removed from the wells, washed with phosphate-buffered saline, and resuspended in HEPES-buffered saline prior to filtration (70 μ m). Hoechst 33258 (4 μ g/mL) was added and the absorbance within the cells measured using a Becton Dickinson LSR II flow cytometer with UV and 440/40 band-pass (BP) (Hoechst 33258), 530/30 BP (H₂DCFDA), and 610/20 BP (DHE). Data were processed using CellQuest (BD Bioscience), and results were reported as the geometric mean $\pm$ SEM.

Statistics

All statistics and curve fittings were calculated using GraphPad Prism, version 5.01c (GraphPad Software, San Diego, CA). Rules of error propagation were used to determine the standard deviation of the fold change of dieldrin concentration for the cellular localization experiments. Significance from control for the thiol and amine reactivity was determined using a two-tailed *t* test (*p* < 0.05). All other statistical analyses were calculated using one-way ANOVA with Newman-Keuls post-test, unless otherwise noted.

RESULTS

Reactivity of Dieldrin (Epoxide) with Cys and Lys

To determine the reactivity of dieldrin, specifically the epoxide, toward the protein nucleophiles Cys and Lys, dieldrin was incubated with *N*-acetyl Lys and Cys and the reaction monitored. The thiol reactivity of dieldrin was assessed using *N*-acetyl Cys, and the concentration of free thiols was monitored via DTNB. No decrease in thiol concentration was observed.

In addition, dieldrin was incubated with *N*-acetyl Lys and the concentration of dieldrin remaining over time measured using HPLC. No significant decrease in dieldrin concentration was observed upon addition of *N*-acetyl Lys, indicating no reaction under these conditions. These results indicate that dieldrin does not covalently modify target proteins under the conditions used and that any observed protein interactions are most likely noncovalent, despite the presence of an epoxide on the organochlorine.

Localization of Dieldrin in Dopaminergic PC6-3 Cells

To determine the cellular localization of dieldrin following the exposure of PC6-3 cells to the organochlorine, cells were fractionated and dieldrin measured using GC/MS. Upon treatment with dieldrin (0–300 μ M), the PC6-3 cells were fractionated giving extracellular, membrane/lipid, and intracellular fractions. Representative chromatograms of the extract of a cellular fraction are shown in Supporting Information, Figure 1. As depicted in Figure 2A, the amount of dieldrin in the membrane/lipid fraction increased with the dose of dieldrin.

Cells treated with 3.8 μg (10 μM), 9.5 μg (25 μM), 19 μg (50 μM), 38 μg (100 μM), and 114 μg (300 μM) dieldrin had measured lipid/membrane organochlorine amounts of 0.022 μg , 0.16 μg , 0.89 μg , 3.1 μg , and 17 μg , respectively. When dieldrin in the intracellular fraction was quantified (Figure 2B), it was found that the amount of dieldrin increased with dosage through 50 μM treatment but not to the same extent as that observed for the membrane/lipid fraction. Cells treated with 3.8 μg (10 μM), 9.5 μg (25 μM), 19 μg (50 μM), 38 μg (100 μM), and 114 μg (300 μM) dieldrin had measured intracellular organochlorine amounts of 0.34 μg , 0.83 μg , 1.3 μg , 0.77 μg , and 2.3 μg , respectively. Such results indicate the limited capacity of the intracellular fraction for dieldrin and that the majority of the organochlorine remains in the membrane/lipid component.

Cell Viability

To assess the structure–activity relationship of dieldrin toxicity, the viability of PC6-3 cells was monitored after 4 h of treatment with various concentrations of each dieldrin analogue via the MTT assay (Figure 4). Results are depicted for dieldrin in Figure 3A, Group 1 analogues in Figure 3B and C, Group 2 in Figure 3D and E, and Group 3 shown in Figure 3F and G. All of the compounds, except for endrin, displayed a dose-dependent decrease in cell viability in the PC6-3 cells after 4 h. On the basis of these results, IC_{50} values were calculated in order to easily compare the potency of each compound (Table 1).

The parent compound dieldrin showed a dose-dependent increase in toxicity as determined via the decrease in cell viability, with an IC_{50} value of 293 μM . Endrin did not exhibit a dose-dependent increase in toxicity (Figure 3B); therefore, no IC_{50} value could be calculated. When the methano bridge was removed, yielding a more flexible structure (desmethylene dieldrin), the compound was substantially more toxic than dieldrin (Figure 3C) with an IC_{50} value of 22 μM .

When dieldrin was compared to Group 2, compounds with changes to the polar moiety, it was found that the substitution of the epoxide for an olefin (aldrin, Figure 3D) or a *cis*-diol (*cis*-aldrin diol, Figure 3E) both resulted in decreased cell viability (IC_{50} values of 46 and 36 μM , respectively), as compared to that for dieldrin. Such a result implies that a moiety capable of being a hydrogen bond donor (i.e., *cis* diol) enhances the toxicity, as does the presence of an olefin.

The last group of compounds takes into account the presence of the olefin, already shown to enhance toxicity, as well as the 3-dimensional orientation of the compound. The isomer of aldrin (isodrin, Figure 3F) showed very little toxicity (IC_{50} value was 578 μM). When the desmethylene analogue of aldrin was assessed (desmethylene aldrin, Figure 3G), it resulted in the most substantial decrease of cell viability of all of the analogues with an IC_{50} value of 16 μM . These results imply that the presence of the olefin augments the toxicity of dieldrin, and when this modification is coupled with the removal of the methano bridge, the toxicity is further enhanced.

Release of Lactate Dehydrogenase

To further assess the structure–activity relationship of dieldrin toxicity, the viability of PC6-3 cells was monitored after 4 h of treatment with various concentrations of each dieldrin analogue via the LDH assay. For each of the compounds, a dose-dependent decrease in cell viability with respect to that of the control was observed, except for endrin, which showed no cytotoxicity, even at high concentrations (900 μ M) (Figure 4). The results from the LDH experiment correlate well to those observed using MTT to monitor cell viability (Figure 3) and data from a previous study.⁷ The relative order of toxicity between each analogue was also comparable, with the desmethylene analogues being the most potent, followed by both compounds in Group 2 (aldrin and *cis*-aldrin), then dieldrin. Isodrin and endrin were the least toxic.

In the LDH results, a deviation from the dose-dependent trend was observed at higher concentrations of organochlorine, particularly with the more toxic derivatives. Each compound showed increased release of LDH until the concentration at which roughly 65% cytotoxicity was achieved. At organochlorine concentrations that would be expected to yield greater than 65% cytotoxicity, the values representing LDH release and activity remained constant (desmethylene dieldrin, Figure 4C) or decreased (*cis*-aldrin diol, Figure 4E). This deviation from the expected dose–response was not seen with the MTT results. It was determined that the compounds did not absorb at the wavelengths monitored or react with the reagents used in this experiment.

Since this experiment was based on enzyme activity, the compounds were then tested for inhibition of the enzyme, LDH. When each compound was incubated with rabbit L-LDH at higher concentrations, a decrease in enzyme activity was observed as shown in Supporting Information, Figure 2. It can be inferred that when the cells were treated with higher concentrations of the dieldrin analogues, the outer membrane of the cells was disrupted, causing a release of LDH. However, due to the substantial amount of the dieldrin analogue still remaining in the extracellular media or that was released from the cell during apoptosis, the LDH was inhibited by the compound. This resulted in the decreased enzyme activity corresponding to the observed deviation from the dose-dependent increase in cytotoxicity for each analogue at higher concentrations (Figure 4).

Dopamine Catabolism

To determine the effect of dieldrin and analogues on DA catabolism, which is a process capable of generating reactive and toxic intermediates, PC6-3 cells were incubated with the organochlorines and extracellular DA metabolites measured, including DOPAL and DOPAC. Extracellular DA metabolites (Scheme 1) were quantified by HPLC, with the results presented in Table 2 and Figures 5 and 6. The concentration of extracellular DOPAL after 4 h of treatment is shown for the control (vehicle-treated) and all organochlorine compounds in Figure 5. When the cells were treated with dieldrin, a time- and concentration-dependent increase in DOPAL was observed. Small changes in the levels for the other DA metabolites, DOPAC and DOPET, were also observed (Figure 6 and Table 2). Such findings were also noted following treatment of the cells with the various isomers of dieldrin.

When endrin, the isomer of dieldrin, was analyzed, a slight decrease in DOPAL and an increase in DOPAC were observed. For desmethylene dieldrin (300 μ M), the analogue of dieldrin lacking the methano bridge, the extracellular DOPAL concentration increased 10-fold. The concentration of DOPAC was significantly reduced from the control following treatment with this analogue (Figure 6A).

Group 2, with changes to the polar group opposite of the chlorine atoms, also showed a profound effect on the extracellular concentration of DOPAL in comparison to dieldrin, with both aldrin and *cis*-aldrin diol resulting in significant and substantial increases in DOPAL levels, as compared to the control (Figure 5B). This observation was accompanied by a marked decrease in the production of DOPAC (Figure 6B).

For the third group of compounds, isodrin and desmethylene aldrin, results similar to those of endrin and desmethylene dieldrin were observed, respectively. Isodrin showed only a small change from the control for both DOPAL (slight increase) and DOPAC (slight decrease), whereas desmethylene aldrin showed a marked increase in DOPAL concentration and a corresponding decrease in DOPAC (Figures 5C and 6C).

Because the PC6-3 cells released low levels of DA metabolites, the cells were spiked with 100 μ M DA 15 min prior to dieldrin exposure in order to more accurately determine the influence of dieldrin on DA metabolism (Figure 7). DOPAL levels were measured as previously described and were expressed as % control [DOPAL] for treatment with 10, 30, 100, or 300 μ M dieldrin. A dose-dependent trend was observed, with concentrations as low as 10 μ M dieldrin generating a nearly 200% increase in extracellular DOPAL levels.

Reactive Oxygen Species

To determine the effect of nontoxic concentrations (<20% cell death) of each dieldrin analogue on the production of ROS, cells were incubated with the organochlorines, and ROS was measured via flow cytometry. General ROS production was determined using H₂DCFDA, and a significant increase in oxidative stress was observed upon treatment with each of the compounds studied (endrin was not included in this experiment) (Figure 8A). When levels of superoxide anion were monitored using DHE, no significant change from the control was observed (Figure 8B), indicating that the accumulation of ROS from dieldrin analogue exposure is most likely from H₂O₂ in the PC6-3 cells.

DISCUSSION

The goal of this study was to elucidate the interaction of the dieldrin with components of dopaminergic cells and determine structure–activity relationships in order to better understand the mechanism of toxicity for this organochlorine pesticide. To complete this goal, a series of experiments were conducted to determine the protein reactivity, localization, effect on DA metabolism, and structure–activity relationship of dieldrin for toxicity using dopaminergic cells. Such work is significant given that the structure–activity relationship of dieldrin is not well-defined in mammals.⁴⁰

Dieldrin contains an epoxide, a moiety that is typically electrophilic and highly reactive toward nucleophiles.⁴¹ It is conceivable that the nucleophilic sites on proteins (i.e., Cys or Lys residues) would be able to react with the epoxide, causing the opening of this three-membered ring and protein modification.⁴¹ Previous studies have shown that dieldrin “binds” to proteins, such as those found in spinal cord homogenate from rats and cockroaches and, to a lesser extent, albumin.^{42,43} While isolated amino acids cannot completely model the unique environment of these amino acids in each possible protein target, they can provide a valuable reactivity profile. In the current study, it was determined that dieldrin was not capable of covalently modifying Lys or Cys residues, supporting the previous assumptions of noncovalent interactions. The dieldrin epoxide could be stabilized due to the rigid polycyclic structure, thereby limiting the likelihood of nucleophilic attack by amino acids such as Lys and Cys.

The cellular localization of dieldrin in PC6-3 cells was investigated using cell fractionation and GC/MS. On the basis of the results (Figure 2), dieldrin was found to accumulate in the cell, particularly in the membrane and lipid fraction (Figure 2A). Saturation of the membrane fraction was not observed with organochlorine concentrations used, and dieldrin levels increased as treatment levels increased. The amount of dieldrin in the intracellular fraction increased as organochlorine treatment concentration (up to 50 μM) increased. At higher treatment concentrations (>50 μM), the amount of dieldrin in the intracellular fraction began to level out, indicating decreased diffusion or saturation of binding sites. From the results in Figure 2, it may be concluded that dieldrin readily enters PC6-3 cells and accumulates in the membrane and lipid-containing portion; however, binding to intracellular components can be saturated at low micromolar levels. This trend is most likely due to the lipophilicity of the organochlorine.

The adverse cellular effects of dieldrin have been previously investigated, particularly with respect to the effect of dieldrin on various aspects of mitochondrial activity.^{7,11,44,45} Bergen et al.⁴⁴ demonstrated dieldrin’s ability to disrupt respiration in rat liver mitochondria and inhibit electron transport. In a dopaminergic system similar to that of the PC6-3 cells (i.e., PC12 cells), dieldrin has been shown to cause a 50% decrease in cell viability (MTT) at 143 μM at 1 h in PC12 cells.⁷ Even with variations in cell line, cell preparation, and time frame, this value is comparable with the IC_{50} value of 293 μM obtained using the MTT assay in the current study.

In an effort to assess the structure–activity relationship of dieldrin, as it relates to its toxicity in dopaminergic cells, six analogues were used (Figure 1). These compounds were chosen based on their chemical structure, allowing for the assessment of three structural characteristics: (1) the importance of the 3-dimensional orientation and presence of the methano bridge (Group 1, endrin and desmethylen dieldrin), (2) the effect of the polar group opposite of the chlorine atoms (Group 2, aldrin and *cis*-aldrin diol), and (3) the interplay between both the polar moiety and the 3-dimensional orientation (Group 3, isodrin and desmethylen aldrin).

Dieldrin contains an epoxide opposite of the chlorine atoms, whereas the compounds in Group 2 contain either an olefin (aldrin) or a *cis*-diol (*cis*-aldrin diol). Aldrin was widely

used as a pesticide around the same time as dieldrin but is slightly less toxic in various species of insects.^{14,46} Aldrin has a shorter half-life than dieldrin, mostly due to increased volatility and rate of metabolism in the environment and in mammals.⁴⁷ Despite these characteristics limiting the adverse effects of aldrin exposure, it is still listed as #25 on the CERCLA priority list of hazardous substances⁴⁸ and has been detected in the post-mortem brains of Parkinson's disease patients.^{2,4-6} *cis*-Aldrin diol is a metabolite of dieldrin that can then be epimerized to *trans*-diol in mammals.⁴⁹ This compound is less toxic to insects, as compared to dieldrin.⁵⁰

The third group aimed to assess the combined effects of altering the polar moiety (epoxide to olefin) along with the 3-dimensional structure. The two compounds in this group are based on aldrin, with isodrin being its isomer, and desmethylene aldrin lacking the methano bridge found in aldrin and dieldrin. Isodrin was never used commercially as a pesticide, but in *Musca domestica*, it is only slightly less toxic than endrin.¹⁴ The toxicity profile of desmethylene aldrin has not yet been investigated. These organochlorines (Groups 1, 2, and 3) were chosen based on their environmental and metabolic relevancy and also to assess the role of dieldrin's epoxide.

The order of potency for each group of compounds on cell viability was assessed by LDH and MTT assays, both showing similar results. Group 2, aldrin and *cis*-aldrin diol, showed moderate toxicity, the dieldrin isomers (endrin and isodrin) evinced little to no toxicity, and the desmethylene compounds were the most toxic in the PC6-3 cells. Dieldrin, while substantially more toxic than endrin and isodrin, was markedly less toxic than the Group 2 analogues. Orientation of the bridge did greatly influence toxicity (the structural difference between dieldrin and endrin and between aldrin and isodrin), but its absence also increased toxicity as the desmethylene compounds were the most toxic. These results demonstrate the importance of the three-dimensional structure in the cytotoxic potency of the cyclodiene pesticides in a dopaminergic model. This structure-activity relationship differs significantly from the structural features found to be important for insect toxicity. The toxicities of many organochlorines have been assessed in various species of insects, including *Musca domestica* (house fly), *Anopheles stephensi* (mosquito), *Glossina austeni* (tsetse fly), and *Periplaneta americana* (American cockroach).^{14-16,46,50} When considering only the compounds investigated in the current study, previous work has shown dieldrin to be the most toxic in insects, usually followed by aldrin, endrin, and then isodrin. *cis*-Aldrin diol, desmethylene aldrin, and desmethylene dieldrin were all found to have minimal toxicity in the insect species investigated. There were many other compounds also investigated in these studies (>51 compounds in total), and some species differences, in terms of the relative potency of each compound, were observed.¹⁴⁻¹⁶

The general characteristics of the structural features important for toxicity in both insects and dopaminergic cells are similar.¹⁴⁻¹⁶ These characteristics include the importance of the 3-dimensional structure and the identity of electronegative/polar moiety opposite of the chlorine atoms (i.e., epoxide or *cis*-diol). For example, while dieldrin and endrin are stereoisomers of each other differing only the position/orientation of the epoxide and methylene bridge (Figure 1), the measured toxicity is significantly different. Similar results were found for the stereoisomers aldrin and isodrin. The mechanistic basis for such

differences in toxicity, e.g., dieldrin versus endrin, are unknown at this point but might include diversity in regards to cellular targets, metabolism, and disposition.^{51,52} In regards to the latter, it is known that unlike dieldrin, endrin is rapidly excreted in humans and does not bioaccumulate.^{51,52}

In addition, it seems the minor structural changes that make a molecule less toxic toward insects increase toxicity toward dopaminergic cells (i.e., orientation and presence of the methano bridge). There are significant differences in the model systems used between these studies, whole insect versus immortalized cell line, that affect the metabolism, localization, and absorption of these compounds. However, based on findings for the organochlorine isomers used in the current study (e.g., dieldrin and endrin), it may still be concluded that there are significant differences in the cellular targets that warrant further investigation. It is these differences that will be useful in developing new, potent insecticides that do not adversely affect the dopaminergic system of humans.

The *cis*-diol metabolite of dieldrin resulting from cleavage of the epoxide was found to be significantly more toxic than the parent compound dieldrin (Table 1).⁴⁹ Such a finding raises the question of whether dieldrin or its metabolites are the toxic insults relevant to neurodegenerative disease development.

Dieldrin has been associated with the disruption of many key processes in dopaminergic neurons, including the metabolism of the neurotransmitter, DA; mitochondrial function; and the balance of oxidative stress. Dysregulation of these processes and exposure to dieldrin have been correlated to PD.^{4–6,53–57}

Previous studies have shown that exposure to dieldrin causes depletion of DA in both ring doves and mallards.^{58,59} This effect has also been shown to be specific to DA, with no corresponding inhibition of GABA.⁶⁰ When this effect was further investigated in mice dosed with dieldrin, a decrease in DOPAC, the carboxylic acid metabolite of DA, was detected, along with a corresponding increase in cysteinyl-DA, cysteinyl-DOPAC, and elevated levels of protein carbonyls. This indicates a disruption in DA metabolism and trafficking, but instead of seeing elevated levels of the DA metabolites, increased protein modification was observed.⁹

Dieldrin has also been found to cause the release of striatal DA and DOPAC in mice.¹² Developmental exposure to dieldrin has also been shown to have long-term, adverse effects on murine DA metabolism. An increase in DOPAC was observed in the striatal region of these mice after they were aged to 12 weeks, and they were much more sensitive to MPTP toxicity.¹⁰ In dopaminergic PC12 cells, treatment with dieldrin resulted in decreased intracellular DA and a corresponding increase in extracellular DA and DOPAC.⁷

Higher extracellular DA and DOPAC levels suggest release of vesicular DA and elevated DA turnover/metabolism to DOPAC. The metabolism of DA to DOPAC proceeds via the activity of monoamine oxidase and involves production of an aldehyde intermediate, DOPAL.⁶¹ DOPAL is an endogenous neurotoxin, capable of covalently modifying proteins.^{30,38,39,62,63} Aberrant levels of this reactive compound have been associated with an increased risk for Parkinson's disease.^{62–67} In the current study, the compounds that

exhibited the greatest toxicity caused the largest increase in extracellular levels of DOPAL (desmethylene aldrin, desmethylene dieldrin, and *cis*-aldrin diol). The least toxic compounds (endrin and isodrin) showed the least effect on DOPAL concentration, and minimal effect on the concentrations of the other DA metabolites quantified (DOPAC and DOPET).

Additionally, pretreatment of the cells with DA demonstrated an even greater increase in DOPAL production following dieldrin treatment as compared to the control (Figure 7). While there appears to be a correlation between toxicity and extracellular levels of DOPAL, it is likely that the generation of DOPAL is a secondary effect of cell death in the current study given the full range of organochlorine compounds used with some concentrations higher than the measured LC₅₀. Further experiments should investigate the correlation between organochlorine treatment and generation of DOPAL, especially for chronic low-level dieldrin exposure.

Of question is how treatment of dopaminergic cells with dieldrin or other organochlorines at concentrations lower than needed for toxicity yield elevated DOPAL. As noted above, exposure of PC12 cells to dieldrin resulted in increased extracellular DA and DOPAC, indicative of organochlorine-mediated release of vesicular DA.⁷ One other mechanism that could account for elevated DOPAL would be the impairment of aldehyde metabolism, and previous works have demonstrated aldehyde dehydrogenases and reductases to be inhibited by low levels of products of oxidative stress.^{30,38} Further work is needed to determine whether either or both pathways (i.e., release of vesicular DA or oxidative stress products) are responsible for elevated DOPAL.

In addition to DA metabolism, the current study also investigated the effect of dieldrin and its analogues on the production of ROS. It was found that treatment of PC6-3 cells with nontoxic doses of each of the organochlorines resulted in a significant increase in H₂O₂ levels. This increased production of ROS was not necessarily indicative of toxicity, such as in the case of isodrin which caused the largest increase in ROS but showed the least toxicity. A similar trend has been observed for the organophosphates, another class of synthetic pesticides that includes compounds such as chlorpyrifos and malathion.^{68,69} It was initially thought that all of the organophosphates had the same mechanism of action: irreversible inhibition of acetyl-cholinesterase. While there were some observed differences in relative potency, these could be easily attributed to structural changes.⁶⁸ It was later determined that there were exceptions to this original hypothesis and observed that organophosphates could produce similar levels of oxidative stress, but the cellular compensations and/or reactions to this insult were different depending on the toxicant.^{34,36,70–76} Therefore, the same degree of initial insult can result in a varied degree of toxicity,³⁶ as observed with the dieldrin analogues investigated here.

At this point, it is not clear why elevated hydrogen peroxide but not superoxide anion was observed following organochlorine treatment. However, exposure of the cells to dieldrin and other analogues resulted in increased DOPAL, indicative of higher DA metabolism via monoamine oxidase, which would produce hydrogen peroxide as a byproduct.⁶¹

The information obtained regarding the reactivity and localization of dieldrin is important to better understand the interaction of this pesticide with proteins in dopaminergic cells. The

major findings of this study are the following: (1) dieldrin readily diffuses into cells and accumulates in the membrane fraction of PC6-3 cells, while the intracellular solution demonstrated saturation. (2) The epoxide of dieldrin is not reactive toward Cys or Lys nucleophiles under conditions used. Therefore, dieldrin most likely associates with proteins noncovalently. (3) Minor structural changes can greatly alter toxicity for dieldrin. The most toxic analogues of dieldrin were the desmethyl isomers and the *cis*-diol metabolite. Interestingly, the *cis*-diol analogue is a metabolite of dieldrin, which raises a question as to whether dieldrin or a metabolite is the insulting species. (4) Treatment of PC6-3 cells with dieldrin and organochlorine analogues yielded elevated levels of the toxic DA metabolite DOPAL. (5) Exposure of PC6-3 cells to dieldrin and analogues resulted in elevated hydrogen peroxide but not superoxide anion. Given that minor structural changes to dieldrin resulted in significant alterations in toxicity and dopamine catabolism implies specificity in regards to molecular targets. Work is in progress to better understand the interaction of the pesticide dieldrin with dopaminergic cells, focusing on mechanisms for disruption of cell processes and identification of novel intracellular targets.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

DCFDA	2',7'-dichlorodihydrofluorescein diacetate
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
DOPAL	3,4-dihydroxyphenylacetaldehyde
DOPAC	3,4-dihydroxyphenylacetic acid
DOPET	3,4-dihydroxyphenylethanol
<i>m</i>CPBA	3-chloroperbenzoic acid
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
ALDH	aldehyde dehydrogenase
ALR	aldehyde reductase
Adn	aldrin
CAD	<i>cis</i> -aldrin diol

dAdn	desmethylene aldrin
dDI	desmethylene dieldrin
DI	dieldrin
DHE	dihydroethidium
DMSO	dimethylsulfoxide
DA	dopamine
End	endrin
GC/MS	gas chromatography tandem mass spectrometer
Idn	isodrin
LDH	lactate dehydrogenase
NGF	nerve growth factor
PD	Parkinson's disease
ROS	reactive oxygen species
SOD	superoxide dismutase

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Figure 1.

Structures and groupings of dieldrin analogues. Compared to dieldrin, each of the three groups have varied structure. Group 1: methano bridge, opposite configuration (endrin) or absent (desmethylene dieldrin). Group 2: epoxide, hydrolyzed to diol (*cis*-aldrin diol) or absent (aldrin). Group 3: no epoxide and opposite configuration of the methano bridge (isodrin) or no epoxide and methano bridge (desmethylene aldrin).

Figure 2.

Amount of dieldrin, normalized to internal standard and standard curve, following hexane extraction of cellular fractions from cells treated with 0–300 μM dieldrin. Each bar represents the fold change in dieldrin concentration compared to the amount extracted after treatment of cells with 10 μM dieldrin ($\pm\text{SD}$, $n = 3$). (A) membrane/lipid fractions; (B) intracellular fractions.

Figure 3.

Cell viability of PC6-3 cells treated with dieldrin, Group 1, Group 2, or Group 3 compounds (% control $\pm$ SD, $n = 4-6$) obtained from MTT assay after 4 h of incubation with (A) dieldrin, (B) endrin, (C) desmethylen dieldrin, (D) aldrin, (E) *cis*-aldrin diol, (F) isodrin, and (G) desmethylen aldrin. The difference between each pair of treated groups is significant ($p < 0.05$) unless otherwise noted (NS).

Figure 4.

Percent cytotoxicity by lactate dehydrogenase release from PC6-3 cells after 4 h of incubation with dieldrin, Group 1, Group 2, or Group 3 compounds (% cytotoxicity $\pm$ SD, $n = 4-6$). (A) Dieldrin, (B) endrin, (C) desmethylene dieldrin, (D) aldrin, (E) *cis*-aldrin diol, (F) isodrin, and (G) desmethylene aldrin. The difference between each pair of treated groups is significant ($p < 0.05$) unless otherwise noted (NS).

Figure 5.

Quantification of the extracellular concentration of DOPAL from PC6-3 cells incubated for 4 h with vehicle control (0.6% DMSO), dieldrin, and Group 1 compounds (endrin and desmethylene dieldrin, A), Group 2 compounds (aldrin and *cis*-aldrin diol, B), or Group 3 compounds (isodrin and desmethylene aldrin, C). The values shown represent the mean $\pm$ SD ($n = 3$, except for controls where $n = 21$), where peak area was converted to concentration using a standard curve.

Figure 6.

Quantification of the extracellular concentration of DOPAC from PC6-3 cells incubated for 4 h with vehicle control (0.6% DMSO), dieldrin, and Group 1 compounds (endrin and desmethylene dieldrin, A), Group 2 compounds (aldrin and *cis*-aldrin diol, B), or Group 3 compounds (isodrin and desmethylene aldrin, C). The values shown represent the mean $\pm$ SD ($n = 3$, except for controls where $n = 21$), where peak area was converted to concentration using a standard curve.

Figure 7.

Percent control of the concentration of DOPAL released from PC6-3 cells pretreated with 100 μM dopamine prior to 4 h of exposure to 10, 30, 100, or 300 μM dieldrin. The values shown represent the mean $\pm$ SEM ($n = 3$ or 4), normalized to the percent control of DOPAL release. The percent control of DOPAL release after treatment with 100 and 300 μM dieldrin was significantly different from that of the control ($p < 0.05$).

Figure 8.

Production of ROS monitored via flow cytometry after 1 h of treatment with nontoxic concentrations of each compound (10 μ M for desmethylene aldrin and desmethylene dieldrin, 25 μ M for dieldrin, aldrin, and *cis*-aldrin diol, and 100 μ M for isodrin). Results depicted as geometric mean ($\pm$ SD, $n = 3$) for (A) H₂DCFDA (general ROS) or (B) DHE (O₂^{•-}). Change in geometric mean upon treatment with each compound in A for H₂DCFDA fluorescence was significantly different from that of the control ($p < 0.0001$), except for desmethylene aldrin; whereas in B, for DHE fluorescence, no significant change was observed with any of the compounds ($p > 0.05$).

Scheme 1. DA Metabolism^a

^aDA is metabolized by monoamine oxidase (MAO) to the aldehyde, DOPAL. This intermediate is oxidized by aldehyde dehydrogenase (ALDH) to the acid metabolite (DOPAC) or reduced by aldehyde reductase (AR) to an alcohol, DOPET.

Table 1

IC₅₀ Values ($\mu\text{M} \pm \text{SEM}$) for Each Compound Based on the Concentration of Each Inhibitor at 50% of the Normalized Cell Viability

compd	IC ₅₀ (μM) ^a
dieldrin	293 $\pm$ 1.08
endrin	ND ^b
desmethylene dieldrin	22.3 $\pm$ 1.05
aldrin	46.4 $\pm$ 1.08
cis-aldrin diol	36.9 $\pm$ 1.11
isodrin	578 $\pm$ 1.22
desmethylene aldrin	15.8 $\pm$ 1.05

^aValues represent the mean $\pm$ SEM.

^bNot determined. The measured toxicity was not dose dependent.

Table 2Concentration of Dopamine Metabolites (DOPAL, DOPAC, or DOPET)^a

compd	group ^b	[DOPAL] (μ M)	[DOPAC] (μ M)	[DOPET] (μ M)
control		0.76 $\pm$ 0.21	2.94 $\pm$ 0.87	1.67 $\pm$ 0.32
dieldrin		1.53 $\pm$ 0.25 ^{NS}	2.87 $\pm$ 1.36 ^{NS}	1.58 $\pm$ 0.49 ^{NS}
endrin	1	0.60 $\pm$ 0.09 ^{NS}	3.51 $\pm$ 0.19 ^{NS}	1.78 $\pm$ 0.14 ^{NS}
des dieldrin	1	7.79 $\pm$ 2.49***	0.54 $\pm$ 0.15***	1.16 $\pm$ 0.27 ^{NS}
aldrin	2	7.75 $\pm$ 1.61***	1.15 $\pm$ 0.20**	1.24 $\pm$ 0.11 ^{NS}
cis-aldrin diol	2	10.7 $\pm$ 5.48***	0.53 $\pm$ 0.11***	0.58 $\pm$ 0.06***
isodrin	3	1.00 $\pm$ 0.19 ^{NS}	2.76 $\pm$ 0.95 ^{NS}	2.12 $\pm$ 0.44 ^{NS}
des aldrin	3	7.02 $\pm$ 2.19***	0.23 $\pm$ 0.20***	0.85 $\pm$ 0.06**

^aConcentrations of dopamine metabolites ($\pm$ SD, $n = 3$, except for controls where $n = 21$) after 4 h of treatment with 300 μ M of each compound or vehicle control (0.6% DMSO). Difference from vehicle control was significant with $p < 0.001$ (**) or $p < 0.0001$ (***), or not significant with $p > 0.05$ (^{NS}). Statistical analysis was performed via ANOVA with Newman-Keuls post-test. The elevation of DOPAL following dieldrin treatment was significantly different from that of the control when analyzed by a two-tailed t test ($p < 0.0001$).

^bSee Figure 1 for group assignments and structures.