

## **PESTICIDES RESIDUES IN EGYPTIAN DIABETIC CHILDREN**

*BY*

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### **ABSTRACT**

*Pesticides exposure has been linked with many childhood diseases including endocrine and immune disorders. The aim of the present study is to monitor the toxic levels of pesticides residues in a group of type 1 diabetic children (T1D) in our locality. One hundred and ten Egyptian children; their ages ranged from 1.2 to 10 years were studied. The control group comprised 35 completely healthy children, while the study group included 75 children (newly diagnosed as T1D). Children were chosen from those attending Mansoura University Children Hospital. Blood samples were collected from both groups for detection of pesticides residues. The results reveal that lindane is the most common organochlorine pesticide detected (70.7%) followed by o,p-DDD and p,p-DDE as DDT metabolites (21.3% each); while the most prevalent organophosphate compound is malathion (65.3%). It could be concluded that Egyptian children have measurable levels of several pesticides residues and there is increased risk of developing T1D in children exposed to some types of pesticides. Additionally, biomonitoring of these toxicants provide clinical toxicologists and physicians with reference values to be compared with other populations and could be correlated in the future studies with diseases claimed to be due to pesticide exposure especially in children.*

**Keywords:** *Pesticides, Organochlorine, Organophosphorus, Diabetic Children.*

### **INTRODUCTION**

Pesticides comprised several chemical compounds, which are used to increase agricultural products by preventing losses due to pests. Among the major groups of pesticides; organochlorine pesticides (OC) are one of the most toxic and more potent due to their persistence and stability. They were prohibited from use throughout the world for more than 20 years ago (Fenik et

al., 2011). Currently, OC are replaced by the less toxic organophosphorus compounds despite of being also restricted for use in many developed countries due to their toxic health effects (Rohlman et al., 2011).

However, many of the banned pesticides are still sold or manufactured for export to developing countries (Bergonzi et al., 2009; Rohlman et al., 2011). Further-

more, OC pesticides are subject to transport over long distances and can be detected even in areas where they have never been used. They can bioaccumulate and biomagnify in food chains. They are lipophilic and persistent with long half-lives (Dahmardeh Behrooz et al., 2009).

In Egypt many studies have been documented the presence of OC in water; milk and its products; vegetables and fruits (Abou - Arab et al., 2008; Sharaf et al., 2008).

Exposure of children to pesticides may occur through placenta during fetal life, lactation and diet, or contact with contaminated house dust, carpets, chemically treated gardens or pets treated with insecticides (Bevacqua, 2011; Fenik et al., 2011).

Children are more vulnerable than adults to toxic effects of environmental pollutants because of their unique behavior and dietary pattern. Pesticide exposure has been linked with many childhood diseases particularly congenital malformations, growth disorders, cancer, malabsorption, immunological dysfunction, neurobehavioural and endocrine diseases (Garry, 2004; Sagiv et al., 2007; Bergonzi et al., 2009).

Although pesticide exposure information is readily available for many areas in

the world, toxicological data regarding pesticides exposure in human especially among Egyptian children remains largely unstudied (Porta et al., 2008). The goal of the present study is to monitor the toxic levels of pesticides residues in a group of type 1 diabetic (T1D) children in our locality.

## **MATERIAL AND METHODS**

### **(1) Subjects:**

This study was conducted on 110 children who were presented with their mothers to Mansoura University Children Hospital, Endocrinology and Diabetes Unit. The study began at September 2008 and ended by April 2010 when the targeted cases were collected. They were divided into two groups:

- A. Study group: 75 children aged more than one year and  $\leq 10$  years who were newly diagnosed as type 1 diabetes (within the first month) and fulfilled the inclusion criteria.
- B. Control group: 35 children who were completely healthy selected from the outpatient endocrinology clinic when they came with their siblings for follow up.
- C. Exclusion criteria:
  1. Any child with associated disease, endocrine disorders, birth defect, physical or mental retardation, or congenital anomalies e.g. cardiovascular or musculoskeletal.

2. Children with family history of diabetes.
3. Allergy, atopy, asthma or any associated autoimmune disease.
4. Infections, any other medical condition associated with diabetes, or high complete blood count (CBC).

**Each child was subjected to:**

- History taking from the mother to get sociodemographic criteria regarding age, sex and residence.
- Complete medical examination.

**(2) Sampling:**

- Approval from the Ethical Committee of Mansoura University-Faculty of Medicine was taken besides an informed consent from mothers of the studied children to participate in the research.
- Five ml whole blood sample was collected from each child in polyethylene tubes without anticoagulant or serum separator. Blood was precipitated for 30 min. and centrifuged at 3600 round per minute (rpm) for 15 min.
- All serum samples were stored in Eppendorf tubes at  $-70^{\circ}\text{C}$ , and then they were put in an ice tank to be transported to the National Research Center for pesticides residues analysis.
- All instruments and vials used during sample preparation were cleaned

with hexane and acetone and stored until usage.

**(3) Measurement of pesticides levels:**

***Pesticide standards:***

Standards of organochlorine (OC) pesticides include: hexachlorobenzene (HCB); lindane; aldrin; heptachlor; endrin; p,p'-DDT: 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane; o,p'-DDT: 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2,2-trichloroethane; p,p'-DDE (1,1-dichloro-2,2-bis(p.chlorophenyl) ethylene; o,p'-DDE: 1-(ochlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane); p,p'-DDD: 1-chloro-2,2-bis(p-chlorophenyl) ethane, p.p. DDA: 2,2-bis-4-chlorophenyl acetic acid and o, p'-DDD: 1-(o-chlorophenyl)-1-(p-chlorophenyl)-2,2-dichloroethane.

Standards for organophosphorus (OP) pesticides include diazinon, chlorpyrifos-methyl, malathion, chlorpyrifos and profenofos. All standards were 97-99 % pure and purchased from Chem. Service, Inc. (West Chester, PA). Standard solution mixtures were prepared in acetone from stock individual standards and stored at  $-18^{\circ}\text{C}$ . Working solutions were prepared by dilution with hexane and stored at  $4^{\circ}\text{C}$ .

***Chemicals:***

All used solvents (hexane and acetone) were reagent grades and purchased from Merck (Merck, Darmstadt, Germany).

### **Extraction and Instrumentation:**

Extraction of pesticides residues from different collected samples were applied according to the method of Liu and Pleil (2002). The extracted samples were analyzed by Hewlett Packard Gas Chromatography (GC) Model 5890 equipped with Ni63 Electron Capture Detector (ECD), and fitted with HP- 101capillary column (Cross linked methyl silicon Gum), 30 m length, 0.25 mm diameter, and 0.25  $\mu\text{m}$  film thicknesses. The oven temperature was programmed to start at 160°C and raised to 220°C with rate of 5°C/min and was held for 30 min. Injection and detector temperatures were 220°C and 300°C, respectively. The flow rate of carrier gas (nitrogen) was obtained by adjusting it at the pressure of 10 psi (pound / in<sup>2</sup>). Concentrations of pesticide residues in different analyzed samples were calculated as nanogram / ml serum.

Blank analysis was performed in order to check interference from the sample. Mean recoveries ranged from 90 to 94 % with S.D < 6 indicating excellent repeatability, with relative standard variation (RSD) is usually more than 10 % for methods involving a simple preparation procedure, the RSD is in the order of 5-10% (Aprea et al., 2002). The limit of detection (LOD) must be around 1 $\mu\text{g}$ /l blood, higher than this limit may be adequate for monitoring occupationally exposed workers or for acute poisoning cases. In the

present investigation, LOD was 2  $\mu\text{g}$  / l for OC pesticides and 5  $\mu\text{g}$  / l for OP pesticides.

### **Statistical analysis:**

The statistical analysis of data was done by using excel program for figures and SPSS (SPSS, Inc, Chicago, IL) program statistical package for social science version 16. Kolmogrov-Smirnov Z test was used for analysis of data and it was significant. Quantitative data were presented as mean, median; minimum; maximum and frequency. Mann-Whitney test was used to test significance between groups. Significance was set at  $p < 0.05$ . Odds ratios and corresponding 95 % confidence interval (CI) were calculated to estimate the magnitude of association between independent variables.

## **RESULTS**

The sociodemographic data of the control and the diabetic children are presented in table (1). No significant difference is found between the studied groups as regards age, sex and residence.

Pesticides residues are detected either as a single compound or mixtures as shown in figure (1).

The frequency and Odds ratio of pesticides residues in the studied groups are shown in table (2). Lindane is the most

common organochlorine pesticide detected in study group (70.7 %); followed by o.p.DDD and p.p.DDE; while the most prevalent organophosphorus compound is malathion. Children exposed to malathion, lindane, p.p.DDE, o.p.DDD, endrin and p.p.DDA have the highest risk to develop T1D than the control healthy group by (4.11, 2.02, 1.59, 1.59, 1.52 and 1.49 times respectively).

The concentrations of pesticides residues in the serum of the studied groups are illustrated in table (3). In the study group; the organochlorine pesticides p.p.DDA; o.p.DDD; endrin and the organophosphorus malathion have the highest concentrations. While in the control group, organochlorines are not detected but the organophosphorus malathion has the highest concentration.

However pesticides residues which are not detected in any of the serum of the test group include: hexachlorobenzene (HCB); aldrin, heptachlor, p,p'-DDT; o,p'-DDE; p,p'-DDD; diazinon and chlorpyrifos as they are below the limit of detection (LOD).

### **DISCUSSION**

Several childhood diseases such as allergic disorders, type 1 diabetes and cancer have been linked to environmental ex-

posures (Garry, 2004). Type 1 diabetes mellitus (T1DM) is the most common chronic metabolic condition seen in children. Its global incidence is rising by around 3.4 % per year but the reasons for this increase remain unclear. Researches that highlight environmental "triggers" of T1DM are on the rise (Shulman and Dane-man, 2010; Todd, 2010).

Pesticides are relevant environmental pollutants. Studies regarding the levels of these toxic chemicals in humans especially in children are scarce (Porta et al., 2008; Schulz et al., 2009). To our knowledge, this work is the first one aiming to monitor the toxic levels of pesticides residues in a group of type 1 diabetic children (T1D) in our locality.

In the present study, many types of OC and organophosphorus pesticides or their metabolites (i.e. lindane, endrin, o.p. DDD, p.p. DDE, o.p. DDT, p.p. DDA) were detected in serum of diabetic children (either single residue or mixed residues). Only the organophosphorus compounds were found in the control healthy children.

Regarding organochlorine pesticides, the current work stated that lindane was the most common in 53 cases (70.7 %) followed by o.p-DDD and p.p-DDE in 16 cases each and endrin in 8 cases. On the other

hand, malathion is the commonest organophosphorus compound detected (65.3%). Those compounds show the highest Odds ratio indicating an increased risk of occurrence of type 1 diabetes in the exposed children.

To our knowledge, this is the first study concerned with the relation between pesticides and T1D.

The present results were consistent with Lopez-Espinosa et al. (2008) who detected p,p-DDT, lindane and aldrin in adipose tissue of 12 % of studied children. Other OC residues are found in different percentages i.e. p,p-DDE (79 %), o,p -DDT (17 %); o,p-DDD (15 %) and dieldrin (8 %). In agreement with this result, Luzardo et al. (2006) reported that endrin was present in 22 % of the children studied from Canary Islands (Spain).

Porta et al. (2010) detected p,p-DDT, o,p. DDT, o,p. DDE, p,p. DDE, p,p. DDD, o,p.DDD and HCH in more than 85 % of the studied population in Spain.

The present findings indicated that although an Egyptian Ministerial Decree prohibited the import and use of OC in 1996, some of these toxic pesticides are still illegally applied making exposure to these compounds unavoidable (Barakat, 2004; Lucena et al., 2007). This can be also attributed to the persistence of these com-

pounds for decades in the environment and food chains (Kirman et al., 2011).

Similarly, in spite of the ban of OC pesticides by the United States and Canada more than two decades ago, DDE was detected in a majority of blood samples collected in the US (1999-2004), and in Canada (2007-2009), while DDT was detected in 5-10 % of samples due to a longer half - life of DDE and the direct exposure to DDE in foods (CDC, 2009; Health Canada, 2010). DDD is generally not detected in serum, due to its greater water solubility and lower persistence (Kirman et al., 2011).

In the present study p,p.DDA; o,p.DDD and endrin had the highest mean concentrations among diabetic children (1.07; 1.04 and 0.91 ng / ml respectively). Regarding organophosphorus compounds; malathion had the highest concentration (0.54 ng/ml).

In agreement, other researchers had reported lindane levels below the limit of detection in most persons (Bates et al., 2004; CDC, 2009). This may be attributed to OC banning measures in the studied areas or due to different analytical methods used.

This finding is consistent with other studies as endrin was detected in the serum of diabetic children (Bates et al., 2004;

Lopez-Espinosa et al., 2008). An explanation for this result is that endrin is detected in the serum when the dose is high and the exposure is very recent (CDC, 2009).

Unfortunately, there are no limits or standards that regulate the pesticide levels in biological samples. This is primarily because biomonitoring of these compounds is relatively uncommon and very little is known about how levels correlate with harmful health effects (CDC, 2009).

In Egypt, many studies were carried out to assess OC concentrations in different matrices including soil, air, water and food, with only very few reports in human restricted to milk samples, hence, it is difficult to compare the values in the present work with other researches in our country (reviewed by Barakat, 2004). On the other hand, making a comparison on the basis of historical reference groups in other societies has several limitations due to many variables such as geography, time, type of samples and dissimilar demographic criteria, diversity of analytical methods and results expression. These factors can bias the level of exposure to the chemicals of interest.

In addition to the aforementioned results, three types of organophosphorus (OP) residues were detected in the serum samples of the studied children in the fol-

lowing order of frequency: malathion, chlorpyrifos-methyl and profenofos with mean serum concentrations (0.54, 0.46 and 0.24 ng / ml respectively).

It is well known that OPs had short half - lives range from hours (12 - 24 hours for malathion) to weeks (ATSDR, 2003). The detection of parent compounds reflects very recent exposure over the previous few days (CDC, 2005). So, the fact that we could measure these chemicals in the studied samples is suggestive of a higher magnitude of exposure than was expected.

In the US, a mixture of OP residues were detected in the blood and / or urine of nearly all persons sampled (Barr et al., 2005). Several birth cohort studies have detected chlorpyrifos and diazinon and other various OP in cord blood (Yan et al., 2009; Barr et al., 2010). Of course, the type of the detected pesticides varied due to different pesticides used in each society and the time of the study.

In accordance with the present findings, many researchers reported OPs exposures in young children. However, most of these studies did not involve measurement of the parent pesticides. Instead, they used urinary metabolites as markers of exposure (Panuwet et al., 2009; Ye et al., 2009; Griffith et al., 2011; Quirós-Alcalá et al., 2011).

Virtually, metabolites cannot be attributed to a specific organophosphate pesticide and they might be previously formed in or on the consumed food. It is also difficult to assign health effects to a certain OP compound (Kuklenyik, 2008). In the present work, measurement of the parent OPs could be considered an advantage and more reliable indicator of recent direct exposure to these compounds.

### **CONCLUSION**

From the current work, it could be concluded that Egyptian diabetic children have measurable levels of several pesticides residues and there is increasing risk in children exposed to pesticides to develop T1D. Additionally, biomonitoring of these toxicants provides clinical toxicologists and physicians with reference values to be compared with other populations and could be correlated in the future studies with diseases claimed to be due to pes-

ticide exposure especially in children.

### **RECOMMENDATIONS**

It is recommended to establish regular surveys to set reference values in our population and to identify highly exposed groups. That, in turn, may assist in studying the toxic effects of pesticides and mechanisms possibly related to the etiology of many diseases linked to environmental contaminants. Governments must put strict legislation to reduce exposure to various toxic pesticides especially in the highly susceptible groups i.e. children.

### **ACKNOWLEDGEMENT**

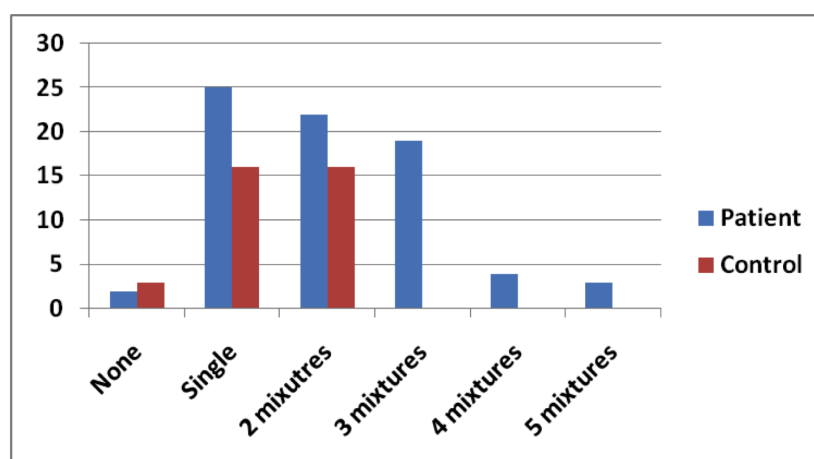
We would like to thank the Pediatric Staff of Mansoura University Children Hospital for their assistance in taking blood samples and for examination of the studied children.



**Table (1):** Sociodemographic data of the control group (n = 35) and the diabetic children (n = 75).

| Demographic data | Studied Groups                   |                   |
|------------------|----------------------------------|-------------------|
|                  | Control (n = 35)                 | Patients (n = 75) |
| 1) Age (years)   |                                  | 6.01 ± 1.9        |
| Mean± SD         | 6.1 ±1.7                         |                   |
|                  | <i>t: 0.356-, p value: 0.722</i> |                   |
| 2) Gender        |                                  |                   |
| Female:          | 19 (54.3%)                       | 39 (52%)          |
| Male:            | 16 (45.7%)                       | 36 (48%)          |
|                  | $\chi^2: 0.050, p\ value: 0.823$ |                   |
| 3) Residence     |                                  |                   |
| Rural            | 19 (54.3%)                       | 35 (46.7%)        |
| Urban            | 16 (45.7%)                       | 40 (53.3%)        |
|                  | $\chi^2: 0.554, p\ value: 0.457$ |                   |

*P* is significant if < 0.05



**Figure (1):** Number of pesticides residues detected in the studied groups.

**Table (2):** The frequency and Odds ratio (OR) of pesticides residues in the studied groups.

| Pesticides residues detected | Control (n = 35) | Patients (n = 75) | Statistical data |                          |       |
|------------------------------|------------------|-------------------|------------------|--------------------------|-------|
|                              | n (%)            | n (%)             | Odds ratios      | 95 % Confidence Interval | p     |
| <b>Organochlorines (OC)</b>  |                  |                   |                  |                          |       |
| Lindane                      | 19 (54.3%)       | 53 (70.7%)        | 2.02             | (0.88-4.65)              | 0.09  |
| Endrin                       | 0                | 8 (10.7%)         | 1.52             | (1.32-1.75)              | 0.04* |
| o.p-DDD                      | 0                | 16 (21.3%)        | 1.59             | (1.36-1.86)              | 0.00* |
| p.p-DDE                      | 0                | 16 (21.3%)        | 1.59             | (1.36-1.86)              | 0.00* |
| o.p-DDT                      | 9 (25.7%)        | 6 (8%)            | 0.25             | (0.08-0.77)              | 0.01* |
| p.p-DDA                      | 0                | 4 (5.3%)          | 1.49             | (1.30-1.70)              | 0.16  |
| <b>Organophosphates (OP)</b> |                  |                   |                  |                          |       |
| Malathion                    | 11 (31.4%)       | 49 (65.3%)        | 4.11             | (1.74-9.69)              | 0.00* |
| Profenofos                   | 6 (17.1%)        | 2 (2.7%)          | 0.13             | (0.02-0.69)              | 0.00* |
| Chlorpyrifos-Methyl          | 12 (34.3%)       | 6 (8%)            | 0.16             | (0.05-0.49)              | 0.00* |

Lindane: (hexachlorocyclohexane isomer:  $\gamma$ -HCH); o,p'-DDT: 1-(o-chlorophenyl) -1- (p-chlorophenyl)-2,2,2-trichloroethane; p,p'-DDE (1,1-dichloro-2,2-bis (p.chlorophenyl) ethylene; o,p'-DDD: 1- (o-chlorophenyl)-1-(p-chlorophenyl) -2,2-dichloroethane; p.p DDA: " 2,2-bis-4-chlorophenyl acetic acid". \*p is significant if  $< 0.05$ .

**Table (3):** Concentrations of pesticides residues (ng / ml) in the serum of the studied groups.

| Pesticides residues detected  | Control (n = 35) |                       | Patients (n = 75) |                       | p      |
|-------------------------------|------------------|-----------------------|-------------------|-----------------------|--------|
|                               | Mean             | Median (Min - Max)    | Mean              | Median (Min -Max)     |        |
| <b>Organochlorines (OC)</b>   |                  |                       |                   |                       |        |
| Lindane                       | < LOD            | < LOD                 | 0.48              | 0.54<br>(0.00 - 0.87) | 0.00*  |
| Endrin                        | < LOD            | < LOD                 | 0.91              | 0.90<br>(0.88 - 0.96) | 0.00*  |
| o.p.DDD                       | < LOD            | < LOD                 | 1.04              | 0.75<br>(0.16 - 4.3)  | 0.00*  |
| p.p.DDE                       | < LOD            | < LOD                 | 0.37              | 0.28<br>(0.1 - 0.97)  | 0.00*  |
| o.p.DDT                       | < LOD            | < LOD                 | 0.20              | 0.00<br>(0.01 - 0.61) | 0.00*  |
| p.p.DDA                       | < LOD            | < LOD                 | 1.07              | 1.07<br>(0.51 - 1.64) | 0.00*  |
| <b>Organo-phosphates (OP)</b> |                  |                       |                   |                       |        |
| Malathion                     | 0.03             | 0.03<br>(0.02 - 0.05) | 0.54              | 0.61<br>(0.11-1.01)   | 0.00*  |
| Profenofos                    | 0.03             | 0.03<br>(0.03 - 0.04) | 0.46              | 0.47<br>(0.47 - 0.47) | 0.034* |
| Chlorpyrifos-Methyl           | 0.02             | 0.03<br>(0.01 - 0.04) | 0.24              | 0.00<br>(0.00 - 0.72) | 0.254  |

LOD = limit of detection; Min = minimum; Max = maximum; \*p is significant if  $\leq 0.05$

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## بقايا المبيدات في أطفال مرضى السكري المصريين

المشتركون في البحث

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تم القاء الضوء على العلاقة المحتملة بين العديد من أمراض الطفولة كاضطرابات الغدد الصماء وخلل الجهاز المناعى وامكانية حدوثها بسبب التعرض للمبيدات السامة.

ويهدف هذا البحث إلى رصد بقايا المبيدات السامة في مجموعة من أطفال مرضى السكري من النوع الأول. واشتملت هذه الدراسة على مائة وعشرة أطفال مصريين تراوحت أعمارهم من ١٢-١٠ سنوات. وتتألف المجموعة الضابطة من ٣٥ طفلاً من الأصحاء أما مجموعة الدراسة تتكون من ٧٥ طفلاً تم تشخيصهم حديثاً كمرضى سكري من النوع الأول. وقد تم اختيار هؤلاء الأطفال من المترددين على مستشفى الأطفال الجامعى. وتم أخذ عينات الدم من الأطفال وفصلها للكشف عن بقايا المبيدات الحشرية. وقد أظهرت النتائج أن الليندان هو من المبيدات الكلورية العضوية الأكثر شيوعاً (70.7%) في دم مرضى السكري تليها مشتقات DDT مثل o.p.DDD و p.p.DDE (21.3% لكل منهما) وكان الملاثيون هو أكثر المركبات الفوسفاتية العضوية إنتشاراً (65.3%).

وبناءً على ما تقدم نستنتج أن الأطفال المصريين لديهم مستويات قابلة للقياس من بقايا المبيدات السامة وان هناك زيادة فى نسبة خطورة الإصابة بمرض السكري النوع الاول فى الأطفال المعرضون لتلك المبيدات. بالإضافة إلى أن الرصد البيولوجي لهذه الملوثات البيئية يوفر للأطباء لا سيما أطباء السموم الاكلينيكية القيم المرجعية لمقارنتها مع الشعوب الاخرى ويمكن أن تستخدم هذه القيم في الدراسات المستقبلية لدراسة الامراض التي يمكن حدوثها نتيجة التعرض للمبيدات وخصوصاً فى الأطفال.