Field and experimental acute toxicity study of diazinon and its break-down products
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SUMMARY

The widespread use of cholinesterase-inhibiting pesticides in the environment presents increasing concerns about their effects on human, wildlife and ecosystem health. Diazinon is one of the most used organophosphorus compounds. In August 2006, at Alexandria governorate a sudden death of 76 cows had been occurred after spraying diazinon in the form of (Sedicone, 60% EC). The clinical signs were excess salivation and acute nervous manifestations. Samples from the concentrated diazinon (Sedicone, 60% EC) and the diluted one used in spraying cows as well as tissues of dead animals were taken for laboratory investigation. Analysis showed that the concentration of diazinon in the sample was 19.82% instead of 60% and the rest had decomposed by hydrolysis into a range of break-down products, including tetraethylidithiopyrophosphate (sulfotepp) and monothionotetraethylpyrophosphate (monothiono-tepp), which are more toxic to mammals than diazinon. Residual amount of diazinon in liver of dead cows was recorded as 3.17ppm. The experimental study revealed that the oral LD50, of diazinon in albino rats was 17.9 mg/kg B.wt. Albino rats given LD50 orally showed residues reached to "3.03 and 1.04" ppm in liver and kidney, respectively. Meanwhile, no residues of diazinon were found in muscles or in spleen.

In conclusion, the present study recorded a case of acute toxicity of decomposed diazinon due to improper storage of diazinon at Alexandria governorate in 2006 as field study and confirmed with further experimental study. In recommendation spot a light on the importance of proper routine checking of stored pesticides and any change in physical or chemical characters must be forbidden its use.
INTRODUCTION

Pesticides are useful tools in agriculture and forestry, but their contribution to the gradual degradation of the aquatic ecosystem cannot be ignored (Konar, 1975). Due to the residual effects of pesticides, important organs like kidney, liver, gills, stomach, brain, muscles and genital organs are damaged (Rahman et al., 2002). Diazinon is a broad-spectrum pesticide widely used in veterinary and agricultural practice throughout the world.

Diazinon is a sulphated organophosphorus insecticide used for lice and fly control in cattle. It is applied by spray concentration 1/1000 for 60% diazinon (Gilman et al., 1996). Diazinon is a non-systemic organo-phosphate insecticide used on home gardens and farms to control a wide variety of sucking and leaf eating insects. It is used on rice, fruit trees, sugarcane, corn, tobacco, potatoes and on horticultural plants. It is also an ingredient in pest strips. Diazinon has veterinary uses against fleas and ticks. Nearly 2.6 million pounds of diazinon were used each year prior to 1983. Some of the older formulations of diazinon were unstable and contained a number of potent impurities such as sulfotepp and monothiono-TEEP. Newer products do not contain impurities which increase the risk associated with diazinon use.

In 1988 EPA cancelled the registration of diazinon for use on golf courses and sod farms. They cited die-offs of birds, which often congregate in these areas (Fabrizi et al., 1999).

Diazinon may be absorbed from the gastrointestinal tract, through the intact skin and following inhalation. Diazinon is oxidized by the microsomal enzymes to cholinesterase-inhibiting metabolites such as diazoxon, hydroxydiazoxon, and hydroxydiazinon (EHC, 1998).

Toxicity of this pesticide has been correlated with its inhibitory effects on acetylcholinesterase, an enzyme needed for proper nervous system function (Paraoanu et al., 2006). Diazinon itself is not a potent cholinesterase inhibitor. However, in animals it is converted to diazoxon (a substitution of oxygen for the sulfur molecule), a compound that is a strong enzyme inhibitor (Fabrizi et al., 1999). TEPP inhibits enzyme acetylcholinesterase via its phosphorylation or phosphorylation at the serine hydroxy group in its active site. Afterwards, AChE is not able to serve its physiological function and intoxicated organism is died due to over stimulation of cholinergic nervous system (Kuca et al., 2006).

Developmental exposure to organophosphorus pesticide diazinon
alters serotonergic synaptic function at dose below the threshold for cholinesterase inhibition (Roegge et al., 2008).

The purpose of the present study was to evaluate the effects of spraying of diazinon decomposed product, as well as detection of its residues, in cow's organs as field study and experimental study in albino rats.

**MATERIALS AND METHODS**

**Pesticide:**
Diazinon: O,O-diethyl-O-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate known economically as Sedicone 60%EC (Tomlin, 2004).

**Animals:**

**Field study:**
In August 2006, a problem arose in El-Amarhya, Alexandria governorate in the form of sudden death of 76 cows after sedicone spraying at the concentration level 1/1000. The animals' suffered from diazinon toxicity.

**Experimental study:**
A total of thirty six adult albino rats of both sexes, were used. 32 albino rats were used for determination of acute oral LD$_{50}$ of diazinon. 4 rats were used for residual analysis.

**Determination of LD$_{50}$:**
Determination of LD$_{50}$ was done in 8 groups of 4 rats each. 4 groups for preliminary trial (for determination of the minimum LD$_{100}$ and the maximum LD$_{0}$) followed by 4 groups for confirmatory determination. The LD$_{50}$ was calculated mathematically according to Spearman-Kerber method as described by Finney (1964).

**Samples:**
**Field samples:**
Diazinon diluted samples 1/1000 which was used for spraying cows and the concentrated one (Sedicone, 60%EC) were performed for chromatographic analysis. Liver from dead cows were taken and immediately frozen after collection for residual analysis.

**Experimental samples:**
Tissue samples were taken after the appearance of toxicity symptoms from rats received 17.9 mg/kg B.wt. (LD$_{50}$ of sedicone). The samples immediately frozen after collection till analysis for diazinon residues and its metabolites.

**Chromatographic analysis:**

**Extraction and clean up of the selected organs:**
20 g of the liver from each dead cow was homogenized with 10 g anhydrous sodium sulphate and 150 ml acetonitrile -acetone (90:10,v/v) were added, then blended for 2 min for complete homogeneity at a high speed blender to extract diazinon from the soft
tissues. Filter through a glass funnel with clean pad of cotton. Evaporate till dryness for the liquid-liquid partitioning and the column clean up according to the method of analysis of organophosphorus pesticide residues in tissues (Felix Hernandez et al., 1996).

**Chromatographic determination:**

High performance Liquid Chromatography (Agilent 1100) equipped with a UV diode array detector and set at 254nm provided with a Zorbox C\textsubscript{18} (150 mm x 4 mm, i.d. x 4.6 µm film thickness) column was used for the determination of diazinon according to the method of the determination of organophosphorus insecticides, their oxygen and metabolites by HPLC (Sultatos et al., 1982). The mobile phase was a mixture of acetonitrile: water (65:35 v/v) at a flow rate of 1 ml/min. under these conditions, the retention time of diazinon standard was 4.49 min., while the detected breakdown products were at 1.43, 1.683, 2.26, 2.36, 2.73, 2.99, 3.45, 3.593, 3.97 min. and finally a short peak with retention time of 4.49 min.

The reliability of the above analytical methods were examined by fortifying untreated organs with known quantities (1 ppm) of diazinon, followed by the same procedure of extraction, clean up and determination. The rate of recovery of the samples was shown in table (1). The results were adjusted by the rates of the recovery percentages.

Table (1): The recovery percentage of diazinon in selected organs.

<table>
<thead>
<tr>
<th>Fortified limit</th>
<th>Cow's liver %</th>
<th>Rat's Liver %</th>
<th>Spleen %</th>
<th>kidney %</th>
<th>muscle %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 ppm</td>
<td>93.56</td>
<td>92.62</td>
<td>93.87</td>
<td>97.79</td>
<td>95.79</td>
</tr>
</tbody>
</table>
Fig (1): Peaks of fresh diazinon (standard).

Fig (2): Peaks of decomposed diazinon (Sedicone, 60% EC).
Fig (3): Mean of diazinon residues (ppm) in liver of dead cows sprayed with diazinon (Sedicone).

Fig (4): Mean of diazinon residues (ppm) in liver of rats given orally LD₅₀ of diazinon (Sedicone).
RESULTS AND DISCUSSION

A great problem occurred in El-Amarhya, Alexandria governorate in August 2006 in the form of sudden death of 76 cows after diazinon spraying. This problem summarized in appearance of salivation and acute nervous manifestation followed by death after sedicone spraying. Sedicone has dark brown color with offensive odor. The technical active ingredient diazinon is a yellow/brown liquid with a slight compound-specific odor (EHC, 1998). These drastic changes in physical characters mostly resulted from change in diazinon composition as a result of its degradation.

Fig (5): Mean of Diazinon residues in Kidney of rats given orally LD$_{50}$ of diazinon (Sedicone).
Determination of LD₅₀:
Table (2): Determination of acute oral LD₅₀ of diazinon in albino rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg B.wt.)</th>
<th>No. of each group</th>
<th>No. of dead animals</th>
<th>Mortality %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>40</td>
<td>4</td>
<td>4</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>4</td>
<td>3</td>
<td>75</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>8</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Calculation:

\[ m = X_k + 1/2d - (dr_1/N) \]

\[ \log LD_{50} = \log 40 + 1/2 \log 2 - 8/16 \]

\[ = 1.600206 + 0.150515 - 0.5 \]

\[ = 1.752575 - 0.5 = 1.252575 \]

\[ LD_{50} = \text{antilog } m = 17.888544 = 17.9 \text{ mg/kb B.wt.} \]

Clinical symptoms included nasal discharge, salivation, vomiting, diarrhea and neuropathy. Symptoms are typical to organophosphate toxicity as mentioned by Smith, 1996; Gfeller and Messonnier, 1998 and Omar Khan, 2001. Exposure of animals to diazinon caused extensive changes in physiological, biochemical, and histopathological parameters as well as histochemical AChE. So, contact exposure of diazinon leads to negative response on animal health (Yehia, 2007).

Oral LD₅₀ of diazinon was calculated to be 17.9 mg/kg b.wt. (Table, 2). By dietary routes, diazinon is classified as moderately acutely toxic to small mammals (Al-Qarawi et al., 1999). This LD₅₀ transfers diazinon from moderately toxic to extremely toxic one.

From this result, the increase in diazinon toxicity could be regarded to diazinon decomposed by hydrolysis into range of breakdown products (Fig, 2), including sulfo-TEPP and monothio-TEPP, which are much more toxic to mammals than diazinon (Sharpe et al., 2006). The corresponding oral LD₅₀ of TEPP in rats 0.5 mg/kg (U.S. Department of Labor Oc-
Several authors reported the LD$_{50}$ of diazinon to be 238-321 mg/kg b.wt. for albino rats (DeProspo, 1972); an average 450 mg/kg b.wt. in male albino rats (Clarke and Myra, 1978) and 150 mg/kg b.wt. for active principle (Clarke, 1984). The increase in diazinon toxicity could be attributed to breakdown of diazinon that showed in scanning pattern of HPLC.

In comparison, Hatjian et al. (2000) describes work performed in 17 subjects with a single or two exposures to a sheep dip containing diazinon. Urine samples revealed OP metabolites dimethylphosphate (DMP), dimethylthiophosphate (DMTP), diethylphosphate (DEP) and diethylthiophosphate (DETP) in 37% of subjects at low levels which were not elevated after exposure. In vitro studies with both authentic diazinon (98%) and diazinon in a sheep dip formulation (45%) showed increased Sister chromatid exchanges and decreased replicative indices, suggesting toxic and genotoxic effects of diazinon.

Phosphorothioate pesticides (OP) such as diazinon are activated to highly toxic oxon metabolites by the cytochromes P450 (P450s), mainly in the liver. Simultaneously, the P450s catalyze detoxification of OP to nontoxic dearylated metabolites. The oxon is then detoxified to the dearylated metabolite by PON1, an A-esterase present in the liver and blood serum (Mutch et al., 2006).

**Residue analysis:**

In the present study the concentration of diazinon in the form of sedicone 60%, was determined and found to be 19.82% of diazinon as active principle instead of 60%. Also, the diluted one used for spraying showed the same concentration of the concentrated one (0.02%) which caused a sudden death of cows present in the farm. Indicating degradation by hydrolysis into a range of break-down products (Fig, 2), including tetraethyl-dithiopyrophosphate (sulfo-Tepp) and monothionotetraethylpyrophosphate (monothiono-Tepp) which, are more toxic to mammals than diazinon (Fig, 2) in comparison to fresh diazinon (Fig, 1) Similar results were recorded by Sharpe et al. (2006).

Acute toxicity resulted from unsuitable storage conditions of the emulsifiable concentrate formulation of diazinon. Diazinon was stored in "tin" containers made of tin-plated sheet steel, the sample represented virtually conversion of diazinon into transformation products. Sulfo-tepp and monothiono-TEPP were two of the identified products in the sample,
both of which are much more toxic than diazinon.

Diazoxon, the active toxicant, which is formed by the oxidation of diazinon through desulfuration was degraded mainly by hydrolysis resulting in the two metabolites diethylphosphoric acid and 2-isopropyl-4-methyl-6-hydroxypyrimidine (Federal Office of Public Health, 1998).

Table (3) represents the residues of diazinon and its metabolites in liver (Fig, 3) of the dead cows in field study and the residues found in liver (Fig, 4) and kidney (Fig, 5) of the tested rats when they exposed to the insecticide sedicone (17.9 mg/kg b.wt.) in experimental study. However, no residue of diazinon could be detected in spleen and muscle of rats.

Table (3) represents the residues of diazinon and TEPP in cow's liver and various tissues in experimental rats (ppm).

<table>
<thead>
<tr>
<th>Animal</th>
<th>Cows</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue</td>
<td>Liver</td>
<td>Liver</td>
</tr>
<tr>
<td>Diazinon</td>
<td>3.17</td>
<td>3.03</td>
</tr>
<tr>
<td>TEPP</td>
<td>Detected +ve</td>
<td>detected +ve</td>
</tr>
</tbody>
</table>

ND= non-detectable

These residues were very low to induce death, but in mammals diazinon was converted to more toxic compounds.

From the mentioned results, it is proved that this problem arising from degradation of diazinon due to transformation during its unsuitable storage. For contradiction of this loss, it is advisable proper usage, storage, application and continuous checking on pesticide and observes any change in its physical and chemical characters as well as contraband of spraying without presence of its antidote (atropine sulphate) to prevent rising this problem in the future. Also, pesticide application in field must be restricted in consideration of risk / benefit value.

REFERENCES
Al-Qarawi, A.A.; Mahmoud, O. M.; Haroun, E.M.; Sobaih,


دراسات حقلية وتجريبية على التسمم الحاد للديازيبون ونتائج تكبيره
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الملخص العربي
في أغسطس 2006 ظهرت مشكلة في محافظة الأسكندرية مركز العامرية وهي عبارة عن نفوق 72 بقرة بعد رش مبيد ديазيبون 20% (سيديكون 20%) وظهرت أعراض التسمم الحاد عبارة عن زيادة أعراض اللامع، وأعراض عصبية أنتهت بالوفاة. تم أخذ عينات من المبيد المسمّد في الرش وكذلك المخزوف كما تم أيضاً جمع عينات كبد من هذه الحيوانات. بالتحليل المعملي لعينة المركزة وجد أن نسبة الديازيبون الموجودة بها 19.82% بدلاً من 20% والباقي تم تكبيره إلى العديد من المركبات والتي هي أشد سمية من الديازيبون مثل مركبات TEPP. كانت نسبة متبقيات المبيد في كبد الإيقار 317 جزء في المليون.

في الدراسة التجريبيه المعملية أثبتت أرتفاع سمية درجة الجرعة النصف المميتة للديازيبون تصل إلى 17.9% مجم/كم من وزن الجسم في الفئران البيضاء مما يدل على تكبير المادة الفعالة. التحليل المعملي أثبت عدم وجود متبقيات للمبيد في العضلات والجلد ولكنها كانت موجودة في كبد وكل الفئران البيضاء.

هناك دراسة أُجريت أن حدوث النفوذ المفاجئ لحيوانات المزرعة التي تم رشها بمبيد الديازيبون هو نتيجة لتبلور المبيد المستخدم في رش هذه الحيوانات إلى متحولاته الفعالة السمية من مركب TEPP المعروض بسمتخدمته الحادة فاتتته السمية التي تؤدي إلى سرعه النفوذ ولا يتفع مبيده استخدام الطرق المتواجدة. لذلك يوصي بضرورة إتباع الشروط الصحية عند استخدام المبيدات خاصة عند التخزين والتدخلي والتطبيق مع مراعاة الطابع المستخدم للمبيدات الفعالة والفعالة للمبيد وملاحظة أي تغييرات تنبأ على المبيد في خواصه الضوئية والكيميائية وملاحظة أي تغيرات في خواصه البيئية والكيميائية وعدم استخدامه في حالة حدوث أي تغيير في هذه الخواص.

المراجع:
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