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Maternal and foetal toxicity induced by exposure to mixture of dimethoate and cypermethrin in albino rats

S. B. Ramon-Yusuf1, Y. O. Aliu2, O. A. Salawu3, I. Chahoud4 and S. F. Ambali5*

1National Universities Commission, Maitama, Abuja, Nigeria.
2Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria.
4WHO Collaborating Centre for Developmental Toxicology, Institute of Clinical Pharmacology and Toxicology, Campus Benjamin Franklin, Charité University Medical School, Berlin, Germany.
5Department of Veterinary Pharmacology and Toxicology, University of Ilorin, Ilorin, Kwara State, Nigeria.

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The use of pesticide mixture to combat increased resistance by pest, to single chemical insecticide has brought about an increase in the use of pesticide mixture from different class, with its attendant toxicity. The objective of this study was to evaluate the maternal and foetal toxicity associated with dimethoate and cypermethrin, the two insecticides that are normally mixed to reduce pest resistance. 100 confirmed-mated (gravid) females were assigned at random among the four groups (control – tap water only): group 1- very low dose-10 mg/kg/day of Cypermethrin (5.3% LD50) + 1.0 mg/kg/day Dimethoate (0.7% of LD50); group 2- intermediate dose- 19 mg/kg/day of Cypermethrin (10% LD50) + 1.4 mg/kg/day Dimethoate (0.9% of LD50); and group 3- high dose- 38 mg/kg/day of Cypermethrin (20% LD50) + 12.8 mg/kg/day Dimethoate (8.5% of LD50). The regimens were administered orally at gestation days (GD) 6-20 to pregnant rats. The dams were examined for mortality, moribundity, pertinent behavioural changes and signs of overt toxicity. Animals were weighed on day 0, and once at every 3-day intervals during the dosing period and on GD 21. The pregnant rats were sacrificed by decapitation on GD 21, the uteri were removed and the fetuses examined for litter weight, size, resorption, sex ratio, absolute and relative organ weight. The results showed that combined pesticide exposure caused overt toxic signs in the dams. However, the maternal body weight and relative organ weight were not adversely affected except in the heart that showed significant alteration. Although, the litter weight was not adversely compromised, the result showed that the prenatal exposure to pesticide mixture caused increased foetal resorption, decreased the litter size and offsprings’ male/female ratio. The study concludes that prenatal co-exposure to cypermethrin and dimethoate has adverse consequences on some maternal and foetal parameters in albino rats.

Key words: Pesticide mixture, litter size, sex ratio, litter weight, maternal relative organ weight.

INTRODUCTION

Pesticides are widely used in food production systems and public health in many countries because of the need to feed the ever-increasing human population and protect them from vector-borne diseases (Bhaskar et al., 2014; Gabr et al., 2015). Pesticide applications have increased dramatically since the 1960s (Olgan et al., 2004). The
wide use of pesticides has made them quite ubiquitous. The reality is that human exposure and the related health effects in humans as well as in wild/domestic animals have become a serious concern (Crumpton, 2001). Human exposure to pesticides is rarely limited to a single chemical. People are exposed daily to a variety of chemicals in foods, drinks, cosmetics and indoor and outdoor pollutants (Marinovich et al., 1996). Agrochemicals are often applied in combinations and it is not uncommon for many classes of pesticides, such as insecticides, herbicides and fungicides, to be used on the same crop (National Agricultural Statistics Service, 2005). Furthermore, the increase incidence of pest resistance to specific pesticide has led to formulations containing two or more pesticides. Each active ingredient in the formulation has a specific mode of action for controlling a pest, and each active ingredient has its own possible side effects on non-target animals and humans exposed to it (Colborn, 2006). In fact, mixed exposures are the rule rather than the exception indicating that exposure assessment, hazard identification, risk assessment and risk characterization should focus on mixtures rather than single chemicals (Liu et al., 2006). Attempts at predicting the toxicology of mixtures based upon the knowledge of individual chemicals often leads to the wrong conclusions (Marinovich et al., 1996). The effect of the interaction between pesticides in combination is not always predictable, as such combinations may result in synergism, additive effects, potentiation or inhibition. Two or more compounds may show additive, antagonistic, or synergistic interaction or each may act on totally different systems and thus not interact (Liu et al., 2006). Synergism of pyrethroids by organophosphorous insecticides has been demonstrated. For instance, Martin et al. (2003) showed significant synergism for mixtures of cypermethrin/ethion; deltamethrin/triazophos and deltamethrin/chlorpyrifos. There is a potential hazard in mixed intoxication by pyrethroids and organophosphorous insecticides, due to the fact that the low toxicity of the pyrethroids on mammals is chiefly due to quick cleavage of molecules by esterases, which can be thwarted by esterase inhibitions (Audegond et al., 1988).

Insecticides are considered as potent pollutants of the environment that have been involved in birth defects and reproductive failure. Due to its widespread use and the rising instability of the foetus and the pregnant mother to toxic exposures, the adverse health effects correlated with exposure to insecticides during pregnancy have become a considerable public health interest (Stillerman et al., 2008). Mothers involved in agricultural activities before conception or during the first trimester of pregnancy have been shown to have an increased risk of having offspring with defect of the nervous system, oral cleft or multiple anomalies (Gary and Ostby, 1998). Garcia et al. (1999) have reported that fathers who were involved in agriculture had an increased risk of having offsprings with defects of the nervous or musculoskeletal systems. Professional applicators of pesticides have been reported to show specific congenital anomalies in their offsprings (Shaw et al., 1999). Chronic exposure to organophosphorous and other pesticides during preconception and perinatal periods can induce a range of adverse birth outcomes (Gomes et al., 2008). In animal models, chronic maternal and paternal exposure to organophosphorous pesticides during the preconception period and early pregnancy can increase the risk of congenital anomalies in offsprings.

Organophosphorous pesticides are reported to induce a variety of symptoms leading to cholinergic morbidity among farm workers and pesticide handlers (Gomes et al., 1999a). These pesticides are also reported to inhibit cholinesterase activity, affect neurological and cognitive function among other health effects in humans and non-target mammalian species (Gomes et al., 1999; Sinha and Shukla, 2003; Young et al., 2006). Some of the organophosphorous pesticides are mutagenic and alter cell division; others are oestrogenic and alter reproductive and central nervous system function (Gomes et al., 2008).

Dimethoate (DM) [(0, 0-dimethyl-S (N-methylcarboxyl methyl) phosphorodithioate)] is an organophosphate insecticide with numerous uses on field, agricultural crops and ornamentals (Gallo and Lawryk, 1991). Its persistence in crops and soils may further enhance its propensity of adverse health consequences in man and other non-target species (IPCS/WHO, 1992). The residue of DM and its analog (omethoate) were found in many food stuffs including cow milk (Srivastava and Raizada, 1996). Although, DM remains one of the most widely used pesticides in the world, there is limited information regarding the developmental toxicity of this pesticide (Farag et al., 2006).

In mammals, the primary site of action of organophosphate pesticides is the central and peripheral nervous systems and is by inhibiting acetylcholinesterase, a biochemical event that results in accumulation of endogenous acetylcholine at the nerve endings (Sarkar and Maitra, 1990; Gore, 2001). Several physiological and behavioral dysfunctions occur in animals after exposure to light doses of organophosphate pesticide (Ambali and Ayo, 2011). Therefore, there is the possibility that DM can affect humans and animals in their natural habitat. DM has been reported to cause developmental toxicity such as decreased number of implantations and live fetuses, incidences of resorptions and decreased foetal body weights (Farag et al., 2006).
Pyrethroid insecticides have achieved widespread agricultural and environmental health applications due to their strong insecticidal properties. They are one of the most frequently used classes of pesticides (Roberts and Hutson, 1999) apparently due low toxicity to mammals than the other classes of insecticides, such as organochlorines, organophosphates and carbamates (Syed et al., 2009). Because of their low acute human toxicity, pyrethroids are widely used to control insects in and around homes (Freeman et al., 2004). The toxicity of pyrethroid insecticides to mammals received much attention in recent years because animals exposed to these insecticides showed changes in their physiological activities besides other pathological features (Glass, 2008). Certain pyrethroids exert hormonal activity that may alter early neurologic and reproductive development, and they are known also to elicit a range of immunotoxic and neurotoxic effects in humans and other mammals. Also, exposure to pyrethroids has been reported to contribute to reproductive dysfunction, developmental impairment and cancer (Garey and Wolf, 1998; Landrigan et al., 1999).

Cypermethrin is a synthetic pyrethroid insecticide widely used all over the world against a varying range of pests in agriculture, public health and animal husbandry (Al-Hamdani and Yajurvedi, 2010). It is a known neurotoxicant and primarily targets sodium channel in the nerve membrane of both central nervous system and peripheral nervous system (Cox, 1996; Narahashi, 1996; Ahmad et al., 2011; Singh et al., 2012). Since its introduction, it has become one of the mostly widely used insecticides in the developing countries (Assayed et al., 2010; Cremonese et al., 2014; Dewailly et al., 2014). Assayed et al., (2010) reported toxic effects of cypermethrin when exposed to either target or non-target organisms.

It can cross the placenta barrier, thereby affecting physiological functions associated with foetal neurological development (Dewailly et al., 2014) and foetal weight (Madu, 2015). The aim of the present study was to evaluate the effect of prenatal co-exposure to cypermethrin and dimethoate on some foetal parameters in male albino rats.

### MATERIALS AND METHODS

#### Chemicals and preparations

Commercial grade Cypermethrin (EC; Nagarjuna Agrochemical limited, Punjaugutta, Hyderabad, India) and commercial grade dimethoate; (VITOATE 40EC; Manufacturer: Asiatic Agricultural Pte Company, Singapore) were obtained from a reputable Agro-Allied Store in Abuja. They were dissolved in tap water to make appropriate concentrations used to dose the various treatment animals (US, EPA, 1989; Galyo and Lawryk, 1991), while those in the control group were given tap water only (2 ml/kg).

#### Test animals

The experimental animals were albino rats obtained from the animal breeding facility at the Department of Pharmacology, University of Jos, Plateau State, Nigeria. The animals were kept in the animal house in the Department of Pharmacology and Toxicology of the National Institute for Pharmaceutical Research and Development (NIPRD), Idu-Abuja, Nigeria. They were housed in groups of not more than five in plastic cages with beddings of wood shavings. All animals in the study were fed rodent diet compounded using growers chick mash. Tap water was made available ad libitum. Animals used in this study were handled and maintained in compliance with the National Institutes of Health Guide for Care and Use of Laboratory Animals (Garber et al., 2011), while the experiment was conducted with the approval of the Institutional Ethical Committee of NIPRD.

#### Mating

Three non-gravid female rats were paired with one male rats of the same strain overnight for mating purpose. The following morning, vaginal swab was taken from each of the females and mating was confirmed by the presence of sperm in a vaginal swab smear under the microscope. The day evidence of mating observed was designated day 0 of gestation (GD 0).

#### Animal groupings and dosing schedule

One hundred (100) confirmed-mated (gravid) females were randomly assigned among the four groups (control, very low dose, intermediate dose and the no-observed-adverse-effect level (NOAEL). The animals were housed in groups of five in plastic cages with stainless steel covers and wood shavings. Every day, from GD6 – GD 20, pregnant rats were treated with the pesticide mixture by gavage once daily according to the regimen indicated in Table 1.

<table>
<thead>
<tr>
<th>Group</th>
<th>Animal assignment</th>
<th>Number of animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Cypermethrin 0 (mg/kg/day)</td>
<td>25</td>
</tr>
<tr>
<td>Very Low</td>
<td>10.0 (5.3% of LD50)</td>
<td>25</td>
</tr>
<tr>
<td>Intermediate</td>
<td>19.0 (10% of LD50)</td>
<td>25</td>
</tr>
<tr>
<td>High</td>
<td>38.0 (20% of LD50)</td>
<td>25</td>
</tr>
</tbody>
</table>

**Table 1. Grouping and dosing schedule.**
Doses were selected based on data from the LD$_{50}$ of 187 and 150 mg/kg for cypermethrin (EPA, 1989) and dimethoate (WHO, 1989), respectively. Doses were selected to ensure that the highest dose should induce some developmental and/or maternal toxicity but not more than 10% maternal mortality. Intermediate dose levels should produce minimum observable toxic effects; and the lowest dose level should not produce any evidence of either maternal or foetal toxicity.

Each animal was observed at least once daily for mortality, moribundity, pertinent behavioural changes and signs of overt toxicity. Animals were weighed on day 0, and once every 3 days during the dosing period and on GD 21. Immediately after the animals were sacrificed, the uteri were removed and the pregnancy status of each of the animals was determined.

**Foetal examination and sex determination**

The abdominal cavity of each pregnant dam was opened, the uterus removed and weighed before it was opened, the number and position of living, dead and resorbed fetuses were determined and recorded. The stage of resorption was divided into three groups: early, intermediate and late. Uterine horns that appeared not to be gravid were stained with ammonium sulphide, in order to be able to demonstrate foetal resorption at an early stage as much as possible.

Briefly, the uterus was inserted in 10% ammonium sulphide for 5 min and then rinsed in water. Implantation sites which appeared as “black points” were thereafter counted. If the entire uterus appears black, then the test is negative, which indicates no implantation had taken place in the uterus. The fetuses were removed and the sex and body weight of each of them were determined. The fetuses were weighed according to their uterine position (top right = 1$^{st}$ foetus; top left = last foetus), numbered according to Standard Operating Procedure (SOP) 6 of the Teratology Laboratory at the Department of Toxicology, Institute for Clinical Pharmacology and Toxicology, Charité University Medical School, Berlin. Before the fetuses were carefully opened by abdominal incision, their sex was determined by judging the anogenital distance (female = smaller, male = greater distance). After opening the abdomen, locating the testes or ovaries, helped to confirm the sex of the foetus. The sex ratio for each group was then calculated.

**Statistical analysis**

Data expressed as mean ± SD was subjected to one-way ANOVA followed by Dunnett test. Statistical analysis was done using SPSS for Windows version 16.0 and the level of statistical significance was set at P<0.05.

**RESULTS**

**Maternal clinical signs**

No evidence of toxicity was seen in any of the dams exposed to a combination of 10 mg/kg body weight of cypermethrin + 1 mg/kg body weight of dimethoate. No mortality was recorded in the group and no significant behavioural changes were observed. Generally, no signs of overt toxicity were recorded at cage side observation. Similarly, no mortality was recorded in the second treatment group exposed to a combination of 19 mg/kg of cypermethrin + 1.4 mg/kg of dimethoate. However, four animals in the group, exhibited hyperactivity, tremors, spasms and hypersalivation. Unlike animals exposed to the first two combined dose levels of the pesticides, rats exposed to a combination of cypermethrin (38 mg/kg) and dimethoate (2.8 mg/kg) showed muscarinic signs characterized by hypersalivation and nasal discharges and a preponderance of signs of nicotinic cholinergic stimulation mainly muscle fasciculation, tremors, spasms and stiff gait. Two animals exhibited hyperactivity and mild clonic convulsion before death supervened.

**Effect on maternal body weight**

No significant differences (P>0.05) were observed in the body weights’ of treated and control animals.

**Effect on uterine contents**

With the exception of one animal which was pregnant in only one uterine horn, no abnormality was observed in the uterine contents of animals exposed to a combination of 10 mg/kg body weight of cypermethrin + 1 mg/kg body weight of dimethoate. There was no evidence of embryonic or foetal deaths/resorption in the uteri of animals in this group. However, in the group of animals exposed to the second combined dose level of 19 mg/kg of cypermethrin + 1.4 mg/kg of dimethoate, the uterus of one of the animals had numerous blood spots indicative of late foetal resorption. Exposure to a combination of 38 mg/kg of cypermethrin + 2.8 mg/kg of dimethoate was found to result in foetal deaths in utero in two animals. In one of such cases, the dead foetus was intact while on the other, foetal disintegration had already taken place.

**Effect on litter size**

The litter size was found to be significantly (P<0.05) lower when animals in the treatment groups were compared with those in the control group. The litter size was lower in the treatment groups than in the control. Those in group 2 exposed to a combination of 19 mg/kg cypermethrin + 1.4 mg/kg dimethoate, recorded the least mean litter size of 7.70. This was found to be statistically significant at P<0.05 (Table 2).

**Effect on litter weight**

No statistically significant difference (P>0.05) was found in the litter weight between the offspring of treated and control animals (Table 2).

**Effect on sex (male/female) ratio**

Although, there was no statistically significant difference
(P>0.05) in the sex ratio between the offspring of treated and control animals, that of the offspring of the former were relatively lower (Table 2).

**Table 2.** Number of fetuses/litter, foetal weight/litter, and sex ratios of fetuses of rats treated with combinations of cypermethrin and dimethoate during pregnancy.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>10 mg/kg cypermethrin + 1.0 mg/kg dimethoate</th>
<th>19 mg/kg cypermethrin + 1.4 mg/kg dimethoate</th>
<th>38 mg/kg cypermethrin + 2.8 mg/kg dimethoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Foetuses/litter</td>
<td>8.95 ± 1.19</td>
<td>8.35 ± 0.99</td>
<td>7.70 ± 1.30*</td>
<td>8.15 ± 1.22</td>
</tr>
<tr>
<td>Average foetal weight/litter(g)</td>
<td>2.56 ± 0.60</td>
<td>2.46 ± 0.51</td>
<td>2.43 ± 0.67</td>
<td>2.45 ± 0.72</td>
</tr>
<tr>
<td>Sex ratio (male/female)</td>
<td>47.3/52.5</td>
<td>47.1/52.6</td>
<td>46.4/53.6</td>
<td>46.8/52.1</td>
</tr>
</tbody>
</table>

**Table 3.** Relative (percent of body weight) organ weights of females treated with combination of cypermethrin and dimethoate during pregnancy (n=25/group).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>10 mg/kg cypermethrin + 1.0 mg/kg dimethoate</th>
<th>19 mg/kg cypermethrin + 1.4 mg/kg dimethoate</th>
<th>38 mg/kg cypermethrin + 2.8 mg/kg dimethoate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain (g)</td>
<td>0.82 ±0.30</td>
<td>0.78 ±0.29</td>
<td>0.78 ±0.32*</td>
<td>0.78±0.30</td>
</tr>
<tr>
<td>Heart (g)</td>
<td>0.45 ±0.15</td>
<td>0.44 ±0.16</td>
<td>0.45 ±0.26</td>
<td>0.37 ±0.18*</td>
</tr>
<tr>
<td>Kidneys (g)</td>
<td>0.67±0.28</td>
<td>0.62±0.29</td>
<td>0.67±0.14</td>
<td>0.68±0.25</td>
</tr>
<tr>
<td>Liver (g)</td>
<td>3.40 ±0.69</td>
<td>3.32 ±0.11</td>
<td>3.60 ±0.15</td>
<td>4.43 ±0.66</td>
</tr>
<tr>
<td>Lungs(g)</td>
<td>0.88 ±0.55</td>
<td>0.86 ±0.62</td>
<td>0.85 ±0.56</td>
<td>0.76 ± 0.22</td>
</tr>
<tr>
<td>Spleen(g)</td>
<td>0.68 ± 0.28</td>
<td>0.38 ± 0.01</td>
<td>0.41 ± 0.03</td>
<td>0.38 ±0.01</td>
</tr>
<tr>
<td>Uterus+ovaries (g)</td>
<td>5.95 ± 2.38</td>
<td>3.43 ± 1.10</td>
<td>1.82 ± 0.59</td>
<td>4.89 ± 1.40</td>
</tr>
</tbody>
</table>

*Significant at p<0.05 for values within the same row.

**Effect on relative maternal organ weight**

No difference was observed in the absolute weights of the brain, kidneys, liver, lungs, spleen, uterus and ovaries of pregnant females in the control animals as well as those exposed to three combinations of cypermethrin and dimethoate. However, when the organ weights were expressed as a ratio of body weight (relative weight), animals in the three treatment groups had relatively smaller hearts than control animals. However, there was no statistically significant difference (P>0.05) in the relative heart weight between control and treatment groups. Only animals exposed to 38 mg/kg cypermethrin + 2.8 mg/kg dimethoate, had statistically significant (p<0.05) difference in relative heart weight as compared to those in the control group (Table 3).

**DISCUSSION**

The lack of evidence of foetotoxicity in the fetuses of dams exposed to the combination of 10 mg/kg of cypermethrin + 1 mg/kg of dimethoate and the group that received 19 mg/kg of cypermethrin and 1.4 mg/kg of dimethoate, in the present study, is consistent with the results obtained from other studies where authors concluded that dosages of anticholinesterases which are not maternally toxic, do not produce embryotoxicity or fetotoxicity (Farag et al., 2000, 2003). It is noteworthy that foetotoxicity manifested as foetal death occurred only in the group that received 38 mg/kg of cypermethrin and 2.8 mg/kg of dimethoate and it was in this same treatment group that two dams exhibited signs of toxicity. It is pertinent to mention that all the previous studies referred to above, involved the use of doses of dimethoate far higher than the low doses used in the present study. In addition, those studies investigated dimethoate as a single pesticide and not in combination with other pesticides.

The present study also revealed that combined exposure to cypermethrin and dimethoate resulted in a reduction in the litter size. This is consistent with the reported effect of dimethoate exposure causing increased foetal resorption and reduced number of live foetuses (Farag et al., 2006). In the same vein, cypermethrin has been shown to also cause foetal resorption and reduced litter size (Madu, 2015).

Maternal co-exposure to cypermethrin plus dimethoate at the doses used in the present study, did not significantly alter litter weight of the fetuses. This shows that the pesticides at the doses used did not adversely
affect the foetus, since body weight alterations is a demonstration of stress exposure. In addition, the exposure did not adversely affect the absolute and relative weights of the brain, kidneys, liver, lungs, spleen, uterus and ovaries of pregnant females in the control animals as well as in the three treatment groups. However, the relative heart weight in animals exposed to 38 mg/kg cypermethrin + 2.8 mg/mg dimethoate these weights were significantly (p < 0.05) lower as compared to that of the animals in the control group. There was no evidence in the literature on the effects of either cypermethrin or dimethoate on the heart/body weight ratio. The fact that there was a significant change in the relative heart weight following exposure to 38 mg/kg cypermethrin + 2.8 mg/mg dimethoate showed that the heart is highly susceptible to injury provoked by the pesticides. The observed reduction in the relative heart weight in the present study may be due to some of the unknown untoward consequences of the interaction between cypermethrin and dimethoate following combined exposure, which had not been hitherto studied. We can however speculate that oxidative injury and/or direct cytotoxic damage to the heart provoked by the pesticide mixture may have been partly responsible for the lower relative heart weight in the present study.

The present study also revealed that maternal exposure caused increased foetal resorption, similar to what was observed following dimethoate (Farag et al., 2006) and cypermethrin (Raees et al., 2010; Madu, 2015) exposure. Exposure to xenobiotic such as dimethoate and cypermethrin that eventually disrupt ovarian steroid secretion would directly result in inadequate uterine decidualization and receptivity (Matt and Borzelleca, 1995). Fetuses were often susceptible to toxicant due to their fragile developmental state and lack of adequate defense mechanisms (Kimberly et al., 2004). Also, the complex nature of the reproductive regulatory process allows for numerous target sites and accounts for the various mechanisms through which toxins operate to exert their adverse effects (Abouamer et al., 2013).

Sex ratio, defined as ratio of number of males to females has been shown to decrease in the groups exposed to the pesticide mixture in the present study, indicating bias towards female sex. Studies have revealed a decrease male to female ratio following maternal exposure of rats to dimethoate (Abouamer et al., 2013) and cypermethrin (Huang and Li, 2014). Garry et al. (2002) have also reported a decrease in sex ratio of children born to pesticide applicators. A trend towards a declining proportion of male births has been noted in many industrialized countries (Astolfi and Zonta, 1999) and many factors including widespread use of environmental endocrine disrupting chemicals such as pesticides have been implicated (Martuzzi et al., 2001). Therefore, the sex ratio alteration in favour of females recorded in the present study may be linked to endocrine disrupting properties of the insecticide mixture.

In conclusion, the present study has shown that maternal co-exposure to dimethoate and cypermethrin even at low doses do adversely alter some maternal and foetal parameters, and therefore, restriction of pregnant mother to pesticide exposure is quintessential to promoting child health.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES


