 Fallout of certain ACCase-inhibitor and ALS-inhibitor herbicides on Culex pipiens larvae and pupae under laboratory conditions in Egypt

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The potential toxicity of five selected herbicides i.e. bispyribac-sodium, penoxsulam, haloxyfop-p-ethyl, pinoxaden, and tralkoxydim, and a pyrethroid insecticide deltamethrin was determined on the early 4th instar larvae (after 24, 48 and 72-h exposure) and pupae (after 24 and 48-h exposure) of Culex pipiens L. in the laboratory. As a result, deltamethrin was count as the most toxic compound on the early 4th instar larvae and pupae of Cx. pipiens compared with all tested herbicides after exposure times. For the tested herbicides, pinoxaden was the most potent herbicide against both tested developmental stages followed by bispyribac-sodium and penoxsulam, which exhibited a moderate toxicity; while haloxyfop-p-ethyl and tralkoxydim were deemed to be the lowest toxic compounds among all tested pesticides after exposure times. In addition, the toxicity of all pesticides against larval and pupal stages of mosquito was increased gradually as exposure time increased especially after 72-h exposure for larvae and 48-h exposure for pupae. In conclusion, this is the first report on the side toxicity effects of haloxyfop-p-ethyl, tralkoxydim, penoxsulam and bispyribac-sodium on immature stages (larvae and pupae) of Cx. pipiens mosquitoes in Egypt.

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Introduction

Modern agricultural systems use different forms of natural and synthetic pesticides such as herbicides and insecticides to control serious pests and to protect human and their animals and crops from these pests. Culex pipiens L. complex (Diptera: Culicidae) is considered one of the most serious dipteran insect pests and is responsible for transmitting dangerous pathogens like Rift Valley fever virus, the West Nile Virus (WNV) and lymphatic filariasis to humans and livestock [7,8]. The developmental stages, larvae and pupae, of Cx. pipiens and other mosquito species spend their life in different pools in agricultural regions or around human buildings [17,27].

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As far as it is known that the aquatic environments which have the mosquito immature stages are polluted by many agricultural pesticides (i.e., insecticides and herbicides) by various ways viz., direct application of pesticides for insect and weed control, spray drift, disposal through wastes, run off, and drain from agricultural areas [20,25]. Deltamethrin is a common second pyrethroid insecticide widely and extensively used for controlling mosquito adults and immature stages at their habitats to reduce disease transmission [15,29].

Herbicides were introduced to agricultural world to control weeds, however various herbicides such as glyphosate, 2,4-D, glufosinate, bromoxynil and atrazine have affected different mosquito species e.g. Aedes aegypti (L.), A. albopictus (Skuse) and Cx. pipiens [3,6,15,17]. Moreover, some acetyl-CoA carboxylase (ACCase)-inhibitor herbicides (i.e., quizalofop-ethyl, sethoxydim, halofop, clodinafop plus pinoxaden and fluazifop-p-butyl) which are a commonly grassy weed killer in agricultural and non-agricultural regions in the world have also caused different effects on many insect species such as cotton leafworm, Spodoptera littoralis Boisd., cabbage white butterflies, Pieris rapae (L.), Bihar hairy caterpillar, Spilarctia obliqua Walker, rove beetle, Aleochara bilineata (Gyll.), bean leaf beetle, Cerotoma trifurcata Forster and Cx. pipiens mosquito [1,9,15,22,24]. Although the mechanisms of most herbicides on animals particularly insect are unclear until now, however, these compounds have been found to affect the survival, morphological shape, growth, behavior, reproduction, and the biochemical reaction [3,4,17].

Bispyribac-sodium and penoxsulam, ALS-inhibitors, haloxyfop-p-ethyl, pinoxaden and tralkoxydim are used commonly for controlling grassy weeds in many agronomic crops, vegetables, orchards, landscape and forestry all over the world. Previous studies have manifested the presence of various herbicide residuals in aquatic systems [25] and a negative impact of bispyribac-sodium and penoxsulam have been detected on fish [4,26].

Yet, no toxicological studies could be found on the effect of pinoxaden, haloxyfop-p-ethyl, tralkoxydim, bispyribac-sodium and penoxsulam on Cx. pipiens, so the study here is to attest the effect of these herbicides in comparison with deltamethrin (as a pyrethroid insecticide used to control mosquito) on the early 4th larval instar and the pupal stage of Cx. pipiens under laboratory conditions.

Materials and methods

Mosquitoes

Larvae and pupae of Culex pipiens were obtained from Arab El-Madabegh region, Assiut city, Egypt, and then transferred into plastic boxes directly to the toxicological laboratory of Plant Protection Department, Faculty of Agriculture, Assiut University for conducting the experiments.

Pesticides

Commercial formulations of five selected herbicides include three ACCase-inhibitors: pinoxaden (Axial 4.5% EC; Syngenta Co.), haloxyfop-p-ethyl (Gallant Super 10.8% EC; Dow Agroscience Co.), and tralkoxydim (Avalanche 40% WDG; Starchem Co.) and two ALS-inhibitors: bispyribac-sodium (Nomine 2% SL; Komia Chemical and Stry) and penoxsulam (Rainbow 2.5% OD; Dow Agroscience Co.) along with a pyrethroid insecticide deltamethrin (Butox 5% EC, Agroseed Co.) as a equivalence control.

Toxicological laboratory bioassay

The standard pesticide bioassay procedures were conducted on the early fourth larval instar and the pupal stage of Cx. pipiens according to [28] Mohamed et al. [15]. Six to eight concentrations of each tested pesticide were freshly prepared using water. Three replicates were prepared for each concentration. Further, a set of ten early 4th instar larvae or pupae of Cx. pipiens, approximately of same size, were transferred to different plastic cups, which contain a 100 ml of appropriate concentrations of each tested pesticide. Controls were only contained tap water. Mixture of dry yeast and grinded bread were given as food for larvae. The bioassay was reserved under laboratory conditions at 25±2 °C and 60±5% relative humidity and 12:12 (light: dark) photoperiod. The experiments were repeated. Mortality was recorded after 24, 48, and 72-h of exposure to the tested concentrations of each pesticide for larvae and after 24 and 48-h exposure for pupae. Larvae or pupae were considered dead if they were unrestrained to the touching with a probe or if they could not reach the surface of the water.

Data analysis

\( \text{LC}_{50} \) and \( \text{LC}_{90} \) values and their corresponding 95% fiducial limits (FLs), and the slope values were estimated using probit analysis (SPSS Inc., Chicago, IL) and expressed in ppm (unit weight of active ingredient of pesticide). Toxicity index was calculated following [31], as Toxicity index— \[ (\text{LC}_{50} \text{ value of the most toxic tested pesticide} / \text{LC}_{50} \text{ value of the tested pesticide}) \times 100 \].
Table 1
Toxicity of selected ALS- and ACCase-inhibitor herbicides compared to deltamethrin insecticide on the 4th larval instar of Culex pipiens after 24-h exposure.

| Pesticides          | LC50 ± SE (ppm) (95% FL) | LC90 ± SE (ppm) (95% FL) | Slope ± SE | Toxicity indexb |
|---------------------|---------------------------|---------------------------|------------|-----------------|
| Herbicide groups    |                           |                           |            |                 |
| 1. ALS-inhibitors herbicides: |                  |                           |            |                 |
| Bispyribac-sodium   | 3.80 ± 0.025              | 8.82 ± 0.215              | 3.51 ± 0.074 | 0.526          |
| (2.37–6.33)         | (5.536–33.734)            |                           |            |                 |
| Penoxsulam          | 5.76 ± 0.139              | 15.47 ± 0.446             | 3.00 ± 0.160 | 0.347          |
| (4.47–7.44)         | (11.257–25.90)            |                           |            |                 |
| 2. ACCase-inhibitor herbicides: |                    |                           |            |                 |
| Haloxyfop-p-ethyl   | 21.71 ± 0.173             | 56.94 ± 0.124             | 3.06 ± 0.019 | 0.092          |
| (12.17–38.99)       | (33.48–308.018)           |                           |            |                 |
| Pinoxaden           | 0.427 ± 0.013             | 1.93 ± 0.141              | 1.96 ± 0.057 | 4.68           |
| (0.244–0.627)       | (1.231–4.598)             |                           |            |                 |
| Tralkoxydim         | 40.81 ± 0.357             | 97.57 ± 6.355             | 3.41 ± 0.220 | 0.049          |
| (32.67–51.14)       | (73.89–151.49)            |                           |            |                 |
| Insecticide         |                           |                           |            |                 |
| Pyrethroid group    |                           |                           |            |                 |
| Deltamethrin        | 0.020                     | 0.377                     | 1.08 ± 0.016 | 100            |
| (0.013–0.028)       | (0.263–0.617)             |                           |            |                 |

a FL: fiducial limits.
b Toxicity index = [(LC50 of the most toxic tested pesticide/LC50 of the tested pesticide) x 100].

Fig. 1. Time-fold increase in toxicity of the tested herbicides and deltamethrin insecticide on the early 4th instar larvae of Culex pipiens after 24, 48, and 72-h exposure.

Results

The potential toxicity of five selected herbicides, bispyribac-sodium, penoxsulam, haloxyfop-p-ethyl, pinoxaden, tralkoxydim and a pyrethroid insecticide deltamethrin was determined on the early 4th instar larvae of Cx. pipiens after 24, 48 and 72-h exposure in the laboratory (Tables 1–3).

Based on the estimated LC50 and LC90 values of deltamethrin and the selected herbicides, results showed that all tested pesticides induced varied toxicity against the early 4th instar larvae of Cx. pipiens, and the toxicity of all pesticides were enhanced gradually as exposure time increased particularly after 72-h exposure (Fig. 1).

Based on the toxicity index values for LC50 values of the tested pesticides (Tables 1–3), deltamethrin was the highest toxic compound on the early 4th instar Cx. pipiens larvae than pinoxaden, bispyribac-sodium, penoxsulam, haloxyfop-p-ethyl and tralkoxydim with 4.68, 0.526, 0.347, 0.092, and 0.049-fold after 24-h (Table 1) and with 2.17, 0.108, 0.133, 0.106 and 0.020-fold, respectively after 48-h exposure (Table 2) while deltamethrin was more effective than all tested herbicides except pinoxaden with values ranged from 0.174 to 0.016 (Table 3).

Toxicity of deltamethrin and the selected herbicides on pupae of Cx. pipiens after 24 and 48-h was listed in Tables 4 and 5. It is evident that deltamethrin insecticide was the highest toxic pesticide against Cx. pipiens pupae with LC50 values of 0.010, and 0.002 mg/l and LC90 values of 0.180, and 0.066 mg/l, respectively after 24 and 48-h exposure. Based on the estimated LC50 values, the toxicities of the tested pesticides on Cx. pipiens pupae were increased after 48-h exposure compared to 24-h data. For example, pinoxaden, deltamethrin and tralkoxydim were increased in toxicity to be 51.3, 5.0 and 1.3-fold after 48-h exposure compared to 24-h values (Fig. 2). The toxicity index values based on LC50 values of the tested pesticides against Cx. pipiens pupae showed that deltamethrin was more effective than pinoxaden, penoxsulam, bispyribac-sodium,
Table 2
Toxicity of selected ALS- and ACCase-inhibitor herbicides compared to deltamethrin insecticide on the 4th larval instar of Culex pipiens after 48-h exposure.

| Pesticides               | LC50 ± SE (ppm) (95% FL) | LC90 ± SE (ppm) (95% FL) | Slope ± SE | Toxicity indexb |
|--------------------------|--------------------------|--------------------------|------------|-----------------|
| Herbicide groups         |                          |                          |            |                 |
| 1. ALS-inhibitors herbicides: |                      |                          |            |                 |
| Bispyribac-sodium        | 1.86±0.166 (0.635–3.2455) | 6.06±0.071 (3.4225–81.346) | 2.50±0.162 | 0.108           |
| Penoxsulam               | 1.50±0.013 (0.601–2.383)  | 11.12±1.082 (7.286–45.123) | 1.48±0.066 | 0.133           |
| 2. ACCase-inhibitor herbicides: |                    |                          |            |                 |
| Haloxyfop-p-ethyl        | 12.31±0.876 (7.20–17.642) | 43.33 ± 2.265 (28.409–105.649) | 2.34 ± 0.035 | 0.016           |
| Pinoxaden                | 0.092±0.010 (0.0215–0.1535)| 0.455±0.082 (0.329–0.724) | 1.91±0.343 | 2.17            |
| Tralkoxydim              | 10.08 ± 0.726 (6.692–14.119)| 58.71 ± 6.519 (37.705–121.77)| 1.73±0.013 | 0.020           |
| Insecticide              |                          |                          |            |                 |
| Pyrethroid group         |                          |                          |            |                 |
| Deltamethrin             | 0.002 (0.0001–0.005)     | 0.105 (0.0635–0.216)     | 0.728±0.166 | 100             |

a FL: fiducial limits.
b Toxicity index = [(LC50 of the most toxic tested pesticide/LC50 of the tested pesticide) x 100].

Table 3
Toxicity of selected ALS- and ACCase-inhibitor herbicides compared to deltamethrin insecticide on the 4th larval instar of Culex pipiens after 72-h exposure.

| Pesticides               | LC50 ± SE (ppm) (95% FL) | LC90 ± SE (ppm) (95% FL) | Slope ± SE | Toxicity indexb |
|--------------------------|--------------------------|--------------------------|------------|-----------------|
| Herbicide groups         |                          |                          |            |                 |
| 1. ALS-inhibitors herbicides: |                      |                          |            |                 |
| Bispyribac-sodium        | 0.574±0.068 (0.1685–0.944) | 1.32±1.324 (2.0425–6.371) | 1.83±0.040 | 0.174           |
| Penoxsulam               | 0.581±0.170 (0.176–0.956) | 2.06±0.512 (1.402–2.664) | 2.31±0.083 | 0.172           |
| 2. ACCase-inhibitor herbicides: |                    |                          |            |                 |
| Haloxyfop-p-ethyl        | 6.34±0.821 (2.37–9.874)  | 13.63±13.292 (18.731–71.94) | 1.98±0.111 | 0.016           |
| Pinoxaden                | NC                       | 28.96±0.924 (21.364–45.891) | NC         | NC              |
| Tralkoxydim              | 4.13±0.068 (2.711–5.578) | 28.96±0.924 (21.364–45.891) | 1.52±0.012 | 0.024           |
| Insecticide              |                          |                          |            |                 |
| Pyrethroid group         |                          |                          |            |                 |
| Deltamethrin             | 0.001 (0.0001–0.0025)    | 0.032 (0.02–0.049)       | 0.905 ± 0.195 | 100             |

a FL: fiducial limits.
b Toxicity index = [(LC50 of the most toxic tested pesticide/LC50 of the tested pesticide) x 100].
c NC: not calculate; the mortality% was 100% at first five tested concentrations of six.

Fig. 2. Time-fold increase in toxicity of the tested herbicides and deltamethrin insecticide on the pupae of Culex pipiens after 24 and 48-h exposure.
Table 4
Toxicity of selected ALS- and ACCase-inhibitor herbicides compared to deltamethrin insecticide on the pupae of Culex pipiens after 24-h exposure.

| Pesticides                      | LC50 ± SE (ppm) (95% FL) | LC50 ± SE (ppm) (95% FL) | Slope ± SE | Toxicity indexa |
|---------------------------------|--------------------------|--------------------------|------------|----------------|
| **Herbicide groups**            |                          |                          |            |                |
| 1. ALS-inhibitors herbicides:   |                          |                          |            |                |
| Bispyribac-sodium               | 4.72±0.534 (3.736–6.293) | 10.25±0.268 (7.687–24.320) | 3.90±0.696 | 0.212          |
| Penoxsulam                      | 3.08±0.253 (1.84–4.587)  | 26.21±2.41 (15.278–67.267) | 1.39±0.113 | 0.325          |
| 2. ACCase-inhibitor herbicides: |                          |                          |            |                |
| Haloxyopyrop-ethyl              | 16.06±0.290 (10.384–26.003) | 35.06±0.788 (22.62–114.662) | 3.78±0.022 | 0.062          |
| Pinoxaden                       | 0.667 ± 0.028 (0.569 – 0.784) | 2.19±0.021 (1.765 – 2.907)  | 2.49±0.107 | 1.50           |
| Tralkoxydim                     | 52.09±3.70 (25.836–30.151) | 94.32±5.11 (40.073–51.727)  | 5.19±1.09  | 0.019          |
| **Insecticide**                 |                          |                          |            |                |
| Pyrethroid group                |                          |                          |            |                |
| Deltamethrin                    | 0.010 (0.000–0.027)      | 0.180 (0.072 – 3.980)     | 1.01 ± 0.007 | 100           |

a FL: fiducial limits.
b Toxicity index = [(LC50 of the most toxic tested pesticide/LC50 of the tested pesticide) x 100].

Table 5
Toxicity of selected ALS- and ACCase-inhibitor herbicides compared to deltamethrin insecticide on the pupae of Culex pipiens after 48-h exposure.

| Pesticides                      | LC50 ± SE (ppm) (95% FL) | LC50 ± SE (ppm) (95% FL) | Slope ± SE | Toxicity indexa |
|---------------------------------|--------------------------|--------------------------|------------|----------------|
| **Herbicide groups**            |                          |                          |            |                |
| 1. ALS-inhibitors herbicides:   |                          |                          |            |                |
| Bispyribac-sodium               | 2.71±0.172 (1.675–4.188) | 7.76±0.118 (4.882–25.311) | 2.18±0.210 | 0.074          |
| Penoxsulam                      | 0.786±0.128 (0.539–1.038) | 5.37±0.636 (4.278–7.1745) | 1.57±0.230 | 0.254          |
| 2. ACCase-inhibitor herbicides: |                          |                          |            |                |
| Haloxyopyrop-ethyl              | 7.51±0.435 (5.581–10.096) | 22.14±1.056 (15.382–41.439) | 2.75±0.269 | 0.027          |
| Pinoxaden                       | 0.013 ± 0.001 (0.004 – 0.024) | 0.098±0.025 (0.071–0.1315) | 1.52±0.144 | 15.39          |
| Tralkoxydim                     | 39.26±2.34 (7.491–360.054) | 87.99±12.97 (22.87–574.5) | 3.97±1.034 | 0.005          |
| **Insecticide**                 |                          |                          |            |                |
| Pyrethroid group                |                          |                          |            |                |
| Deltamethrin                    | 0.002 (0.000–0.085)      | 0.066 (0.022–0.4085)     | 0.832±0.016 | 100           |

a FL: fiducial limits.
b Toxicity index = [(LC50 of the most toxic tested pesticide/LC50 of the tested pesticide) x 100].

haloxyopyrop-ethyl and tralkoxydim with 1.50, 0.325, 0.212, 0.062, and 0.019-fold after 24-h (Table 4) and with 15.39, 0.254, 0.074, 0.027 and 0.005-fold, respectively after 48-h exposure, respectively (Table 5).

Discussion

The current results agreed with that of Mohamed et al. [15], when they evaluated the toxicity effects of deltamethrin and some selected herbicides on the fourth instar larvae of Cx. pipiens. Their findings indicated that deltamethrin was the most toxic pesticide on Cx. pipiens larvae with LC50 values of 0.0062, 0.0025, and 0.0019 mg/L post 24, 48, and 72-h exposure compared with the toxicity of the tested herbicides in the laboratory. Indeed, Saba et al. [23] indicated that thiamethoxam+ chlorantraniliprole insecticide was more effective on Cx. pipiens larvae than other tested herbicides. Deltamethrin also exhibited variable toxicity against different populations of Cx. tritaeniorhynchus Giles larvae with LC50 values ranged from 0.0058 to 4.12 mg/L after 24-h of treatment [30]. Deltamethrin exhibited a highly effective larvicide and adulticide against various mosquito species like Culex, Aedes and Anopheles [14,21]. Deltamethrin is counted as a highly toxic compound to range of various aquatic invertebrates including mosquitoes and water flea, Daphnia magna Straus as well as fish [21]. In the present study the highly efficacy of deltamethrin against larvae and pupae of Culex mosquito might due to the disruption action of the insecticide on sodium channels function of nervous system of the insect as reported by Haug and Hoffman [11].

In the present study, pinoxaden was the most toxic herbicide against the early fourth instar larvae and pupae of Cx. pipiens after exposure time (particularly after 72-h exposure for larvae) followed by penoxsulam and bispyribac-sodium.
which exhibited moderate toxicity against the target stages than haloxyfop-p-ethyl and tralkoxydim which were the least toxic compounds. Similar to our results, toxicity effects of some ACCase-inhibitors (i.e., fenoxaprop-p-ethyl, diclofop, clodinafop-p-pinoxaden, fluazifop-p-butyl and clethodim), ALS-inhibitors (mesosulfuron-methyl-iodosulfuron-methyl), amino acids synthesis-inhibitors (glufosinate-ammonium and glyphosate-isopropyl ammonium) and photosynthesis-inhibitor (bromoxynil octanoate) were detected on the larval stages of Cx. pipiens [15,23]; glyphosate and thiobencarb against three mosquito species (Anopheles quadrimaculatus Say, Cx. salinarius Coquillett, and Psorophora columbiae (Dyar & Knab) [12] and 2,4-D, pentachlorophenol (PCP) and butachlor against Cx. pipiens fatigans larvae [6]. Mohamed et al. [15] indicated that the ACCase-inhibitors, fenoxaprop-p-ethyl and fluazifop-p-butyl, were counted to be the most potent compounds against larvae of Cx. pipiens followed by clethodim while glyphosate was the least toxic herbicide compound. Saba et al. [23] found that mesosulfuron-methyl-iodosulfuron-methyl an ALS-inhibitor herbicides exhibited highly toxicity on the Cx. pipiens larvae than ACCase-inhibitor herbicides.

Furthermore, the toxicity of herbicides against insects including mosquito was varied based on different factors belonging to herbicides (i.e., herbicide type, group, mechanism of action and their chemical structure of formulations), insects (i.e., species, sex, age and stages) and time of exposure. In the present study, pupae were more sensitive to most tested pesticides than larvae after 24 and 48-h exposure time. In addition, the toxicity of all pesticides against larvae and pupae of mosquito was increased gradually as exposure time increased especially after 72-h exposure for larvae and 48-h exposure for pupae. Plentiful studies of Mohamed et al. [15], Saba et al. [23], and Ahmed and Saba [2] were in harmony with this study as they demonstrated that the efficacy of tested insecticides, herbicides and fungicides was increased drastically against mosquito larvae by increasing the time of exposure particularly after 48-h and 72-h exposure.

In this study, the toxicity of pinoxaden, haloxyfop-p-ethyl and tralkoxydim against larval and pupal stages of culex mosquito may attributed to the toxicity effects of these herbicides on ACCase enzyme and fatty acids biosynthesis in the insect particularly the target site of these herbicides are found in plants and animals [5,10]. Indeed, Jelenska et al. [13] demonstrated that the growth of Toxoplasma gondii endo-parasite have been inhibited in human blood using low doses of ACCase-inhibitor herbicides, as clodinafop-propargyl and haloxoyfop. The larval stage development and growth of Diatraea grandiosella (Walker) were disrupted by inhibiting the activity of ACCase enzyme that caused a disruption to lipid biosynthesis in both insect [9,19]. Furthermore, in this study both ALS-inhibitors, penoxsulam and bispyribac-sodium, exhibited toxicity on larvae and pupae of culex mosquitos, however the target site of both herbicides is not found in insects and other animals [23]. Probably, both herbicides might affect other enzymes or metabolic pathways in the insects.

For examples, Bispyribac-sodium and penoxsulam induced inhibitory effects on the activities of glutathione S-transferases (GSTs), catalase (CAT) and acetylcholinesterase (AChE) which is the primary target site of carbamate and or ganophosphate insecticides on fish [4,26]. In addition, both imazethapyr and imazapic (an ALS-inhibitor herbicides), and oxyfluorfen were found to reduce the activity of GSTs in fish [16,18].

In conclusion, based on our knowledge, this is the first report on the side toxicity effects of haloxyfop-p-ethyl and tralkoxydim (ACCase-inhibitor herbicides) and penoxsulam and bispyribac-sodium (ALS-inhibitor herbicides) on immature stages (larvae and pupae) of Cx. pipiens mosquitos in Egypt. Furthermore, larvae and pupae of Cx. pipiens mosquito can be used as a stander aquatic insect model like water flies for evaluating the side effects of herbicides on aquatic toxicological studies. Moreover, field and semi-field trails in rice fields were needed to illustrate the potentially of these herbicides as well as biochemical investigations should be conducted to determine the unclear mechanisms of many herbicides on mosquitos and other insects.

Declaration of Competing Interest

None.

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