RESIDUE BEHAVIOR OF THE TWO HERBICIDES, CINMETHYLIN AND ANILOFOS IN WATER AND FISH Oreochromis niloticus

El-Bouze, M. F. R.*, Hala R. Abdel - Rahman** and Gamila A.M. Kotab.***
* Pesticides Analysis Res. Division, Central Agric. Pesticide Lab. Agric. Res. Center, Dokki, Giza, Egypt
** Department of Economic Entomology and Pesticides, Faculty of Agric., Cairo Univ., Egypt
*** Mammalian Toxic. Department, Central Agric. Pesticide lab. Agric., Res. Center, Dokki, Giza, Egypt.

ABSTRACT

The present investigation was carried out to evaluate the residual behavior of sublethal concentrations of two herbicides, cinmethylin (0.74 and 1.47 ppm) and anilofos (0.98 and 1.96 ppm) on the Nile fish Oreochromis niloticus for 28 days and left for 28 days after withdrawal both herbicides as depuration period. Uptake and accumulation of the tested herbicides are of major concern for monitoring their residues in tissues of treated fish and water as well. Determination of cinmethylin and anilofos residues in fish tissues was mainly done as it is important point for human consumption. Cinmethylin and anilofos residues in water were gradually decreased throughout the experimental course. The percentage losses of cinmethylin residues at the end of depuration period reached 94.8% and 98.4% at 0.74 and 1.47 ppm, respectively. The residues level was undetected in fillet (meat) during the exposure period except at 28 days of treatment. A complete recovery of the fillet residues was obtained in fish at the depuration period for the two tested concentrations. While in brain, these residues were detected 3 days after treatment followed by gradually decrease until it reached 1.78 and 3.98 ppm at the two concentrations at the end of depuration period, respectively. Anilofos showed distinguish fast degradation in water compared with cinmethylin. Anilofos had residues of 0.96 and 1.93 ppm one hour after treatment using 0.98 and 1.96 ppm, respectively. After 28 days, anilofos residues reached 0.06 and 0.046 ppm for the same concentration, respectively. No detectable residues of anilofos were found in water during the depuration period. In fish brain and fillet, anilofos residues gradually increased through 0-time to 7 days for the two tested concentrations, respectively. Then, the residues decreased during the remaining period. Anilofos accumulation in fish showed that the amounts uptaked by fish were greater than 100% in both brain and fillet for both concentrations. These values obviously declined and reached 60.63% and 16.43% loss in fillet and 15.52% and 60.61% loss in brain 28 days after treatment when 0.98 and 1.96 ppm of anilofos were used. Anilofos residues showed the same trend of cinmethylin in fish fillet and brain during the depuration period.

Keywords: Herbicides, cinmethylin, anilofos, residue analysis, Nile fish Oreochromis niloticus.

INTRODUCTION

The unwise use of synthetic toxicants may create problems that can be more serious than those were originally recommended for application.

Adverse effect on non-target organisms is one of these problems. The effect of pesticides on fish generally increased especially in the last two decades. Water may be also contaminated by direct applications for control
aquatic weeds, undesired fish, aquatic insects, percolation and run-off from agricultural land and drift from aerial and land applications, discharge of industrial waste or from cleaning up equipment used for pesticides formulations. On the other hand, fish is an important part of aquatic system as non-target organisms, and so are one of the major sources of protein for human not only in Egypt but also worldwide. Moreover, fish are often at the top of the aquatic food chain and may concentrate large amounts of some pesticides, especially herbicides (Grande et al., 1994).

Thus, several researches had been conducted to evaluate this situation (Anderson and Apollonia, 1978; Lee et al., 86, 88,1990; Grayson et al., 1987; Ohyama et al., 1987; Assano et al., 1989; Woodward et al., 1989; El-Sheamy et al., 1991; Elezovic et al., 1994; Bayoumi et al., 1995; Begum and Vijayaraghavan 1995a and El-Dieb et al., 1996).

Therefore, the residues behavior of sublethal concentrations of the two herbicides cinmethylin (Argold 10%) and anilofos (Aniloguard 30%) was evaluated in water and Oreochromis niloticus, which is considered a representative of susceptible residents in Nile water and fish culture industry.

MATERIALS AND METHODS

A- Herbicides Used

1- Cinmethylin

Commercial name: Argold

Common name: Cinmethylin 10% EC

Chemical name: exo - (±)-1-methyl -4-(1-methyl ethyl)-2-[(2-methylphenyl) methoxy]-7-oxabicclo(2,2,1)heptan

2- Anilofos

Commercial name: Aniloguard

Common name: Anilofos 30% EC

Chemical name: 2- S-[N-4-chloro-N-isopropylcarbomethyl] O, O - dimethyl phosphorodithioate

B- Experimental animals

The present studies were performed on fresh water fish Oreochromis niloticus weighting 85±5 g each and 14.5 ± 0.4 cm in length. They were purchased from a commercial fish supplier (Fokk center farm, Kalubia) and transported to the laboratory in dechlorinated - oxygenated water to keep the fish alive. Nile tilapia O. niloticus were reared in aerated glass aquarium (50 x 50 x 50 cm and 100L capacity). The fish were acclimatized for two weeks under laboratory conditions in dechlorinated tap water. The physico - chemical properties of the water used were PH 7.5±0.07; temperature 27±0.5°C, total hardness 97.8 ±2.7 mg/L as CaCO3 dissolved oxygen 6.4 ± 0.24 mg/L; alkalinity 2.43±0.16 mg/L as CaCO3 and electrical conductivity 432.8±13.0 μmhs/cm.

Fish were fed once a day with a commercial dry pellets (25% protein) at a rate 2% of the body weight (Sprague, 1969). Aquariums were siphoning before the feeding day to remove any feces and unused food from the previous day. Following the two weeks of acclimation, the fish were
transferred randomly into experimental tanks till starting the exposure to tested herbicides.

C- Experimental protocol

Fish acute-toxicity tests (96 hr LC$_{50}$) according to the OECD Guidelines (1984) for Testing of Chemicals No.203 and estimated with Weil (1952) fish – prolonged toxicity test (28-day study) according to OECD (1992) for testing of chemicals No. 204.

Fish were exposed in water to different concentrations. These concentrations are fraction (1/6 96 hr – L C$_{50}$ as 0.74mg/L and 1/3 96 hr- LC$_{50}$ as 1.47 mg/L) of cinmethylin. For anilofos, these values were 1/6 96 hr LC$_{50}$ as 0.98 mg/L and 1/3 96 hr LC$_{50}$ as 1.96 mg/L.

D- Experimental procedures

D.1. Tissue sampling

Groups of five fish each from experimental and control were killed at the end of each period. Some internal organs i.e., muscles (fillet) and brain were collected and frozen at - 40°C and stored for the residue determination. Samples of either untreated or treated fish and water were taken at one hour and 1, 3, 7, 14, 21 and 28 days after treatment with cinmethylin and then after 28 days for the depuration period. The samples treated with anilofos were taken one hour and 1, 2, 3, 7, 14, 21 and 28 days for the depuration period.

D.2. Extraction procedure:

The extraction of cinmethylin and anilofos residues from water with a solvent mixture of hexane: acetone (1:1v/v), while they were extracted from fish tissues with methylenechlordhe according to the method of El-Sheamy et al. (1991).

D.3. Clean-up procedure

Plates of thin layer chromatography using silica gel GF 254, 0.25mm thickness were used for both identification and clean-up steps. The developing solvent system consisted of hexane: acetone (9:1v/v) according to the method of El-Sheamy et al. (1991). The Rf values for cinmethylin was 0.74 and 0.588 for anilofos.

D.4. Determination of residues

HPLC instrument fitted with UV detector, wavelength 264 nm and methanol as mobile phase was used. The retention time of the tested compounds was 2.18 min and 2.17 min for cinmethylin and anilofos, respectively.

D.5. Recovery percentage

The same technique of extraction and clean-up was used for recovery tests of samples of water and fish. A known amount of herbicide was added to each sample and then extracted, cleaned-up and determined by HPLC. The recovery percentages were 82.20% and 80.66% for cinmethylin and 84.5% and 81.7% for anilofos in water and fish tissues, respectively.

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RESULTS AND DISCUSSION

The behavior of the two herbicides cinmethylin (Argold 10% EC), and anilofos (Aniloguard 30% EC) were used at selected sublethal concentrations. The residual levels of cinmethylin and anilofos were also determined in water, fillet (meat) and brain of treated fish.
Data showed that the calculated value of acute toxicity (96 hr-LC50) for cinmethylin was 4.42 ppm. Therefore, the lowest and highest sublethal concentrations used were 0.74 and 1.47 ppm, respectively (which represent 1/3 and 1/6 LC50).

The amounts of cinmethylin equivalent residues in water, fillet, and brain during the uptake and depuration phase are summarized in Table 1. There was a general trend for cinmethylin residues in water where they were decreased from 0-time to 28 days after treatment with the tested concentrations 0.74 and 1.47 ppm. Cinmethylin residues decreased from 0.74 to 0.142 ppm with the low concentration and from 1.513 to 0.461 ppm with the higher one, respectively. Data also showed gradual degradation of cinmethylin at both concentrations in contaminated water. The percentage rates of loss after 28 days were 81.07% and 69.53% when 0.74 and 1.47 ppm were used, respectively. After the depuration period of 28 days, the residues reached 0.039 and 0.024 ppm, respectively (Table 1).

Concerning cinmethylin residues in fish tissues, data clearly showed that there were no detectable residues were found in fillet at both concentrations during the whole tested intervals 0, 1, 3, 7, 14 and 21 days in addition to the depuration period. While, the residues in tissue were 0.586 and 0.692 ppm for the two tested concentrations 0.74 and 1.47 ppm 28 days after treatment, respectively. The corresponding bioconcentration factor (BCF) for the above values was 4.0 and 2.0, respectively as shown in Table 1. On the other hand, the fillet residues were completely eliminated and reached about 100% in the exposed fish after the 28-day depuration period.

In addition, residue levels of cinmethylin in brain were not detectable after 0-time (one hour after treatment) and 1 day after treatment with the two concentrations. While, these residues were accumulated after 3 days where they were 36.99 and 48.92 ppm for the two concentrations, respectively and followed by a gradual decrease until the end of experiments. On contrast, the loss percentages revealed increasing values by the laps of time (from 7-28 days). The bio-concentration factors for the uptake phase range from 76 to 118 for 0.74 ppm and from 51 to 60 for the higher concentration 1.47 ppm, respectively.

During the depuration period, the residue levels in brain were 1.78 (as loss of 89.39%) and 3.98 ppm (as loss of 85.65%) when the two concentrations 0.74 and 1.47 ppm were used, respectively.
Generally, it can be concluded that there was significant decomposition of the herbicide cinmethylin over 28 days. Data showed that the decomposition rates were 81% and 70% for the tested concentrations 0.74 and 1.47 ppm, respectively (Table 1). This may be attributed to the hydrolytic stability of cinmethylin at 25°C and pH range of 3-11, and its stability in biotic aqueous
solutions under dark conditions. Similar results were obtained by Grayson et al. (1987).

The lack of total cinmethylin equivalent residues that were detected in fillet (meat) may support the conclusion that cinmethylin residues do not accumulate and are extensively metabolized in fish. The physicochemical properties of cinmethylin such as its water solubility (0.394 mg/L) and KOW n-octanol-water partition coefficient (630) suggested a relatively high bioconcentration potential in lipophilic tissues. Data also showed that the maximum bio-concentration factors of cinmethylin in brain averaged 118 and 40 when 0.74 and 1.47 ppm were used, respectively. These findings are in agreement with those obtained by Lee et al. (1990) who suggested that cinmethylin metabolized in the fish tissue via hydroxylation and oxidation reactions giving α-carboxy cinmethylin and 8-hydroxy α-carboxycinmethylin.

These conjugates have a higher lipid solubility than the free molecule as consistent with those observed in the rat (Lee et al., 1988 and Woodward et al., 1989). Moreover, Lee et al. (1986) stated that other cleavage reaction occurred with the parent molecule (cinmethylin) giving O-toluic acid, which subsequently conjugated with amino acid (glycine). This reaction predominated when test animals were under the high-dose treatment. As well as other cleavage occurred on the major hydroxylated/oxidized metabolic products to yield O-(hydroxymethyl) benzoic acid and phthalic acid which were subsequently with glucuronic acid.

Concerning the depuration of the tissue residues (fillet and brain), approximately 100% (for fillet) and 89% - 85% (for brain) were eliminated within 28 days as a depuration period when the exposed fish were placed in the uncontaminated water. It can be also concluded that the low toxicity and rapid and extensive degradation of cinmethylin, significant impact of cinmethylin to the aquatic environment is not anticipated. These findings are in agreement with those of Lee et al. (1990).

Concerning anilofos, data in Table 2 clearly showed that its residues in treated water showed a similar trend of cinmethylin. Anilofos showed a distinguish and fast degradation compared with cinmethylin. Its residues reached 0.98 and 1.96 ppm for the two tested concentrations 0.98 and 1.96 ppm just one hour after treatment, respectively. While these residues in water became 0.060 and 0.046 ppm for the same concentrations 0.98 and 1.96 ppm during the period from 28 days after treatment, respectively. During the depuration period of 28 days, data in table 2 clearly indicated that no detectable residues were found. Anilofos loss percentages in water clearly increased from 93.8% to 93.8% and from 21.24% to 97.5% for the tested concentrations 0.98 and 1.96 ppm during the period from 1 to 28 days after treatment, respectively (table 2). On the other hand, data obviously cleared that anilofos residues in fish meat (fillet) and brain gradually increased throughout the whole experiment. The accumulated residues of anilofos residues in brain were 11.79 and 19.28 ppm at 0-time (one hour after treatment) when the two tested concentrations 0.98 and 1.96 ppm were used, as shown in table 2, respectively. However, no
Table 1: Residue behavior of two concentrations of chlorothalonil in treated water and their bioconcentration in fish tissues Oreochromis niloticus.

<table>
<thead>
<tr>
<th>samples</th>
<th>Water</th>
<th>Fillet</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc.</td>
<td>0.74 ppm</td>
<td>1.47 ppm</td>
<td>0.74 ppm</td>
</tr>
<tr>
<td>Time interval (Days)</td>
<td>ppm</td>
<td>Loss %</td>
<td>ppm</td>
</tr>
<tr>
<td>1</td>
<td>0.719</td>
<td>4.13</td>
<td>1.283</td>
</tr>
<tr>
<td>3</td>
<td>0.671</td>
<td>23.67</td>
<td>0.562</td>
</tr>
<tr>
<td>7</td>
<td>0.356</td>
<td>52.27</td>
<td>0.883</td>
</tr>
<tr>
<td>14</td>
<td>0.237</td>
<td>63.50</td>
<td>0.891</td>
</tr>
<tr>
<td>21</td>
<td>0.264</td>
<td>64.80</td>
<td>0.870</td>
</tr>
<tr>
<td>28</td>
<td>0.142</td>
<td>81.07</td>
<td>0.461</td>
</tr>
<tr>
<td>Depuration for 28 days</td>
<td>0.038</td>
<td>94.8</td>
<td>0.024</td>
</tr>
</tbody>
</table>

0-time = One hour after treatment  
N.D. = Not detected (Residue less than 0.007 ppm)  
BCF = Bio-concentration factor obtained by dividing the tissue concentration by water concentration
Table 2: Residue behavior of two concentrations of asilofos in treated water and their bioconcentration in fish tissues *Oreochromis niloticus*.

<table>
<thead>
<tr>
<th>Samples</th>
<th>0.98 ppm</th>
<th>1.96 ppm</th>
<th>0.98 ppm</th>
<th>1.96 ppm</th>
<th>Fillet 1.96 ppm</th>
<th>BCF ppm</th>
<th>Loss%</th>
<th>BCF ppm</th>
<th>0.98 ppm</th>
<th>Brain 1.96 ppm</th>
<th>BCF ppm</th>
<th>0.98 ppm</th>
<th>1.96 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc.</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
<td>ppm</td>
</tr>
<tr>
<td>Time intervals (Days)</td>
<td></td>
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<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>0 Time</td>
<td>0.980</td>
<td>-</td>
<td>1.933</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
<td>N.D.</td>
<td>-</td>
<td>11.79</td>
<td>-</td>
<td>12.0</td>
<td>19.26</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>0.828</td>
<td>15.83</td>
<td>1.519</td>
<td>21.42</td>
<td>1.337</td>
<td>-</td>
<td>2.0</td>
<td>3.353</td>
<td>2.0</td>
<td>15.69</td>
<td>134.75</td>
<td>20.0</td>
<td>112.4</td>
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<tr>
<td>2</td>
<td>0.853</td>
<td>16.67</td>
<td>0.886</td>
<td>54.22</td>
<td>2.245</td>
<td>187.80</td>
<td>3.0</td>
<td>4.349</td>
<td>129.70</td>
<td>5.0</td>
<td>18.13</td>
<td>153.77</td>
<td>23.0</td>
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<tr>
<td>3</td>
<td>0.886</td>
<td>28.64</td>
<td>0.414</td>
<td>57.68</td>
<td>2.282</td>
<td>169.45</td>
<td>3.0</td>
<td>4.934</td>
<td>147.15</td>
<td>8.0</td>
<td>19.08</td>
<td>167.83</td>
<td>28.0</td>
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<tr>
<td>7</td>
<td>0.892</td>
<td>27.71</td>
<td>0.762</td>
<td>60.58</td>
<td>3.652</td>
<td>275.15</td>
<td>54.6</td>
<td>4.949</td>
<td>147.51</td>
<td>7.0</td>
<td>14.30</td>
<td>121.29</td>
<td>55.0</td>
</tr>
<tr>
<td>14</td>
<td>0.267</td>
<td>88.85</td>
<td>0.262</td>
<td>86.45</td>
<td>2.714</td>
<td>232.98</td>
<td>25.6</td>
<td>3.594</td>
<td>104.50</td>
<td>13.0</td>
<td>10.66</td>
<td>6.58</td>
<td>97.0</td>
</tr>
<tr>
<td>21</td>
<td>0.291</td>
<td>90.52</td>
<td>0.132</td>
<td>93.17</td>
<td>1.091</td>
<td>186.40</td>
<td>32.2</td>
<td>3.459</td>
<td>104.25</td>
<td>25.0</td>
<td>10.40</td>
<td>11.79</td>
<td>108.0</td>
</tr>
<tr>
<td>28</td>
<td>0.800</td>
<td>95.75</td>
<td>0.046</td>
<td>97.62</td>
<td>0.259</td>
<td>80.63</td>
<td>4.9</td>
<td>2.852</td>
<td>16.43</td>
<td>81.0</td>
<td>8.960</td>
<td>15.62</td>
<td>179.0</td>
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<tr>
<td>Preparation for 28 Days</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

N.D. = Not detected (Residue less than 0.007 ppm)

BCF = Bio-concentration factor obtained by dividing the tissue concentration by water concentration.
accumulated residues were detected in fillet one hour after treatment. The decomposition of anilofos showed different pattern. Data listed in table 2 showed that the loss percentages of residues uptake in fish reached about 100% and then declined to 80.6% and 16.0% for 0.98ppm in fillet, while in brain they were 15.52% and 69.61% for 1.96ppm, respectively. During 28 days of depuration period, anilofos residues averaged 0.015ppm in fillet and 1.458ppm in brain, which presented loss percentages of 94.21% and 85.36% when a concentration of 0.98ppm was used, respectively. These residues were 0.069ppm in fillet and 0.592ppm in brain presenting 96.82% and 89.9% loss using 1.96ppm, respectively.

As for the bio-concentration factor (BCF), data in table 2 clearly showed that the uptake phase ranged from 2.0 to 4.0 and from 2.0 to 61.0 in fish fillet exposed to .98 and 1.96ppm of anilofos during 28 days. These values ranged from 20.0 to 178.0 and from 14.0 to 127 in brain, respectively. From the previous results, it can be concluded that the herbicide anilofos had a rapid and high degradation value in water. That may be due to the low level of persistence of anilofos as an organophosphorus compound. Similar results were obtained by EL-Sheamy et al. (1991), Bayoumi et al. (1995) and Dogheim et al. (1995). Generally, the high residues of anilofos in fish tissues may attribute to its rapid penetration and binding of its residues in tissues (EL-Sheamy et al., 1991). Abo Arab et al. (1999) also reported that OP products such as anilofos were found with the fat phase in fish and that may be due to their physico-chemical properties (lipophilic compounds). Moreover, Holden (1962) found that gills in fish heads contained a high amount of pollutants since they are the main entrance, after which pollutant transportation to the natural tissues took place directly via the vascular system.

From the previous results, it can be noticed that the general features of these experiments show that the two tested herbicides had different fate in water and fish tissues. As the data show that cinmethylin didn’t accumulate in fillet tissues in which BCF values were 4 and 2 at the two levels of exposure. In the same time, the BCF values in brain reached to the maximum at 28 days after treatment, which reached to 118 and 60 for the two tested concentrations, respectively. However, in case of anilofos it was noticed that the BCF values were gradually increased and reached to 4 and 61 in fillet and 178 and 127 in brain tissues at the two levels of treatment, respectively. Generally, the residue levels for both chemicals were higher in brain tissues than fillet and this may be due to the nature of each tissue in relation to the lipophilic affinity for the tested herbicides. Moreover, it is of great interest that cinmethylin residues in fillet tissues were completely disappeared under the experimental analysis and level of detection at the end of depuration period.

Accordingly, it is not recommended to eat fish heads where a high percentage of toxicants can be accumulated.
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دراسات في تقدير سلوكيات ميدي الحشائش سيميتايلين و آنيلوغوس في
سمك البلطي النيلي
محمود فهيمي رياضي البيوض* ، هالة نoha عبد الرحمن صادق **
جميلة أحمد محمد قطب ***
قسم محوّلات المبيدات ـ المعمل المركزي للمبيدات مركز البحوث الزراعية الدفيئ- جزيرة- مصر
قسم الحشرات الاقتصادية والمبيدات ـ كلية الزراعة- جامعة القاهرة
قسم مبيدات الأحياء المائية ـ المعمل المركزي للمبيدات مركز البحوث الزراعية- الدفيئ- جزيرة- مصر

تم في هذا البحث دراسة سلوكيات التركيزات تحت السمية لميدي السيميتايلين وآنيلوغوس في سمك البلطي النيلي Oreochromis niloticus، وجزء من السمية في الملوس أو آنيلوغوس وفي الماء. تم استخدام فصيلة الأسماك السماكة الذرة لمدة 28 يوم يبدأ هذا التفاعل في مياه ملوثة بالبياد لمدة 28 يوم أخرى، حيث تم تكرار التفاعلات على التكتيكات المستخدمة والتراثية بها في الأسماك لتحيي درجة واسعة من المنتجات في الأسماك، وفي أنسجة الأسماك (العضلات) والمادة وقد وجدت النتائج التالية:

أولاً: سمية السيميتايلين: كانت نسبة الميتاتي من ميدي السيميتايلين في الماء تخضع تدركيًا أثناء فترة التفاعل حيث وصلت إلى 100% في الظروف الساكنة، و100% خلال فترة التفاعلات في المياه السماكة. في نهاية فترة التفاعلات لم تكن وجود أي متكسات للسماكة حيث وصلت نسبة الفقد إلى 100% لكل من التركيزات المستخدمين. أما فيما يتعلق بمدى المتغيرات من هذا الفصيلة في ميدان الأسماك المريحة فإنها تلاحظ أحيانًا بعد ساعة وذلك بعد 1 يوم من بداية التفاعلات بينما بدأت في الظهور في بداية من اليوم الأول حيث 48%، 42%، 37%، 33%، 29%، 25%، 21%، 17%، 13%، 9% وكما أن نسبة الأحياء المائية قد تصل إلى 27.23%، 45.29%، 63.36%، 71.43%، 79.50%، 87.56%، 95.62%، 100% للتركيزات المستخدمين.

ثانيًا: سمية آنيليغوس كان لمتكسات هذا السمك السماكي في السماكة، وكانت نسبة الفقد في السماكة 97.05% عند التركيز 98%، 96% عند التركيز 96% في الماك و 94% عند التركيز 94% في الماء. كما وجدت نسبة الفقد 100% عند التركيز 100% في المياه، وتلاحظ نسبة الفقد 100% عند تركيز 98%، 92% عند تركيز 92%، ونسبة الفقد 96% عند تركيز 96% في الماء. ونسبة الفقد 100% عند تركيز 100% في الماء. ونسبة الفقد 97% عند تركيز 97%، ونسبة الفقد 100% عند تركيز 100% في الماء. ونسبة الفقد 93% عند تركيز 93%، ونسبة الفقد 100% عند تركيز 100% في الماء. ونسبة الفقد 94% عند تركيز 94%, 95% عند تركيز 95%, ونسبة الفقد 100% عند التركيز 100%. ومعظم الأسماك على التفاعلات.

وبعد فحص عصات الأسماك أمان للإستخدام الأدمى والأسماك غير آمان للمستهلكين.