Analysis on the Comparative Neurotoxicity of Parathion and Chlorpyrifos from the Animal Model

Yue Yin [1, *, †], Yuhong Zhang [2, †]

[1] School of Changwai Bilingual School, Changzhou, China

[2] Department of Clinical Medicine, Dalian Medical University, Dalian, China

* Corresponding author: 3100601109@caa.edu.cn

†These authors contributed equally to this work.

Abstract. Recently there are many different types of the researches on organophosphates. The results on neurotoxicity can make people find out the treatments on organophosphate-poisoning. The parathion and chlorpyrifos are two typical types of organophates to be examples of the researches. The aim of researches is to find out the neurotoxicity of organophosphates to the mammalian animals. In vitro model, the aggregating cell culture of fetal rat telencephalon, has been used to investigate the different factors of neurotoxicity. The results show that the inhibition of parathion to acetylcholinesterase (AChE) is more effective than the chlorpyrifos. It shows that 2qthe in immature culture, the effect of parathion tends to be more effective. The cell culture treated by parathion is more sensitive than the neuro-specific enzyme activities. On the contrast, the chlorpyrifos have week induced reaction and are only effective on the influenced concentration. In general, the immature culture is more sensitive to OPs treatment than the differentiated culture. Moreover, the general cytotoxicity of chlorpyrifos and parathion are both low. The results show that there are many differences between neurotoxicity of parathion and chlorpyrifos. By taking look of the differences, the separative treatment can be found in the future.

Keywords: Neurotoxicity, parathion, chlorpyrifos.

1. Introduction

Two common examples of the diethyl phosphorothioate pesticides are chlorpyrifos and parathion. Chlorpyrifos is a pesticide with a moderate acute toxicity, which is world-widely used. Parathion is a broad-spectrum highly toxic insecticide, which has acaricidal effect. They are both types of the organophosphates (OPs). As they are widely used, it is very important to figure out the toxicity of parathion on the mammalian animals. For the similar use of pesticides, the OPs show the similar toxic mechanism to each other, and the parathion and chlorpyrifos are also involved. The main idea is to denature the acetylcholinesterase (AChE) and cause the accumulation of acetylcholine in target tissues. Then, the activity of acetylcholinergic receptor will process a dysregulation. After that, the disorder of function of the AChE receptor organs occurs. The process of denaturation can be separated into two parts. Firstly, the organic phosphorus part binds reversibly to AChE. Secondly, the phosphate part binds irreversibly to AChE to produce the phosphorylate AChE. Once the irreversible binding occurs, the AChE will not generate, so the receptor organs must synthesize the new enzyme. The synthesis of new enzymes takes a long time, so that the toxicity process occurs. There are many factors that affect the separated neurotoxicity of parathion or chlorpyrifos, such as the intervention of glial cells. The glial cells are type of neuron cells. In the following investigation, the glial cells that were used are astrocytes. The recent studies focus on the neurons more and there are only few studies on glial cells, so that in order to find out the effects of invention of glial cells on the neurotoxicity of parathion and chlorpyrifos and the influences on the activity of glial cells of the neurotoxicity of parathion and chlorpyrifos, the glial cells are involved in the investigations.

To explain the neurotoxicity and compare the differences of parathion and chlorpyrifos, this article is going to focus on the effect of the inhibition of AChE, the maturation-dependent factors and the cytotoxicity on the neurotoxicity of these two OPs. The involvement of the glial cells in the mechanism of the neurotoxic effects is also under discussion. Cytotoxicity refers to cell death,

cytolysis and inhibition of cell growth caused by products, materials and their extracts. There are two types of cytotoxicity, one is the selective cytotoxicity, the other is the general cytotoxicity. The cytotoxicity below is the general toxicity, which means an adverse reaction caused by the action of a chemical substance on basic cellular structures and physiological processes, such as cellular metabolic processes, synthesis, degradation or release of cellular components or products and the ion regulation and cell division resulting in disruption of cell proliferation and function on all the cells. The maturation-dependent factors mean that the inhibition of AChE of parathion and chlorpyrifos show differences between the immature cultures and the differentiated culture.

2. Comparative Neurotoxicity of Parathion and Chlorpyrifos

2.1. Neurotoxicity of CPF (chlorpyrifos)

2.1.1. General cytotoxicity to nerve cell

CPF does not significantly affect total protein content in immature and differentiated aggregating brain cell cultures according to the Fig. 1, which implies that the general cytotoxicity of CPF is low.

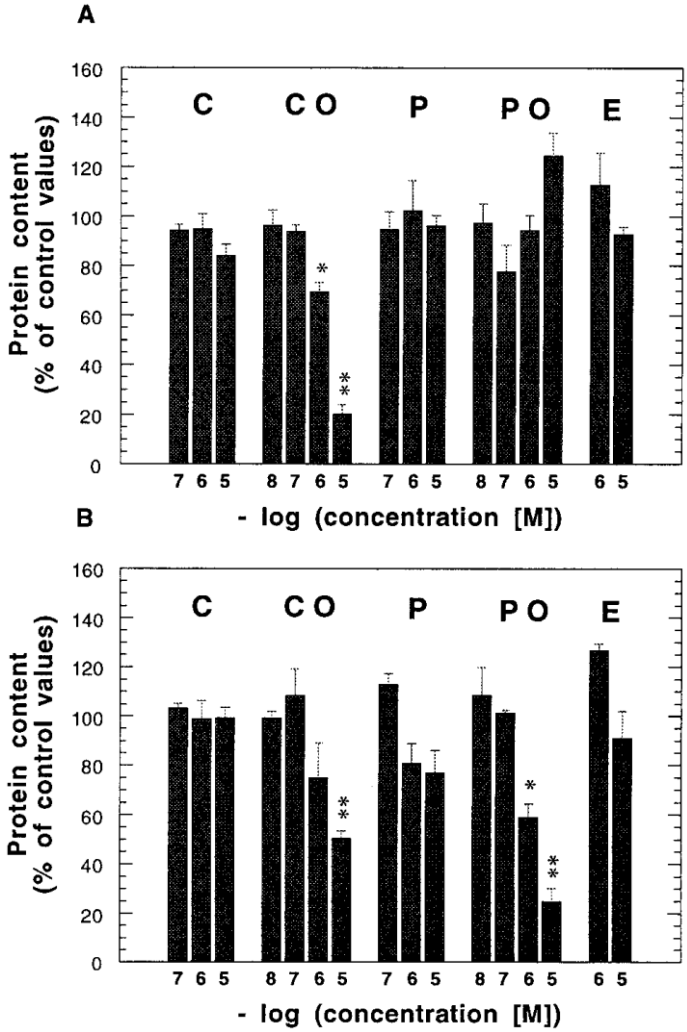

Figure 1. Total protein concentrations were measured in immature (A) and differentiated aggregated brain cell cultures (B) that had been treated for 10 days before to DIV 15 and DIV 35 collection, respectively. The percentages are presented as a proportion of untreated controls. The untreated controls of DIV 15 and 35 had the following results (equal to 100%): 8.260.19 mg/culture flask and 9.740.54 mg/culture flask, respectively [1].

2.1.2. Chlorpyrifos causes changes in the activity of various neurotransmitters and its receptors and associated esterases.

CPF significantly inhibits activity of AChE in rat telencephalon cells [1]. Combined with the experimental result on mice [2], in which mice have typical M-like and N-like symptom, suggest that CPF has an effect on the degradation of acetylcholine, and induce neurological disorders. In addition to the result above, after administered CPF [10 mg / (kg d)] to hens for 4 d-20 d, achieved 58%-70% and 49%-80% inhibition for AChE and BuChE. NTE was inhibited by 18% at 10 d and was more obvious at 20 d, but still did not reach the threshold for triggering OPIDN. Continue dosing until 60 d, the activity of AChE and BuChE was significantly inhibited, while the activity of NTE was not significantly altered [3]. It suggested that the toxic effect of CPF on neurotransmitter related enzymes was mainly acute toxicity (Fig. 2).

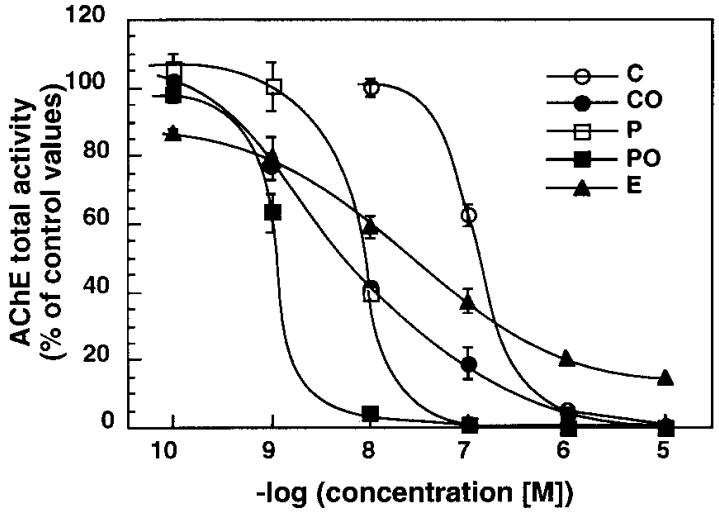

Figure 2. Acetylcholinesterase was suppressed by chlorpyrifos, parathion and its oxon derivatives, and eserine. Immature aggregated brain cell cultures were handled continually for 10 days, from DIV 5 to DIV 15, and then harvested. The inhibition curves were fitted using Bezier curves [1]

2.1.3. Affect glial cell development

According to Garcia [4], CPF has specific effects on glial cells. Subcutaneously administered CPF to pregnant dams and neonatal rats at three periods of development. Postnatally born mice have subnormal levels of MBP, NF68, and NF200, indicating defective myelination and disrupted axon formation. After treatment, GFAP have subnormal level first but higher than normal level second. GFAP reduction suggests a delay in the differentiation of astrocytes and / or a reduction in mature astrocytes. Elevation of GFAP, in turn, is a hallmark of gliosis that occurs in response to neural cell injury [5,6]. The above results suggested that glial cells were damaged by CPF.

2.2. Neurotoxicity of parathion

2.2.1. General cytotoxicity of parathion

From the researches, the general cytotoxicity of parathion is studied using FG-9307, a cell line obtained from a gill of the flounder, Paralichthys olivaceous (Hongyan Li, Shicui Zhang, 2001).

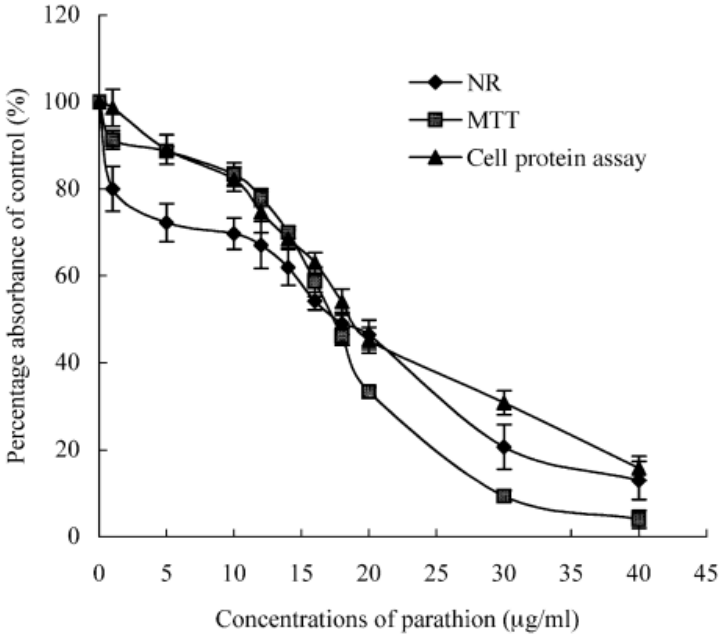

Figure 3. Parathion's in vitro cytotoxicity to FG cells as assessed by the NR test, MTT assay, and cell protein test. The arithmetic average percentage of controlS.E. is used to express the individual data points [7].

In Fig. 3, the cytotoxic findings are displayed. Regardless of the hazardous endpoints used, cytotoxicity was comparable across all systems. The FG-9307 cells were toxic to parathion at the lowest tested concentration (1 mg/ml), and toxicity increased when parathion concentration was gradually raised. Under a light microscope, no obvious alterations in the exterior appearance and attachment characteristics of the examined cells were found.

2.2.2. Parathion causes the inhibition of AChE

The dose-response curves were used to determine the IC50 value, or the concentration of parathion that results in 50% inhibition of cytotoxicity parameters after 48 hours (Fig. 3). For the NR, MTT, and cell protein tests, the IC50 values were 17.7, 17.4, and 19.0 mg/ml, respectively.

It is known that the parathion can cause the inhibition of the AChE and cause the neurotoxicity. The recent researches show that the involvement of menadione can cause the toxicity of AChE.

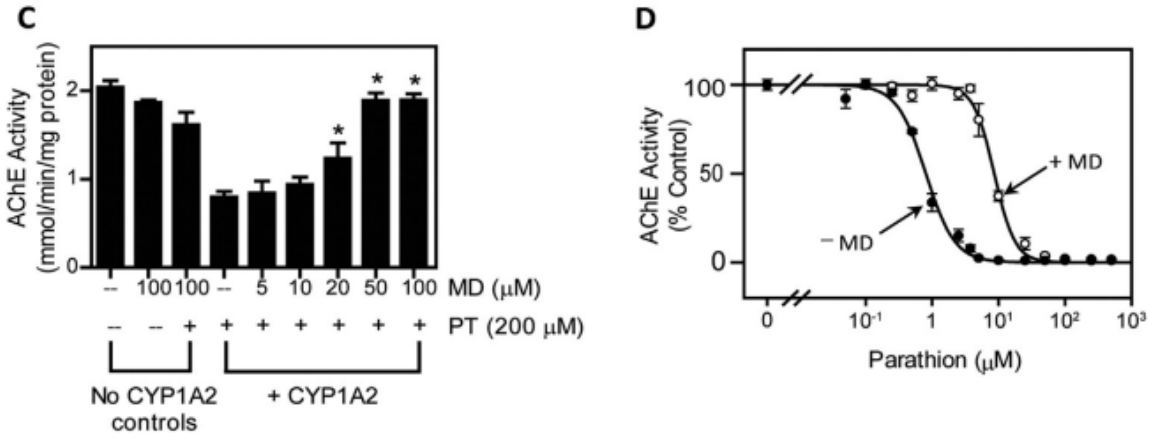

Figure 4. Menadione's effects on acetylcholinesterase inhibition [8]

HPLC was used to evaluate paraoxon in reaction mixtures at the appropriate periods. Menadione did not impede the metabolism of parathion to have an inhibiton on AChE in the control reactions. Parathion (200 M), a NADPH generation system, and the specified amounts of menadione were incubated with CYP1A2. AChE activity was determined using the Ellman assay after 10 minutes at 37°C with the addition of AChE (80 ng/ml), acetylthiocholine (1 mM), and 5,5′-dithiobis (2-

nitrobenzoic acid) (0.33 mM) to the reaction mixture. The results are mean SE (n=3) and significantly different from the mice (p b 0.05) that significantly being fed with CYP1A2. The effect of methadone on the inhibition of AChE by the parathion and its oxon derivatives produced by rat liver microsomes is depicted in Figure D. Rat liver microsomes (0.5 mg/ml) were treated with parathion (100 M) in the absence or presence of methylnaphthalenone. At 37°C, the process was initiated in the presence of NADPH (100 M) and a NADPH regeneration device. The reaction mixture was treated with acetylcholinesterase after 1 hour, and AChE activity was measured using the Ellman test [8].

From Fig.4, it is found that me naphthoquinone decreased AChE inhibition following parathion activation by rat liver microsomes while also increasing IC50 by a factor of 10 in microsomal culture mixes containing me naphthoquinone. CYP1A2-mediated parathion activation and AChE inhibition are completely reversed by menaphone. These findings show that menadione can impede CYP-mediated parathion metabolism, reducing paraoxon's ability to inhibit AChE.

2.2.3. Effect of involvement of glial cells on neurotoxicity of parathion

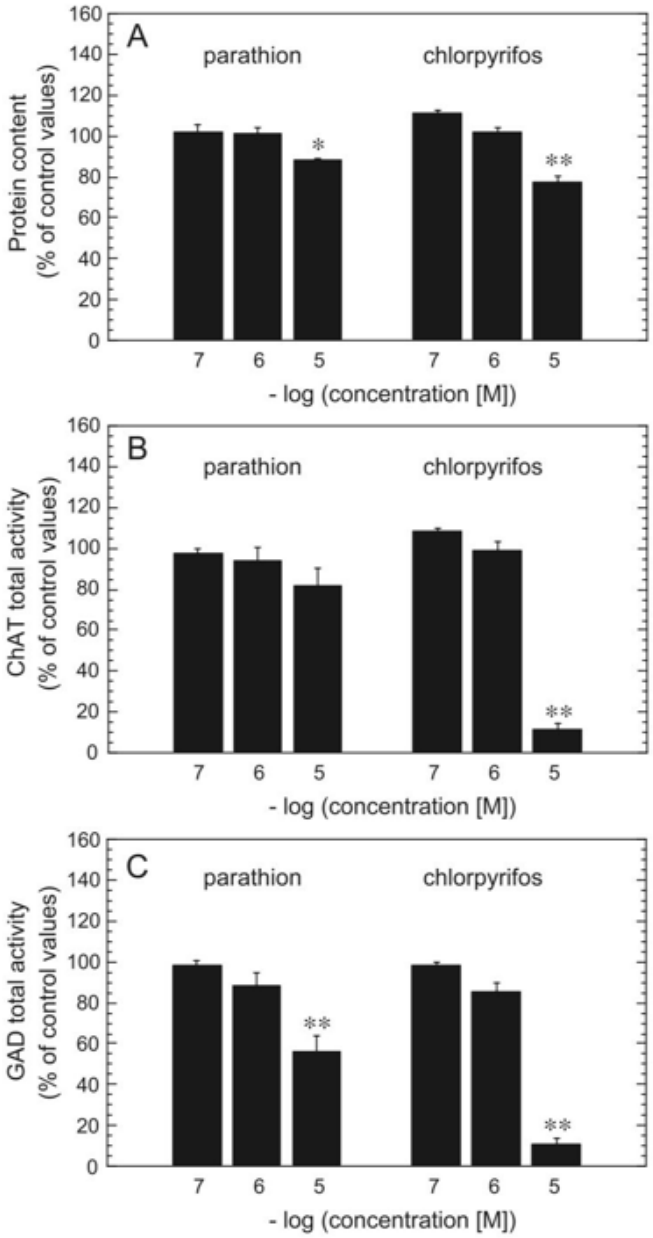

Figure 5. Parathion and chlorpyrifos' neurotoxic effects on neuron-enriched aggregating cultures [9]

From this investigation, we can find that parathion was more potent in the induction of astrogliosis. And the results also show that the glial provide protection against OPs toxicity, and parathion has lower ability to maintain its toxicity.

3. Comparative Neurotoxicity of Parathion and Chlorpyrifos

3.1. The comparative general cytotoxicity to the immature brain cell aggregating culture and the differentiated culture

From Fig. 5 above, it is found that both the chlorpyrifos and parathion have the low general cytotoxicity to the aggregating brain cell cultures. And it is also found that the cytotoxicity of parathion is time-dependent in the differentiated culture. As time went by, the toxicity become larger. Moreover, the oxon derivatives of the chlorpyrifos and parathion cause the larger cytotoxicity than the parent compounds. With time past, the oxygen analogs of chlorpyrifos shows the high time-dependent and strong cytotoxicity to both the immature culture and the differentiated culture. However, the paraoxon shows the lower cytotoxicity in immature culture than in differentiated. It shows that the general cytotoxicity of the parathion is maturation-dependent. In general, the general cytotoxicity of paraoxon is lower than chlorpyrifo-oxon. It means that the ability of the oxon derivatives of parathion and chlorpyrifos to kill cells directly without the mechanism of the inhibition of AChE is better than the parent compounds parathion and chlorpyrifos.

3.2. The inhibition of AChE of parathion and chlorpyrifos

According to Monet-Tschudi's researches, the parathion and chlorpyrifos and their oxon derivatives can be directly put in the brain cell cultures of rat to cause the inhibition of AChE. It means that the brain cells have cytochrome P450 activity to cause the formation of the AChE inhibition. From Fig. 2, it is found that in the immature culture, the parathion causes better inhibition of AChE than the chlorpyrifos. According to Monet-Tschudi, in the differentiated culture, the chlorpyrifos is better inhibitor than parathion. The oxon derivatives of parathion and chlorpyrifos are better inhibitors than the parent compounds. The inhibition of parathion and its oxon derivatives tends to be more effective in immature cultures. However, the chlorpyrifos and its oxon derivatives are proved to be not maturation-dependent.

3.3. Involvement of glial cells in the neurotoxicity of parathion and chlorpyrifos

According to current in vivo and in vitro research, OPs may stimulate glial cells in addition to neurons. So, in order to evaluate the effect of parathion on neurons, we analyze the effect of parathion on glial cells. In an earlier in vitro study [10], it was discovered that parathion was less damaging to neurons than chlorpyrifos, so that this time, the chlorpyrifos is chosen to be the comparison OP to be involved into the investigation on the neurotoxicity of Op. Scientists use the cell cultures of the rat brain and use different analyses such as the immunostaining and the biochemical analyses. The cell is aggregating in the different ways. There is the mixed-cell aggregating and the neuron-enriched aggregating. The mixed one has the spherical structures composed of all the different types of the neural cells but the neuron-enriched one only has the neurons. The differences are set to make the results more obvious. The results analysis is shown together.

Depending on the antibodies employed, cultures were treated in one of two methods for immunocytochemical studies [9]. The immunostaining also has two parts, the astrocytes and the neurons, to make a comparison. For the biochemical analyses, the scientists measured the activity of choline acetyltransferase (ChAT), glutamic acid decarboxylase (GAD), glutamine synthetase (GS) and intracellular lactate dehydrogenase (LDH) and the protein content in homogenates for analysing the influences of parathion and chlorpyrifos on the cell structure.

Fig. 6 shows the outcomes of the biochemical studies. Both chlorpyrifos and parathion had no effect on the amount of total protein at the conclusion of the trial. ChAT and GAD activities are not impacted by parathion in any way (Fig. 6.B, C).

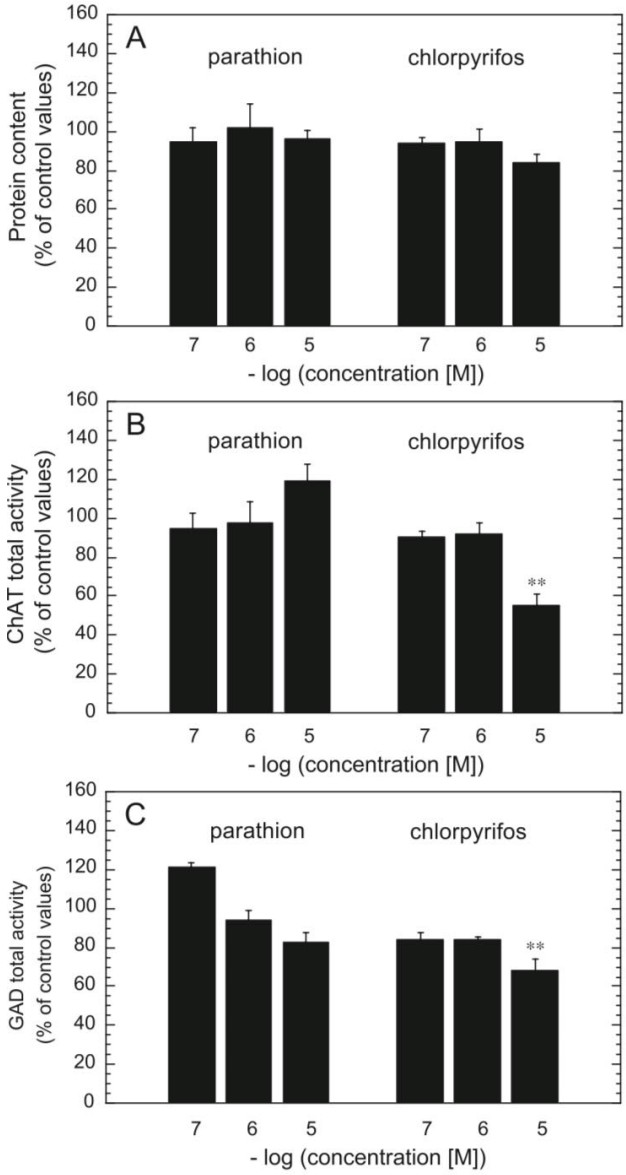

Figure 6. On mixed-cell aggre-gating cultures, parathion and chlorpyrifos's neurotoxic effects [9]

Parathion increased GFAP immunostaining in a concentration-dependent manner and enlarged astrocytic processes, both of which are signs of reactive gliosis. Parathion also reduced MAP-2 immunostaining. Again, after parathion treatment, neuros with strong MAP-2 staining displayed thicker process.

The neuron-enriched aggregate cultures are utilized to investigate whether neuronal sensitivity to OPs is affected in the absence of glial cells.

As can be seen from Fig. 6, both OPs, but especially parathion, displayed greater neurotoxicity when comparing to neuron-glial mixed-cell cultures when glial cells were removed.

4. Conclusion

These investigations can help give the deeper understanding of the neurotoxicity of parathion and chlorpyrifos. From the comparation of the inhibition of AChE of the parathion and chlorpyrifos and the toxic effect, it is found that the oxon derivatives of the parathion and chlorpyrifos have stronger toxic effect and stronger inhibition of AChE. However, it also shows that although the parathion is a stronger inhibitor of AChE. From the results it is found that the high inhibition of AChE does not mean the high neurotoxicity. It means that the future researches can focus on the study on the relationship of the inhibition of AChE of OPs and the neurotoxicity effects. In addition, it is proved

that the glial cells can to some extent protect the neurons from the neurotoxicity effects of the parathion and chlorpyrifos. It means that the scientists can have more deeper studies on the mammalian animals' neuro systems' self-protection on OPs. In addition, the general cytotoxicity of parathion and chlorpyrifos can be compared with their neurotoxicity effects caused by their inhibition to the AChE. The parathion is the better inhibitor to the AChE. However, the chlorpyrifos-oxons have the stronger general cytotoxicity. This gives scientists the idea of investigating the slight differences between different mechanisms of neurotoxicity of different parent OPs. It also states there are differences between the mechanism of neurotoxicity of the parent compounds and the oxon derivatives. The maturation-dependent general cytotoxicity and inhibition of AChE of parathion and the not maturation-dependent inhibition of AChE of chlorpyrifos shows that the maturation-dependent factors of neurotoxicity of parathion and chlorpyrifos are different. From this, it can be inferred that the maturation-dependent factors are different in different OPs. These findings are helpful for the scientists to give different treatment to mammalian animals' different OPs poisoning. The invention of the future drugs and treatments can take advantages of results of the investigations. These researches provide ideas on how to use these pesticides better in agriculture and reduce the harms to human.

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