Food and Chemical Toxicology 130 (2019) 154–160

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Neurobehavioral toxicity of triclosan in mice

Shaghayegh Alizadeh Tabari[a], Mahsa Loran Esfahani[a], Seyed Mohammad Hosseini[b], Atena Rahimi[c,*]

[a] Student Research Committee, Babol University of Medical Sciences, Babol, Iran
[b] Department of Pathology, Islamic Azad University, Babol Branch, Babol, Iran
[c] Cellular and Molecular Biology Research Center, Health Research Institute, Babol University of Medical Sciences, Babol, Iran

ARTICLE INFO

Keywords:
Triclosan
In vivo
Neurotoxicity
Brain
Behavioral tests

ABSTRACT

Triclosan (TCS) is a broad-spectrum antibacterial compound which is used in many cosmetic products, medical devices and house hold products. Toxicity attributed to TCS has recently become a focus of research. Recent studies showed that TCS can easily migrate into the human brain and animal tissues and cause adverse changes in various target organs. Our knowledge of the neurotoxicity of TCS is largely based on very limited data. In this regard, adult male NMRI mice were administered TCS (1000, 2000, and 4000 mg/kg) by gavage for 14 consecutive days, whereas the control animals were given corn oil. At the end of the exposure, all mice were evaluated for locomotor activity, motor coordination and anxiety behaviors through the use of an open-field test, rotarod test, and elevated-plus maze (EPM) test, and for muscle strength in a grip strength test. A significant change in locomotor activity and coordination was observed in TCS treated mice. In parallel anxiety-like behaviors and muscle strength were affected by TCS. Haematoxylin-eosin staining also showed significant adverse effects in brain tissue of the TCS exposed mice. Based on these results, we conclude that a 14-day TCS exposure resulting in some behavioral disturbances in mice.

1. Introduction

Triclosan [5-Chloro-2-(2,4-dichlorophenoxy)phenol; TCS] also known as Irgasan, is an antimicrobial agent that has been added to many personal care products such as toothpastes, hand soaps, mouthwashes, shampoos, deodorants, and cosmetics since the 1960s (Cherednichenko et al., 2012; Dayan, 2007). It is also found in numerous consumer products, including toys, bedding, fabrics, clothes, cleaning supplies, detergent and medical devices (Rodricks et al., 2010). TCS imposes a destructive impact on wide range of organisms, including bacteria, plants, fish, birds, protozoa and mammals with aquatic ecosystems being among the most sensitive to its deleterious effects (Yueh and Tukey, 2016). Bioaccumulation of this compound and its lipophilic metabolite, methyl-triclosan (M-TCS) in algae and snails and their relative stability are growing concerns about the harmful and negative effects it can cause on the environment (Coogan et al., 2007).

Many studies have been published on the human health effects that may result from exposure to TCS. It can reaches the systemic circulation via oral mucosa and gastrointestinal tract (GIT), dermal, and inhalation routes (Bagley and Lin, 2000). Dermal absorption of TCS is considered to be less than 10%, while it is readily and almost completely absorbed after oral exposure. Following absorption, TCS is widely distributed in blood, urine and most of the soft tissues. A number of studies have indicated that significant amounts of TCS are found in urine, breast milk, plasma, brain samples, and adipose tissue (Adolfsson-Erici et al., 2002; Allmyr et al., 2006; Sandborgh-Englund et al., 2006). It is metabolized by conjugation to glucuronide and sulfate and renal excretion is the predominant route of excretion (Dann and Hontela, 2011; Rodricks et al., 2010). Due to wide variety sources of triclosan exposure, the levels of human exposure cannot be accurately estimated (Dann and Hontela, 2011). Previous studies reported that this compound was detected in wide concentration range in human samples. For example it was detected in urine samples at a concentration range of 2.4–3,790 μg/L (Calafat et al., 2007). It was determined in human milk at concentrations ranging from 100 to 2,100 g/Kg (Dayan, 2007). Plasma concentrations of TCS ranged from 0.010 to 38 ng/g by Allmyr et al. (2006). Although metabolism and excretion restrict TCS brain accumulation to a rather large extent; however, there is considerable concern about its harm even in trace amounts.

TCS possesses intrinsic estrogenic and androgenic activity and it has been mentioned with endocrine disrupting potential capable of altering sex hormone function and thyroid hormone homeostasis (Olaniyan

* Corresponding author.
E-mail address: atena.rahimi@yahoo.com (A. Rahimi).

https://doi.org/10.1016/j.fct.2019.05.025
Received 30 January 2019; Received in revised form 17 April 2019; Accepted 15 May 2019
Available online 18 May 2019
0278-6915/

et al., 2016). Besides, Rodricks et al. reported "when evaluated in chronic oncogenicity studies in mice, rats and hamsters, treatment-related tumors were found only in the liver of male and female mice" (Rodricks et al., 2010) through both constitutive androstane receptor (CAR) and peroxisome proliferator activated receptor α (PPARα) activation resulting in significant augmentation of DNA synthesis and cell proliferation (Wang et al., 2017). Recent studies have also concluded that TCS may also negatively alter the immune system response (Anderson et al., 2016; Rees Clayton et al., 2010).

Neurotoxic effect of TCS has been reported in several in vitro studies. Assessment of the developmental neurotoxicity of TCS by Muth-Köhne et al. showed that TCS at high concentration (2.8 μM) could slightly delay development of secondary motor neurons (SMNs) in zebrafish (Muth-Köhne et al., 2012). TCS (10 μM) contributed to induce DNA fragmentation and formation of apoptotic bodies in primary cultures of neocortical neurons through caspase-3, caspase-8, and Fas receptor (FasR) signal activation (Szychowski et al., 2015). As demonstrated by Kyung Park et al. TCS (50 μM) could increase expression of pro-apoptotic markers such as Bax and Bcl-2. It also significantly reduced antioxidant defenses by GSH reduction and ROS homeostasis alteration. Their research on cultured rat neural stem cells (NSCs) showed that TCS can induce neurotoxicity in developing rat brains through mentioned mechanisms (Park et al., 2016). Furthermore, exposure of TCS to *Pangasianodon hypophthalmus* altered behavioral parameters and normal neurological functions that may be due to augment ROS generation (Sahu et al., 2018). Szychowski et al. reported that TCS at environmentally relevant concentrations could induce neurotoxicity in mouse neocortical neurons via enhanced caspase-3 activation and ROS production that may be related to transient degradation of N-Methyl-D-aspartate receptors (NMDARs) subunits (Szychowski et al., 2019). Taken together, Oxidative stress, inflammation, and apoptosis pathways have been purported as potential pathomechanisms involved in the neurotoxicity induced by TCS (Ruszkiewicz et al., 2017).

It has been shown that exposure to triclosan could put the plasma concentration of this compound in a concentration range of 74.5–94.2 μg/ml (Lin, 2000). There is also evidence report that triclosan administration at a dose of 3000 mg/kg could result in plasma concentration of 54 or 86 μg/ml in male and female rats (DeSalva et al., 1989).

However, even though many studies have reported that TCS induced adverse changes in various target tissues, few researchers have addressed the behavioral neurological activity of this compound. So this study attempts to evaluate the neurotoxic effect of TCS at doses 1000, 2000 and 4000 mg/kg on brain using in vivo experimental models.

2. Materials and methods

2.1. Animals

All experiments were performed on male NMRI mice (BUMS, Babol, Iran) weighing 20–25 g (12 weeks). The animals were housed under a 12 h light/dark cycle in a temperature-controlled (22 ± 2 °C) environment and standard mouse diet and water were provided ad libitum. The mice were used after one week adaptation and handling period. All experimental procedures were accomplished in accordance with National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

2.2. Chemicals and treatment

Triclosan (Irgasan, ≥97.0%, CAS#3380-34-5) was obtained from Sigma-Aldrich Chemical Company (St Louis,MO, USA). All other chemicals were of the highest commercial grade.

TCS (1000, 2000, and 4000 mg/kg) was dissolved in corn oil as vehicle and given via oral gavage for 14 consecutive days (Crofton et al., 2007); the experiments were held on the day 15 (n = 8 for all groups). Dosing volume was 1.0 ml corn oil/kg body weight and solutions were prepared fresh weekly and stored at room temperature. The selected doses are lower than LD50 (4350 mg/kg in mice) (Fang et al., 2010).

2.3. Behavioral assessments

2.3.1. Open-field activity

The open-field test was carried out to assessment of locomotor activity, exploratory and anxiety-like behaviors. Assessment was performed in a cage made of transparent Plexiglas (40 cm × 40 cm × 40 cm). After habituation to the testing room, mice were placed gently and individually in the center of the arena and allowed to move freely for 10 min while being monitored by automated tracking system (EthoVision® XT, Version 8, Noldus, the Netherlands). Between trials, the surface of the arena was cleaned with 10% alcohol. For analysis, the arena was subdivided into central and peripheral zones. Distance moved and peripheral and central zone spent time were recorded (Farzanehfar et al., 2016).

2.3.2. Rotarod test

The rotarod apparatus (Harvard, United States) was used to evaluate motor coordination and balance. Fixed speed rotarod was performed at a constant speed of 20 rpm with a 300-s maximum time limit. After acclimation, all mice received training for 2 consecutive days. On the test day, the mice were tested in three consecutive trials of 1 min each, with 1 min rest between trials. The latency to fall during each of the three trials was averaged to give the overall time for each mouse (Di Pardo et al., 2012; Terrando et al., 2011).

2.3.3. Elevated plus maze

The elevated plus maze (EPM) test was employed to assess anxiety-like behavior in mice. The apparatus was arranged as across with two open arms (50 cm × 10 cm) facing each other and two other arms enclosed by high walls (50 cm × 10 cm × 40 cm) and that originated from a common central platform (5 cm × 5 cm). The entire maze was elevated to a height of 50 cm above the floor (Vieira et al., 2013). To begin a test session, mice were placed on the center square region of apparatus with the head facing towards an open arm and allowed to move through apparatus for 5min. The number of entries and the proportion of time spent in the open and closed arms were recorded by automated tracking system (EthoVision® XT, Version 8, Noldus, the Netherlands). The maze was cleaned with 10% alcohol between trials and dried with napkin.

2.3.4. Grip strength

The grip strength test is a simple non-invasive method designed to assess mouse muscle strength in vivo. The experiment was conducted using a digital force-gauging apparatus (GS 5000, Borj Sanat, Iran). Then the mouse was gently pulled parallel away from the bar by the tail until the forelimbs released the bar. The maximum force prior to release of the mouse's paw from the bar was recorded. The test was repeated 3 times and the mean value was reported as the final grip strength (De Luca et al., 2008).

2.4. Histological analysis

Histological analysis of entire brains sections was used to determine severity of pathological changes. At the end of the behavioral experiments, mice were anesthetized with ketamine and xylazine then sacrificed and brain tissue was removed rapidly. All brains were fixed for 48 h in 10% buffered formalin, and embedded in paraffin. Then consecutive sections (10-μm diameter) were obtained using a microtome device (Leica, Vienna, Austria), and mounted on glass slides.

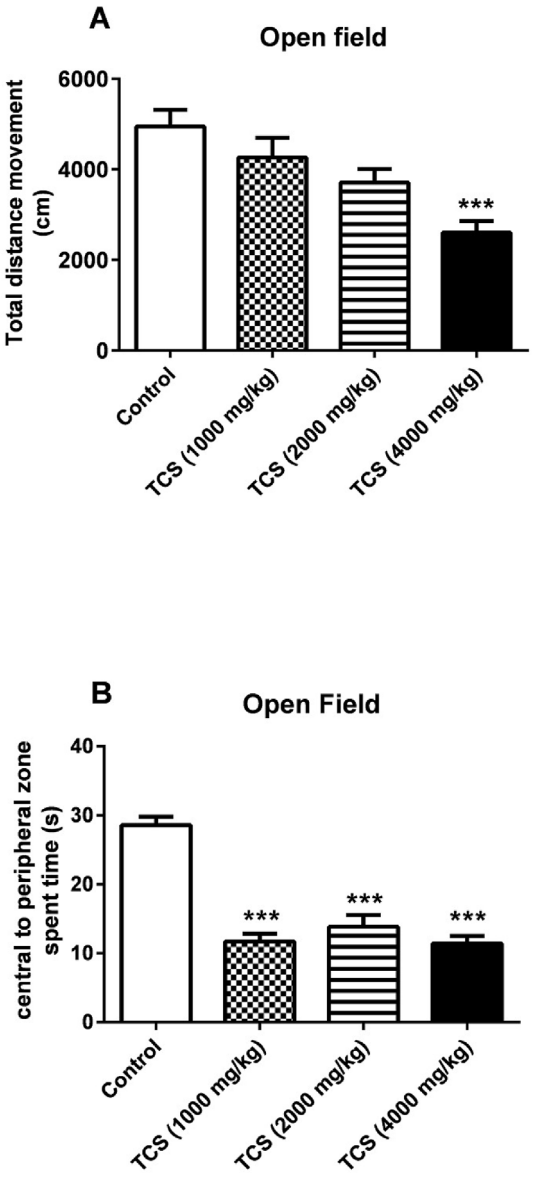

Fig. 1. Effects of triclosan on total distance movement in open field test (A). Effects of triclosan on central to peripheral zone spent time in open field test (B). Each bar represents mean ± SEM. Each group consisted of 6–10 mice. ***$p < 0.001$ significant difference compared to the control group.

Subsequently, the sections were deparaffinized, rehydrated, and stained with Haematoxylin and Eosin (H & E; Sigma– Aldrich, Steinheim, Germany). Then the number of neuron cells in each region (brain cortex, hippocampus, and cerebellum) was counted. The thickness of cerebellar layers were measured using Axio version 4.8 software (Rahimi et al., 2015).

2.5. Statistical analysis

Results were statistically analyzed in GraphPad Prism (version 6.0, GraphPad Software Inc., San Diego, CA) and presented as mean ± SEM. A one-way analysis of variance (ANOVA) followed by Tukey's test were used for multiple comparison. For all statistical analyses, $p < 0.05$ was considered significant.

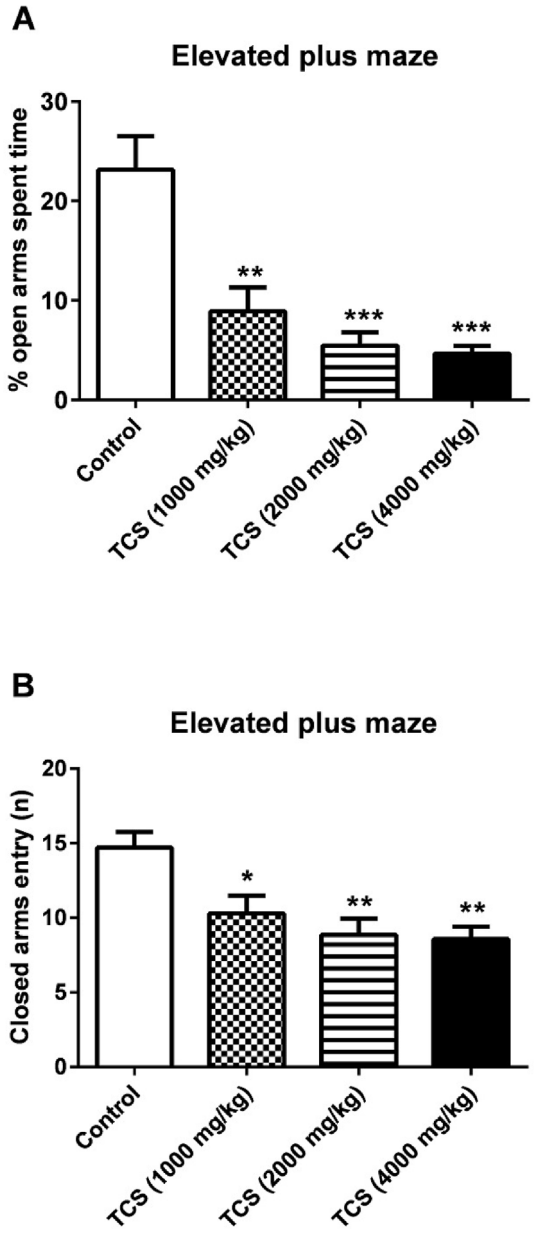

Fig. 2. Effects of triclosan on percentage of open arms spent time in EPM test (A). Effects of triclosan on closed arms entry in EPM test (B). Each bar represents mean ± SEM. Each group consisted of 6–10 mice. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ significant difference compared to the control group.

3. Results

3.1. Open-field test

The open-field test was performed for evaluation of locomotor activity of animals in a novel environment. It is apparent from Fig. 1A that although high dose of TCS (4000 mg/kg) significantly decreased distance movement comparing to the control group which was received vehicle ($p < 0.001$), post-hoc analysis by Tukey's multiple comparisons test revealed no significant change in total distance moved between mice treated with either TCS 1000 mg/kg or TCS 2000 mg/kg compared to control group. In addition, the significant decrease in the central to peripheral zone spent time observed in all TCS groups compared to the control mice ($p < 0.001$, Fig. 1B) and there were no differences between groups treated with TCS.

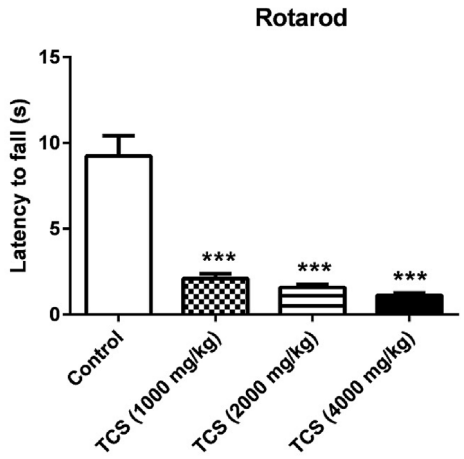

Fig. 3. Effects of triclosan on latency to fall in rotarod test. Each bar represents mean ± SEM. Each group consisted of 6–10 mice. ***$p < 0.001$ significant difference compared to the control group.

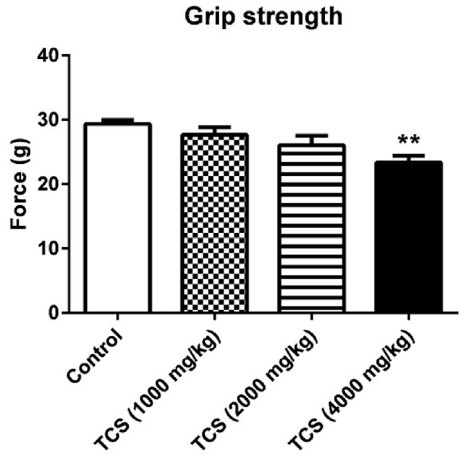

Fig. 4. Effects of triclosan on grip force (gram) in grip strength test. Each bar represents mean ± SEM. Each group consisted of 6–10 mice. **$p < 0.01$ significant difference compared to the control group.

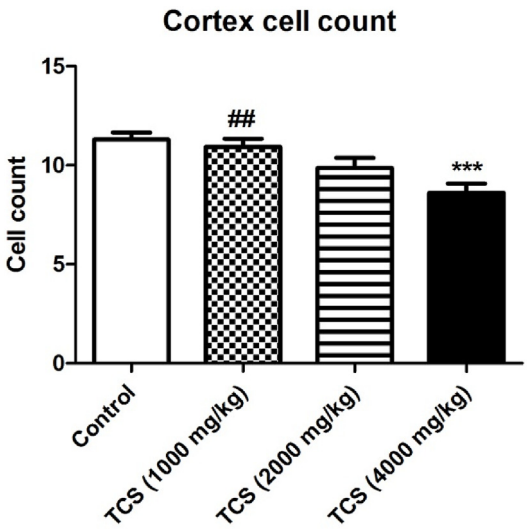

Fig. 6. Effects of triclosan on cell count in cerebral cortex. Each bar represents mean ± SEM. Each group consisted of 6–10 mice. ***p < 0.001 significant difference compared to the control group. ##p < 0.01 significant difference compared to the TCS (4000 mg/kg) group.

3.2. Elevated plus maze test

In the elevated plus-maze test one-way ANOVA showed, TCS significantly reduced the percentage of open arms spent time in mice receiving TCS (1000, 2000, and 4000 mg/kg) comparing to the control group as shown in Fig. 2A ($p < 0.01$ in TCS 1000 mg/kg and $p < 0.001$ in TCS 2000 and 4000 mg/kg). There were also showed a significant reduction in the number of entries into the closed arms when TCS administered mice compared to control group ($p < 0.05$ in TCS 1000 mg/kg and $p < 0.01$ in TCS 2000 and 4000 mg/kg, Fig. 2B). There were no differences between groups treated with TCS.

3.3. Rotarod test

Motor coordination was assessed using the Rotarod apparatus. At fixed rotarod speed (20 rpm), a significant difference was observed in latency to fall between the control and treated mice ($p < 0.001$; Fig. 3). No significant differences were observed among groups were received TCS in various doses.

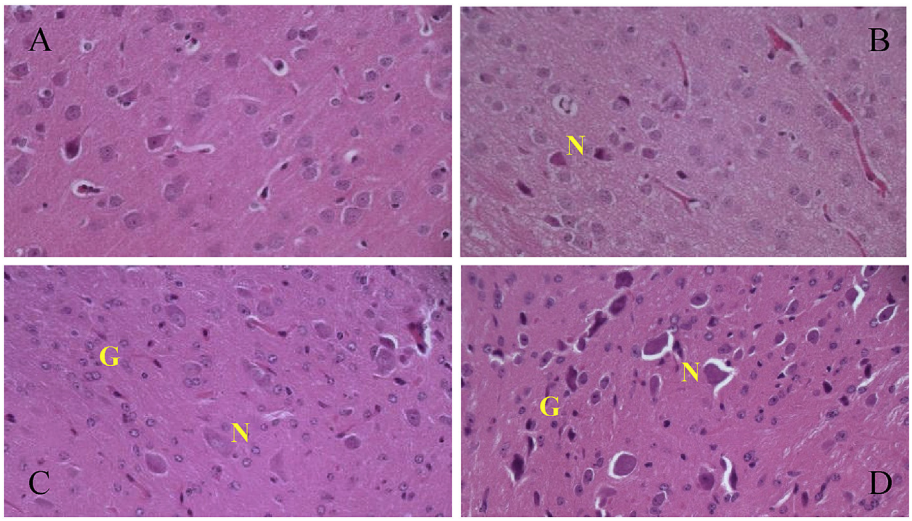

Fig. 5. Effects of TCS in the brain tissue of the control (A) and TCS (1000, 2000 and 4000 mg/kg/day) treated mice (B, C and D). N and G indicate neuronal necrosis and gliosis respectively. (H&E staining X 40).

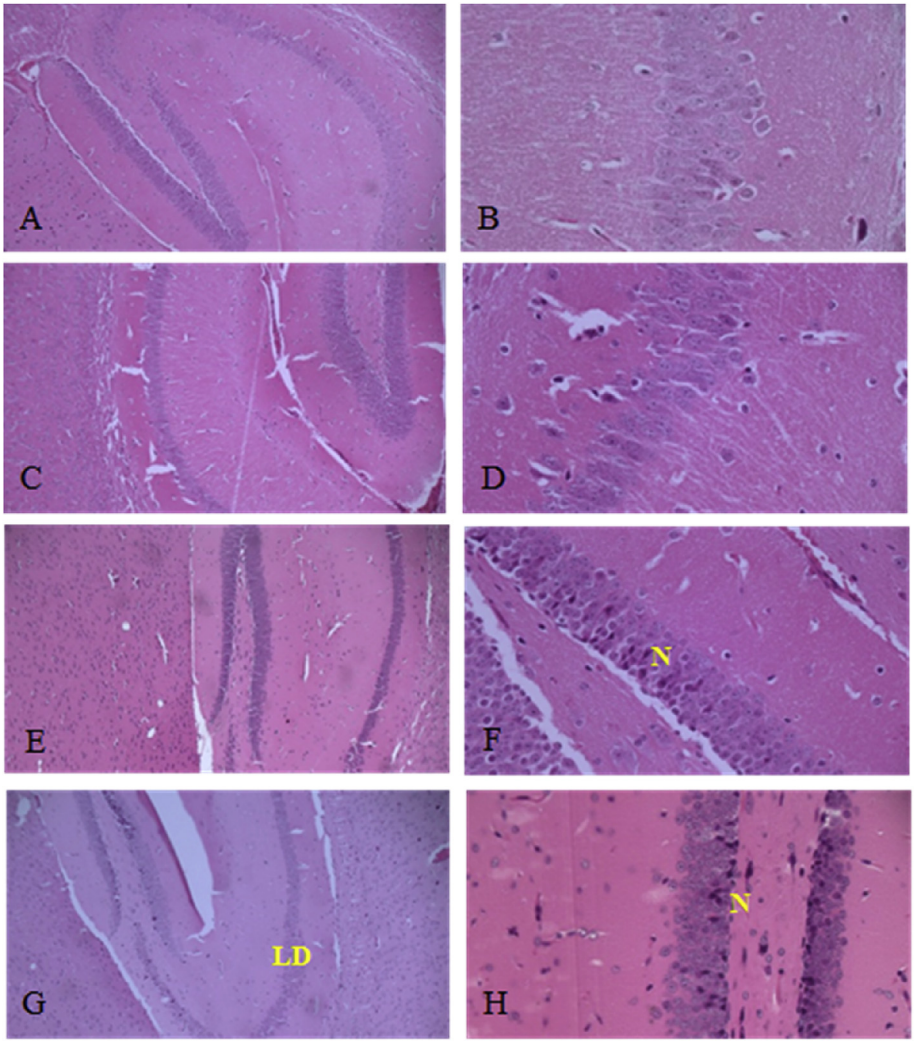

Fig. 7. Effects of TCS in the hippocampus of the control (A,B) and TCS (1000 mg/kg; C,D), TCS (2000 mg/kg; E,F), TCS (4000 mg/kg; G,H). N and LD indicate neuronal necrosis and low density respectively. (H&E staining X 40).

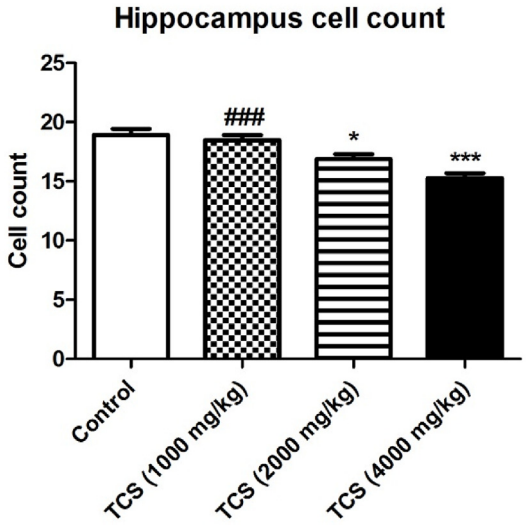

Fig. 8. Effects of triclosan on cell count in hippocampal region. Each bar represents mean ± SEM. Each group consisted of 6–10 mice. *p < 0.05, ***p < 0.001 significant difference compared to the control group. ###p < 0.001 significant difference compared to the TCS (4000 mg/kg) group.

3.4. Grip strength test

No significant difference was observed in grip strength force between the control group and TCS treated mice (1000 mg/kg and 2000 mg/kg), however the grip force significantly decreased in TCS (4000 mg/kg) compared to the control group ($p < 0.01$, Fig. 4).

3.5. Histopathological changes

Pathological changes in the brain, hippocampus, and cerebellum of TCS intoxicated mice were examined under light microscopy. Fig. 5 A, B, C and D shows the haematoxylin-eosin stained neuronal cells of brain tissue in the control and TCS exposed mice (1000, 2000, and 4000 mg/kg/day) respectively. Sever necrosis and reactive gliosis were obviously seen in tissue brain of the TCS treated mice comparing to the control mice. The number of neuron cells in brain cortex significantly decreased in mice treated with TCS 4000 mg/kg ($p < 0.001$) comparing to the control mice (Fig. 6). The hippocampus is a key brain area for many forms of learning and memory. The hippocampal neurons of mice in control and TCS (1000 mg/kg) groups were in distinct and regular structure (Fig. 7A–D). In contrast, higher doses of TCS (2000 and 4000 mg/kg) altered morphologic features of neurons and karyopyknosis and necrosis were increasingly observed in hippocampus (Fig. 7E–H). The neuronal density slightly decreased in TCS (4000 mg/kg) group (Fig. 7G and H). . The number of neuron cells in hippocampal

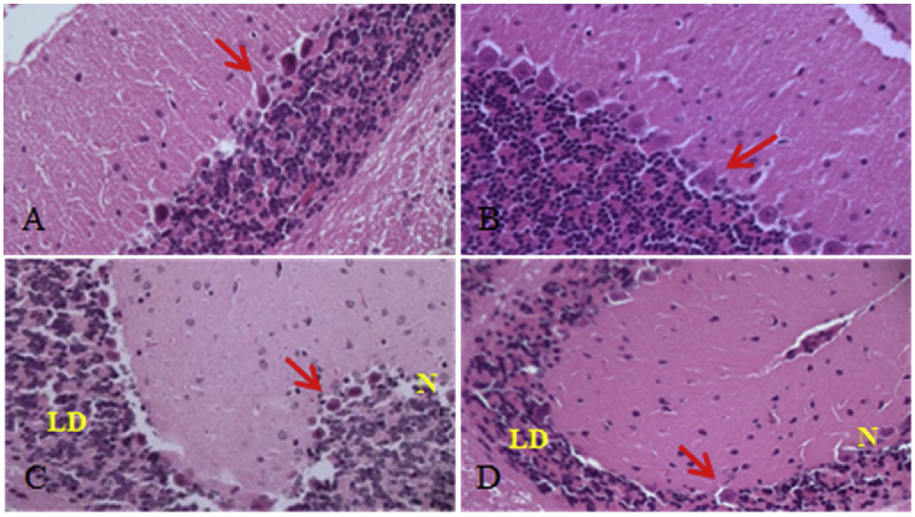

Fig. 9. Effects of TCS in the cerebellum of the control (A) and TCS (1000, 2000 and 4000 mg/kg/day) treated mice (B, C and D). N and LD indicate neuronal necrosis and low density respectively. The arrows indicate Purkinje cells. (H&E staining X 40).

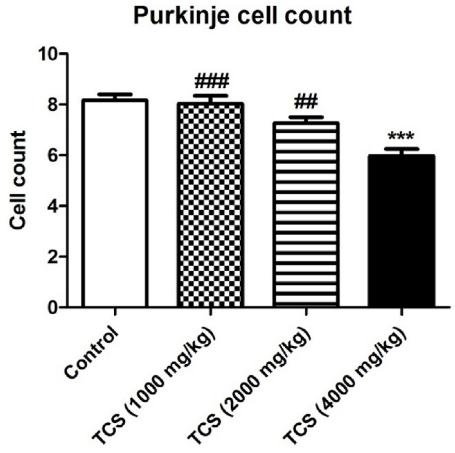

Fig. 10. Effects of triclosan on Purkinje cells count in the cerebrum. Each bar represents mean ± SEM. Each group consisted of 6–10 mice. ***p < 0.001 significant difference compared to the control group. ##p < 0.01, ###p < 0.001 significant difference compared to the TCS (4000 mg/kg) group.

region (CA1-CA4) in mice treated with TCS 2000 mg/kg ($p < 0.05$) and TCS 4000 mg/kg ($p < 0.001$) was lower than control mice (Fig. 8). Histological analysis in the cerebrum showed the sever loss of Purkinje cells and necrosis in mice exposed to high doses of TCS (2000 and 4000 mg/kg) comparing to the control mice (Fig. 9). . The number of Purkinje cells in the cerebrum significantly decreased in mice treated with TCS 4000 mg/kg (p < 0.001) comparing to the control group (Fig. 10). Furthermore, there was a significant difference between control group and TCS (2000 and 4000 mg/kg) groups when the average thickness of the molecular and granular layers as well as that of the total cortex was compared. The total cortical thickness in TCS (2000 mg/kg) and TCS (4000 mg/kg) groups decreased by 24.2% and 33.44% respectively in compared with control group (Table 1). The thickness of molecular and granular layers in mice treated with TCS also showed statistical significance; in the molecular layer the thickness decreased by 25.53% in TCS (4000 mg/kg) group and in the granular layer by 44.27% and 41.87% in TCS (2000 mg/kg) and TCS (4000 mg/kg) groups respectively (Table 1). The diameter of white matter also significantly decreased in all groups received TCS compared with control group ($p < 0.0001$; Table 1).

4. Discussion

To the best of our knowledge, the present study reports the effects of TCS on behavioral changes in mice for the first time. Based on the locomotor activity results, high dose of TCS (4000 mg/kg) reduced total distance movement in open field test. In parallel, the ability of triclosan to modulate the anxiety-related behavior in mice was assessed by the open-field and the elevated-plus maze, both of which are widely used behavioral tests for the study of anxiety in rodents (Hoffman, 2016). In the open-field, TCS-exposed mice were more anxious than controls, as reflected by the significant decrease in central to peripheral zone spent time (Fig. 1B). In this test, TCS treated mice revealed the tendency to remain close to walls and showed less exploration behaviors in the central zone. Concomitantly, the results of the EPM test indicated that the TCS treated mice spent more time in the enclosed arms than the control mice. The findings from these two mazes suggest the capacity of TCS to induce anxiety in mice.

In addition, the neurotoxic effect of this compound was observed when administration of TCS resulted in significant differences in mobility, motor coordination and muscle strength as assessed through the rotarod test and the grip strength test. Since impaired rotarod performance is referable to cerebellar dysfunction (Furnari et al., 2014), it might be concluded that the data on open field test and EPM test could

Table 1
The thickness of cerebellar layers in control and TCS-exposed mice.

	Control	TCS (1000 mg/kg)	TCS (2000 mg/kg)	TCS (4000 mg/kg)
The thickness of cerebellar cortex (μm)	0.3098 ± 0.0104	0.2764 ± 0.0101	0.2348 ± 0.0131[c]	0.2062 ± 0.0037[c]
The thickness of molecular layer (μm)	0.1598 ± 0.0048	0.1486 ± 0.0059	0.1512 ± 0.0081	0.1190 ± 0.0067[b]
The thickness of granular layer (μm)	0.1500 ± 0.0123	0.1278 ± 0.0063	0.0836 ± 0.0064[c]	0.0872 ± 0.0037[c]
The thickness of white matter (μm)	0.1078 ± 0.0072	0.0722 ± 0.0042[b]	0.0662 ± 0.0054[c]	0.0530 ± 0.0052[c]

Data are presented as mean ± SEM. [b]$p < 0.01$, [c]$p < 0.001$ significant difference compared to the control group.

be related to lack of motor coordination, muscle weakness or neuromuscular function impairment in the TCS treated mice. Our results are consistent with Schultz et al.'s findings stating that TCS had attenuated predator-avoidance performance in larvae and decreased activities in behavioral aggression assays (Schultz et al., 2012). It also impaired swimming behavior of larval fathead Minnows (Fritsch et al., 2013) and altered behavioral patterns and normal neurological functions in *Pangasianodon hypophthalmus* (Sahu et al., 2018).

Histological examination of haematoxylin and eosin stained sections revealed that TCS particularly with high doses induced profound morphological abnormalities in brain tissues (cerebrum and cerebellum) including necrosis, gliosis, karyopyknosis, and cell density alteration. The reduction in neuronal count has also seen in mice treated with TCS. The significant loss of Purkinje cells in cerebrum also observed in TCS treated mice. Purkinje neurons, the sole integrating center of cerebellar cortex, have a critical role in coordination, neuromuscular strength, learning functions, and motor movement (Zhang et al., 2014). So the results obtained from open field, grip strength, and rotarod tests may relate to Purkinje cells loss in cerebellar cortex of mice treated with TCS. Due to the pivotal role of the hippocampus in anxiety-related behaviors (Cominski et al., 2014), our findings from histopathological examination confirm the results of anxiety response in the EPM test. Our results are in agreement with the results of previous in vivo and in vitro studies that have shown TCS exerted highly damaging impacts on rodent hippocampal neuronal function (Arias-Cavieres et al., 2018).

5. Conclusion

The evidence from this study intimates that TCS might be considered as a toxicant that impacts the neurobehavioral parameters in mice. Considering the broad usage of this compound as an antiseptic in several commercial products and its detection in human samples, further studies are required to investigate whether TCS cause neurobehavioral toxicity in humans.

Conflicts of interest

There is no conflict of interest.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.05.025

References

Adolfsson-Erici, M., Pettersson, M., Parkkonen, J., Sturve, J., 2002. Triclosan, a commonly used bactericide found in human milk and in the aquatic environment in Sweden. Chemosphere 46, 1485–1489.

Allmyr, M., Adolfsson-Erici, M., McLachlan, M.S., Sandborgh-Englund, G., 2006. Triclosan in plasma and milk from Swedish nursing mothers and their exposure via personal care products. Sci. Total Environ. 372, 87–93.

Anderson, S.E., Meade, B.J., Long, C.M., Lukomska, E., Marshall, N.B., 2016. Investigations of immunotoxicity and allergic potential induced by topical application of triclosan in mice. J. Immunotoxicol. 13, 165–172.

Arias-Cavieres, A., More, J., Vicente, J.M., Adasme, T., Hidalgo, J., Valdés, J.L., Humeres, A., Valdes-Undurraga, I., Sanchez, G., Hidalgo, C., 2018. Triclosan impairs hippocampal synaptic plasticity and spatial memory in male rats. Front. Mol. Neurosci. 11, 429.

Bagley, D., Lin, Y., 2000. Clinical evidence for the lack of triclosan accumulation from daily use in dentifrices. Am. J. Dent. 13, 148–152.

Calafat, A.M., Ye, X., Wong, L.-Y., Reidy, J.A., Needham, L.L., 2007. Urinary concentrations of triclosan in the US population: 2003–2004. Environ. Health Perspect. 116, 303–307.

Cherednichenko, G., Zhang, R., Bannister, R.A., Timofeyev, V., Li, N., Fritsch, E.B., Feng, W., Barrientos, G.C., Schebb, N.H., Hammock, B.D., 2012. Triclosan impairs excitation–contraction coupling and Ca2+ dynamics in striated muscle. Proc. Natl. Acad. Sci. Unit. States Am. 109, 14158–14163.

Cominski, T.P., Jiao, X., Catuzzi, J.E., Stewart, A.L., Pang, K.C., 2014. The role of the hippocampus in avoidance learning and anxiety vulnerability. Front. Behav. Neurosci. 8, 273.

Coogan, M.A., Edziyie, R.E., La Point, T.W., Venables, B.J., 2007. Algal bioaccumulation of triclocarban, triclosan, and methyl-triclosan in a North Texas wastewater treatment plant receiving stream. Chemosphere 67, 1911–1918.

Crofton, K.M., Paul, K.B., DeVito, M.J., Hedge, J.M., 2007. Short-term in vivo exposure to the water contaminant triclosan: evidence for disruption of thyroxine. Environ. Toxicol. Pharmacol. 24, 194–197.

Dann, A.B., Hontela, A., 2011. Triclosan: environmental exposure, toxicity and mechanisms of action. J. Appl. Toxicol. 31, 285–311.

Dayan, A., 2007. Risk assessment of triclosan [Irgasan®] in human breast milk. Food Chem. Toxicol. 45, 125–129.

De Luca, A., Tinsley, J., Aartsma-Rus, A., van Putten, M., Nagaraju, K., de La Porte, S., Dubach-Powell, J., Carlson, G., 2008. Use of grip strength meter to assess the limb strength of mdx mice. SOP DMD_M 2.

DeSalva, S., Kong, B., Lin, Y., 1989. Triclosan: a safety profile. Am. J. Dent. 2, 185–196.

Di Pardo, A., Maglione, V., Alpaugh, M., Horkey, M., Atwal, R.S., Sassone, J., Ciammola, A., Steffan, J.S., Fouad, K., Truant, R., 2012. Ganglioside GM1 induces phosphorylation of mutant huntingtin and restores normal motor behavior in Huntington disease mice. Proc. Natl. Acad. Sci. Unit. States Am. 109, 3528–3533.

Fang, J.-L., Stingley, R.L., Beland, F.A., Harrouk, W., Lumpkins, D.L., Howard, P., 2010. Occurrence, efficacy, metabolism, and toxicity of triclosan. J. Environ. Sci. Health, Part C 28, 147–171.

Farzanehfar, V., Naderi, N., Kobarfard, F., Faizi, M., 2016. Determination of dibutyl phthalate neurobehavioral toxicity in mice. Food Chem. Toxicol. 94, 221–226.

Fritsch, E.B., Connon, R.E., Werner, I., Davies, R.E., Beggel, S., Feng, W., Pessah, I.N., 2013. Triclosan impairs swimming behavior and alters expression of excitation-contraction coupling proteins in fathead minnow (Pimephales promelas). Environ. Sci. Technol. 47, 2008–2017.

Furnari, M.A., Saw, C.L.-L., Kong, A.-N., Wagner, G.C., 2014. Altered behavioral development in Nrf2 knockout mice following early postnatal exposure to valproic acid. Brain Res. Bull. 109, 132–142.

Hoffman, K., 2016. 3—modeling disorders of fear and anxiety in animals. Modeling Neuropsychiatric Disorders in Laboratory Animals. Woodhead Publishing, pp. 87–160.

Lin, Y., 2000. Buccal absorption of triclosan following topical mouthrinse application. Am. J. Dent. 13, 215–217.

Muth-Köhne, E., Wichmann, A., Delov, V., Fenske, M., 2012. The classification of motor neuron defects in the zebrafish embryo toxicity test (ZFET) as an animal alternative approach to assess developmental neurotoxicity. Neurotoxicol. Teratol. 34, 413–424.

Olaniyan, L., Mkwetshana, N., Okoh, A., 2016. Triclosan in water, implications for human and environmental health. SpringerPlus 5, 1639.

Park, B.K., Gonzales, E.L.T., Yang, S.M., Bang, M., Choi, C.S., Shin, C.Y., 2016. Effects of triclosan on neural stem cell viability and survival. Biomol. Ther. 24, 99.

Rahimi, A., Faizi, M., Talebi, F., Noorbakhsh, F., Kahrizi, F., Naderi, N., 2015. Interaction between the protective effects of cannabidiol and palmitoylethanolamide in experimental model of multiple sclerosis in C57BL/6 mice. Neuroscience 290, 279–287.

Rees Clayton, E.M., Todd, M., Dowd, J.B., Aiello, A.E., 2010. The impact of bisphenol A and triclosan on immune parameters in the US population, NHANES 2003–2006. Environ. Health Perspect. 119, 390–396.

Rodricks, J.V., Swenberg, J.A., Borzelleca, J.F., Maronpot, R.R., Shipp, A.M., 2010. Triclosan: a critical review of the experimental data and development of margins of safety for consumer products. Crit. Rev. Toxicol. 40, 422–484.

Ruszkiewicz, J.A., Li, S., Rodriguez, M.B., Aschner, M., 2017. Is Triclosan a neurotoxic agent? J. Toxicol. Environ. Health, Part B 20, 104–117.

Sahu, V.K., Karmakar, S., Kumar, S., Shukla, S., Kumar, K., 2018. Triclosan toxicity alters behavioral and hematological parameters and vital antioxidant and neurological enzymes in Pangasianodon hypophthalmus (Sauvage, 1878). Aquat. Toxicol. 202, 145–152.

Sandborgh-Englund, G., Adolfsson-Erici, M., Odham, G., Ekstrand, J., 2006. Pharmacokinetics of triclosan following oral ingestion in humans. J. Toxicol. Environ. Health, Part A 69, 1861–1873.

Schultz, M.M., Bartell, S.E., Schoenfuss, H.L., 2012. Effects of triclosan and triclocarban, two ubiquitous environmental contaminants, on anatomy, physiology, and behavior of the fathead minnow (Pimephales promelas). Arch. Environ. Contam. Toxicol. 63, 114–124.

Szychowski, K., Sitarz, A., Wojtowicz, A., 2015. Triclosan induces Fas receptor-dependent apoptosis in mouse neocortical neurons in vitro. Neuroscience 284, 192–201.

Szychowski, K.A., Wnuk, A., Rzemieniec, J., Kajta, M., Leszczyńska, T., Wójtowicz, A.K., 2019. Triclosan-evoked neurotoxicity involves NMDAR subunits with the specific role of GluN2A in caspase-3-dependent apoptosis. Mol. Neurobiol. 56, 1–12.

Terrando, N., Eriksson, L.I., Kyu Ryu, J., Yang, T., Monaco, C., Feldmann, M., Jonsson Fagerlund, M., Charo, I.F., Akassoglou, K., Maze, M., 2011. Resolving postoperative neuroinflammation and cognitive decline. Ann. Neurol. 70, 986–995.

Vieira, L.F.d.A., Reis, M.D.d.S., Brandão, A.R.A., Viana, I.M., Silva, J.P.d., Barreto, E., Smaniotto, S., 2013. Anxiolytic-like effect of the extract from Bowdichia virgilioides in mice. Revista Brasileira de Farmacognosia 23, 680–686.

Wang, Z., Li, X., Klaunig, J.E., 2017. Investigation of the mechanism of triclosan induced mouse liver tumors. Regul. Toxicol. Pharmacol. 86, 137–147.

Yueh, M.-F., Tukey, R.H., 2016. Triclosan: a widespread environmental toxicant with many biological effects. Annu. Rev. Pharmacol. Toxicol. 56, 251–272.

Zhang, L., Chung, S.K., Chow, B.K.C., 2014. The knockout of secretin in cerebellar Purkinje cells impairs mouse motor coordination and motor learning. Neuropsychopharmacology 39, 1460.