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Oxidative stress response after prolonged exposure of domestic rabbit to a lower dosage of extracted microcystins

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ABSTRACT

Oxidative stress response after prolonged exposure to a low dose of microcystins (MCs) was studied in liver, kidney and brain of domestic rabbits. Rabbits were treated with extracted MCs (mainly MC-LR and MC-RR) at a dose of 2 MC-LReq. μg/kg body weight or saline solution every 24 h for 7 or 14 days. During the exposure of MCs, increase of lipid peroxidation (LPO) levels were detected in all the organs studied, while antioxidant enzymes responded differently among different organs. The enzyme activities of superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR) in liver decreased in the MCs treated animals. In brain, there were obvious changes in glutathione peroxidase (GPx) and GR, while only CAT was obviously influenced in kidney. Therefore, daily exposure at a lower dosage of MCs, which mimicked a natural route of MCs, could also induce obvious oxidative stress in diverse organs of domestic rabbits. The oxidative stress induced by MCs in brain was as serious as in liver and kidney, suggesting that brain may also be a target of MCs in mammals. And it seems that animals may have more time to metabolize the toxins or to form an adaptive response to reduce the adverse effects when exposed to the low dose of MCs.

1. Introduction

Cyanobacterial blooms in natural waters, causing serious water pollution and public health hazard to humans and livestock, have become a worldwide problem (Cohen, 1989; Carmichael, 1997). Of all the algal toxins, microcystins (mainly microcystin-LR, RR and YR) are the most toxic and abundant species (Carmichael, 1994). Microcystins (MCs) are cyclic heptapeptides, composed of seven amino acids. More than 80 variants are known (Sivonen and Jones, 1999), many of which are potent hepatotoxins. And it has been demonstrated that MCs had toxic effects to diverse organs in animals (Cazenave et al., 2006).

Acute MCs poisoning in mammals is characterized by disruption of hepatic architecture, leading to massive intrahepatic haemorrhage and death in a few hours (Carmichael, 1994). One of the well-studied toxic mechanisms of MC is their ability to inhibit protein phosphatases 1 and 2A (Yoshizawa et al., 1990). And the resulting imbalance in protein phosphorylation leads to the destruction of cytoskeleton, directly causing cytotoxic effects, deregulation of cell division, and probably also increasing tumor-promoting activity (Carmichael, 1994). Cyanobacterial extracts can also enhance intracellular production of reactive oxygen species (ROS) (Ding et al., 1998). It is widely reported that MCs induce oxidative stress in many animals: mice, rat and aquatic organisms such as crab and fish (Moreno et al., 2003; Pinho et al., 2005; Prieto et al., 2006; Jayaraj et al., 2006).

Oxidative stress may occur either due to the over production of ROS or to the decrease of cellular antioxidant levels (Ding et al., 1998). Free radicals and ROS generated in tissues and sub-cellular compartments are efficiently scavenged by the antioxidant defence system, which constitutes antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR) (Filho, 1996). These antioxidant defenses can protect cells against DNA damage, protein oxidation and lipid peroxidation (LPO) (Prieto et al., 2006).

Many researches have reported the oxidative stress response after different exposure route of MCs in fish and murine animal (Maidana et al., 2006; Jayaraj et al., 2006; Cazenave et al., 2006). And most of these researches were limited under the high dose of MCs exposure or in the acute tests. Moreno et al. (2005) reported the effect of acute exposure of intraperitoneal (i.p.) injection of

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MC-LR on antioxidant enzymes and LPO in liver and kidney of rats treated with high doses of MC-LR (100, 150 and 230 mg/kg body weight). And Jayaraj et al. (2006) also described the significant decrease in the activity of antioxidant enzymes GPx, SOD, CAT, GR and glutathione-S-transferase (GST) under an acute exposure of 1 LD_{50} (76.62 μg/kg) of MCs.

However, the prolonged natural exposure to wild and domestic animals of lower levels of MCs is likely to be unnoticed. This exposure is important since it could become an important form of exposure which is similar with the natural route of potential presence of MCs in daily drinking water. Guzman and Solter (1999) have pointed out that, in the pathogenesis of chronic MC-LR toxicosis, oxidative stress played a significant role. So the aim of this study was to detect the oxidative stress response in domestic animals under the prolonged sub-chronic exposure of low dose of MCs, and to get a broader understanding on the response of different organisms exposed to microcystins in a different exposure way.

2. Material and methods

2.1. Toxin

The cyanobacterial material used in this experiment was collected from surface blooms of Lake Dianchi, Yunnan in China. Freeze-dried crude algae were extracted three times with 5% acetic acid and were suspended in saline solution for the toxic experiment. Quantitative analysis of MCs was performed using a reverse-phase high-performance liquid chromatography (HPLC, LC-20A, Shimadzu Corporation, Kyoto, Japan). MCs concentrations were determined by comparing the peak areas of the test samples with those of the standards available (MC-LR and MC-RR, Wako Pure Chemical Industries, Japan). The toxin-containing solution was finally diluted with saline solution to 136.5 μg/ml MC-RR and 22.7 μg/ml MC-LR.

2.2. Animals

Healthy male rabbits (*Oryctolagus cuniculus*) weighing about 1500–2000 g were obtained from a local rabbit warren in Wuhan City, China. Animals were housed three per cage and maintained in a mass air-displacement room with a 12-h light–dark cycle at 20–26 °C with a relative humidity of 50–70%. Animals were allowed to acclimate for 1 week prior to experimentation and were fed with commercial rabbit food at a rate of 6.0% of body weight per day and offered with water.

2.3. Exposure

Three acclimated rabbits without any administration were expressed as 0 day. Other rabbits were divided at random into two groups. Each group had three MCs-treated animals and three controls. In both groups, the treated animals were injected intraperitoneally (i.p.) at a dose of 2 MC-LReq. μg/kg body weight every 24 h for 7 days (group 1) and 14 days (group 2), and equal volume of saline solution for control ones. All animals were injected at the same hour. On the following day after the final toxin dose was administered (on day 7 for group 1 and day 14 for group 2), rabbits were sacrificed immediately, livers, kidneys and brains were quickly removed. To minimize diurnal variations, animals were routinely killed between 12:00 and 13:00 h. Tissue samples were immediately homogenized, aliquoted into Eppendorf tubes and frozen at −80 °C for enzyme activity analysis. The protein concentration was measured with Bio-Rad protein assay dye based on the method of Bradford (1976) with BSA as the standard.

2.4. Lipid peroxidation

The thiobarbaturic acid (TBA) method described by Fatima et al. (2000) was used to evaluate the peroxidation of lipids (LPO) in liver, kidney and brain of exposed and control rabbits with little modifications. 1.0 mL homogenate was incubated during 1 h at 37 °C with

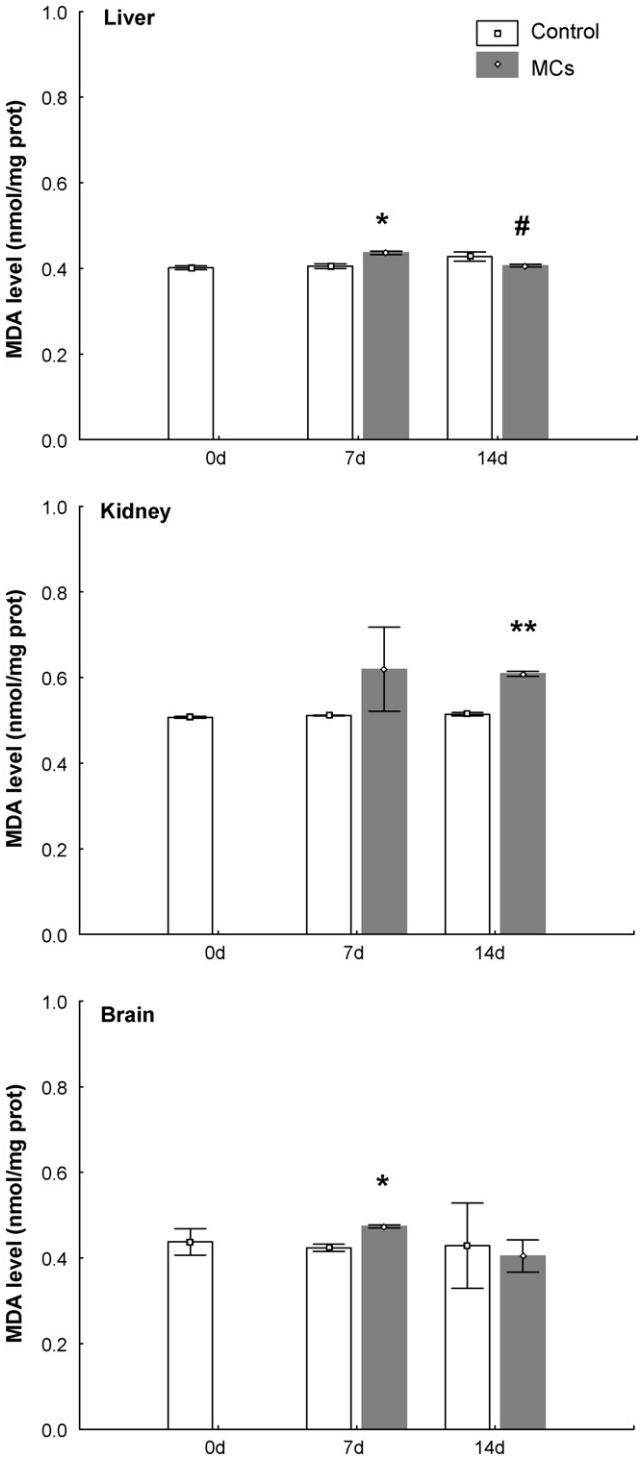

Fig. 1. Lipid peroxidation (LPO) values in liver, kidney and brain of domestic rabbits exposed to MCs. The values are expressed as mean ± S.E. The number of measurements performed in each group was three. LPO values are expressed as nmol MDA/mg prot. The significance levels observed are $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison to control group values and $^{\#}P < 0.05$ compared to 7 days exposure of MCs.

continuous shaking. Afterwards, 1.0 mL of 5% trichloroacetic acid, and 1.0 mL of 0.67% thiobarbituric acid were added to each sample and mixed. Then, each vial was centrifuged at 3000 rpm for 10 min. The supernatant was separated and placed in a boiling water bath for 40 min, cooled to room temperature, and measured at 532 nm. The rate of lipid peroxidation was expressed as nanomoles of substances reactive to thiobarbituric acid (TBA) formed per hour, per milligram of proteins (nmol TBA/(mg prot)).

2.5. Enzyme activity assays

GR was measured spectrophotometrically by measuring the oxidation of NADPH at 340 nm (Carlberg and Mannervik, 1975). GPx activity was estimated by the method of Flohe and Gunzler (1984). The decrease in absorbance was monitored at 340 nm. CAT activity assay was performed following the method of Prieto et al. (2007). The decrease in absorption was measured spectrophotometrically at 240 nm. SOD activity assay was based on the method described by McCord and Fridovich (1969) with slight modifications. SOD activity was monitored spectrophotometrically at 550 nm. One unit of SOD activity is defined as the amount of enzyme that gave 50% inhibition of the control rate of cytochrome c reduction.

2.6. Statistical analysis

The data from MC-administration and control rabbits were presented as mean ± S.E. The data were tested for statistical differences by one-way ANOVA followed by Duncan's multiple comparison test using STATISTICA software package (Version 6.0, Statsoft, Inc.) to compare data of control rabbits and the ones exposed to extracted microcystins. Statistical differences were determined at the $P < 0.05$ and $P < 0.01$ level for all analyses.

3. Results

3.1. Effects of MCs on lipid peroxidation

Liver and brain showed significant increase of lipid peroxidation (LPO) in rabbits exposed to MCs at 7 days (8 and 12%, respectively) compared with the control animals. At this time point, kidney had no obvious change of LPO. At 14 days, LPO in kidney increased about 18%, while liver and brain maintained basal values (Fig. 1). From day 7 to day 14, a significant decrease in LPO was observed in liver.

3.2. Effect of MCs on antioxidant enzymes

Fig. 2 shows no obvious change of SOD activity in all organs of rabbits after MCs exposure at two time points, except a decrease of 8% in liver was observed on day 14.

On day 7, CAT activity decreased 20% in liver and increased 9% in kidney in the MCs-treated animals compared with the control ones. And no significant changes were observed in CAT activity in brain in any of the experimental groups (Fig. 3).

An enhancement in GPx activity was observed in brain of rabbits on days 7 and 14 (14 and 17%, respectively) (Fig. 4). However, no noticeable changes were observed in liver and kidney of the animals exposed to MCs.

GR activity decreased significantly (25%) only in liver of rabbits on day 7. In brain, an obvious elevation (23%) was observed in the animals exposed to MCs on day 14 (Fig. 5). There was no significant change in enzyme activity in kidney in comparison to the control group.

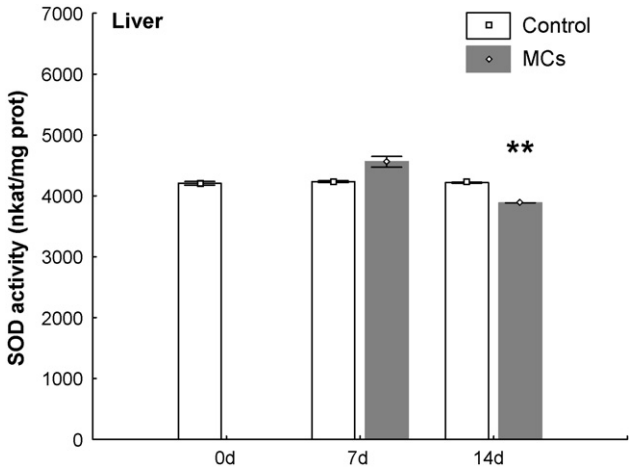

Fig. 2. Superoxide dismutase (SOD) values in liver of domestic rabbits exposed to MCs. The values are expressed as mean ± S.E. The number of measurements performed in each group was three. The significance levels observed are $^{**}P < 0.01$ in comparison to control group values.

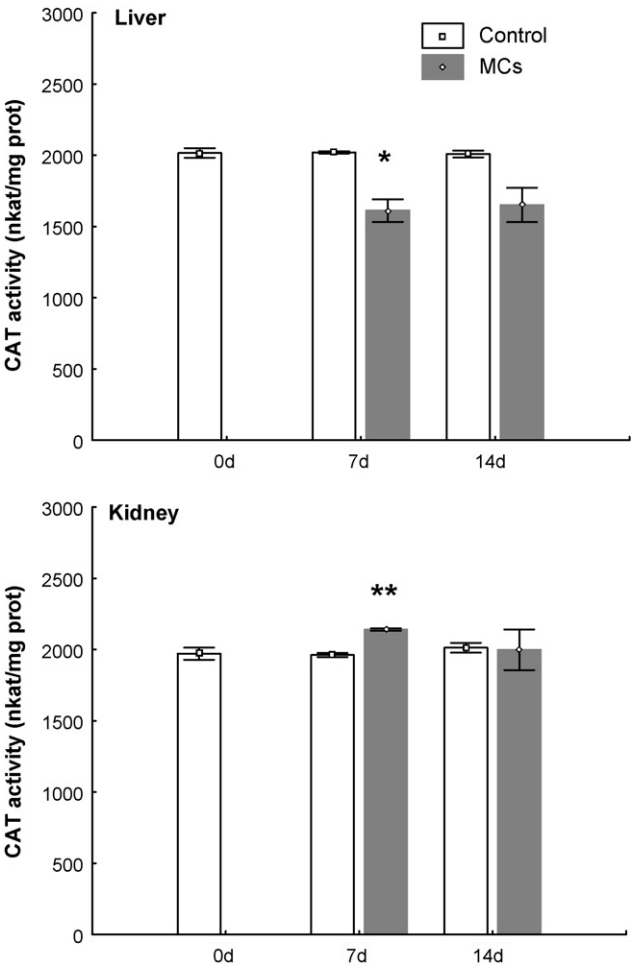

Fig. 3. Catalase (CAT) values in liver and kidney of domestic rabbits exposed to MCs. The values are expressed as mean ± S.E. The number of measurements performed in each group was three. The significance levels observed are $^{*}P < 0.05$ and $^{**}P < 0.01$ in comparison to control group values.

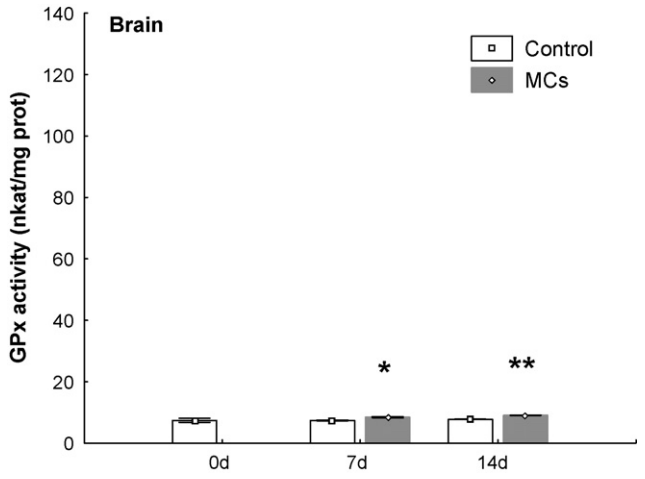

Fig. 4. Glutathione peroxidase (GPx) values in brain of domestic rabbits exposed to MCs. The values are expressed as mean ± S.E. The number of measurements performed in each group was three. The significance levels observed are $^{*}P < 0.05$ and $^{**}P < 0.01$ in comparison to control group values.

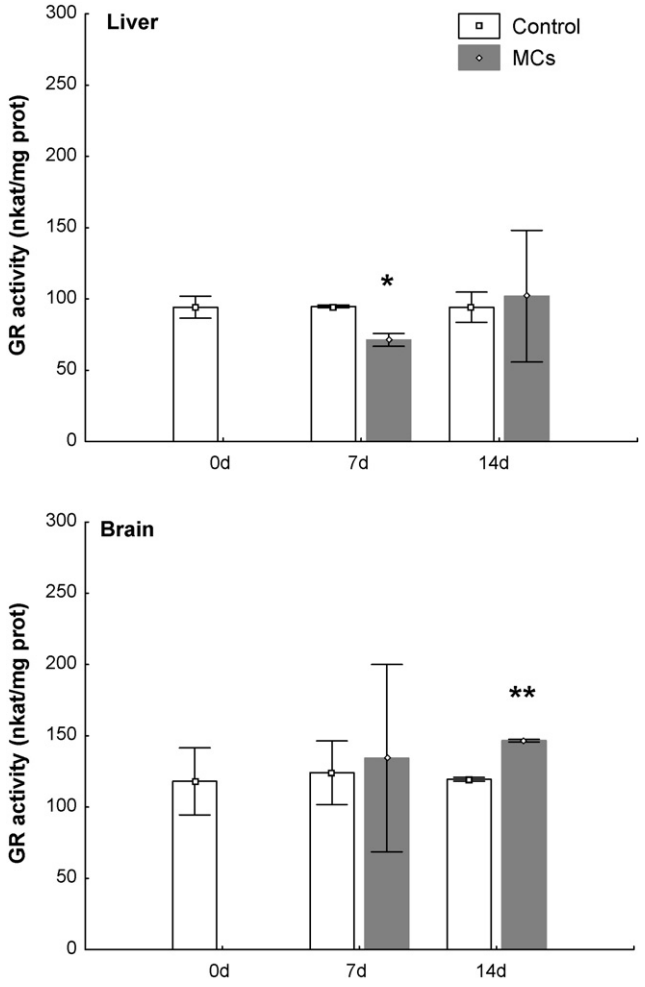

Fig. 5. Glutathione reductase (GR) values in liver and brain of domestic rabbits exposed to MCs. The values are expressed as mean ± S.E. The number of measurements performed in each group was three. The significance levels observed are $^{*}P < 0.05$ and $^{**}P < 0.01$ in comparison to control group values.

4. Discussion

Oxidative stress is usually defined as an adverse reaction resulting from the exposure of molecules, cells, or tissues to excess level of oxidants, particularly reactive oxygen species (Ding and Ong, 2003). Oxidative stress can damage cells or tissues by increasing LPO. There are several reports of microcystin-induced LPO in the cells of rats or mice (Ding et al., 1998; Weng et al., 2007). Thus, it is believed that LPO might be one of the important events responsible for the hepatotoxicity of microcystins (Towner et al., 2002). Usually, cellular oxidative stress is established when the pro-oxidant forces overwhelm the antioxidant defenses (Packer, 1995). Some acute MC exposure tests showed serious oxidative stress in different organs in mammals (Guzman and Solter, 1999; Moreno et al., 2005). In the present study, we detected increased LPO and significant changes of antioxidant enzymes in liver, kidney and brain of domestic animals under prolonged extracted microcystins exposure at a lower dosage.

As to liver and kidney, our results were in agreement with previous reports on oxidative stress of animals treated with extracted MCs in a high dose (Cazenave et al., 2006; Weng et al., 2007; Xu et al., 2007). However, compared with these acute tests at a high dose of MCs, oxidative stress in our experiment was relatively light. Moreno et al. (2005) reported significant increases in LPO levels in liver (121 and 196% for 100 and 150 mg/kg, respectively) and kidney (48 and 58% for 100 and 150 mg/kg, respectively), while the average increase was less than 20% in the present study. It is likely that the animals had enough time to reduce the toxic effects in our sub-chronic test although they were exposed to extracted MCs daily.

Both ROS and LPO are known to be reduced by the activity of antioxidant enzymes, such as GPx, GR, CAT and SOD (Packer, 1995). Some acute tests have shown obvious alterations in these enzymatic activities in liver and kidney of mammals exposed to a higher dosage of MC (Moreno et al., 2005; Jayaraj et al., 2006). However, in the present study, antioxidant enzymes responded differently in diverse organs after a sub-chronic exposure at a lower dosage of MCs. In liver, mainly SOD, CAT and GR showed changes, while in brain, mainly GPx and GR activities were influenced, while only CAT changed in kidney. Apparently, there was a significant difference in the response of antioxidant enzymes in liver and kidney between acute and sub-chronic MC exposures.

Previous studies have demonstrated that liver is the main target organ in animals after exposure of MCs (Falconer, 1994). In terms of oxidative stress, it has been demonstrated that liver is the most affected organ under the acute exposure of MCs (Moreno et al., 2005; Prieto et al., 2006, 2007). Prieto et al. (2006) reported that, with regard to LPO values, liver was the most affected organ in all the organs studied by an acute exposure of MC-LR. While in the present study, we did not find that liver was the most affected organ with regard of LPO. And even, LPO level had an obvious decrease in liver with prolonging exposure of MCs. It could be taken into account the possible depuration of MCs in liver as the exposure time prolonging. It has been reported that MC in animal liver could be converted to a more polar compound with glutathione enzymatically via soluble glutathione-S-transferase (Kondo et al., 1992). On the other hand, liver is the organ with a higher level of antioxidant enzymes which are responsible for the reactive oxygen species clearance. Based on these facts, animals may have more time to metabolize the toxins or to form an adaptive response to reduce the adverse effects when exposed to the low dose of extracted MCs.

To our extent of knowledge, there were only limited studies about the toxic effects of MC on the brain of mammals. In the present study, the oxidative stress induced by MCs in brain was as serious as in liver and kidney, suggesting that brain may also be a target of MCs in mammals (Maidana et al., 2006).

In the present study, the oxidative stress response induced by a lower dose of extracted MCs showed differently in organs of rabbit. The effects of MCs on different organs might be related to the mode of uptake, biotransformation and bioaccumulation capabilities by different organs (Cazenave et al., 2006). Differences of the oxidative stress response following MC exposure between different previous studies can be due to different experimental systems, exposure times, routes and doses. Mechanisms for oxidative stress are complex. Mitochondrial dysfunction induced by MC is considered as a key factor for free radicals production (Ding and Ong, 2003). It has also been supposed that under MCs exposure a block in electron flow along the respiratory chain results in an increased production of free radicals, and LPO products (Turrens, 2003). Recently, our researches have shown the obvious oxidative stress response followed the mitochondrial damage induced by extracted MC in rabbits (Zhao et al., 2008). In our present study, the oxidative stress response showed obviously in different organs. But the exact mechanism for oxidative stress and the difference in organs still needs further researches.

In conclusion, this kind of exposure which mimics a natural route of exposure of MCs assessed the possible damage to domestic animals. Sub-chronic exposure of lower dosage of MCs could also induce oxidative stress in diverse organs in domestic rabbits, although this did not induce fatal damage to animals compared with other acute tests. Daily exposure of a low dose of MCs may stimulate certain adoptive response in animals to resist the adverse effects.

Conflict of interest

There are no conflicts of interest.

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