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The intake of water containing a mix of pollutants at environmentally relevant concentrations leads to defensive response deficit in male C57Bl/6J mice

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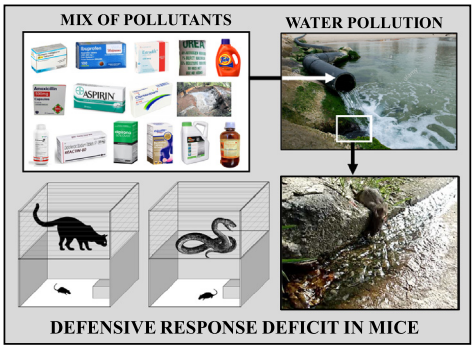
HIGHLIGHTS

- Water containing a mix of pollutants (Mix) leads to behavioral disorders in mouse.
- Defensive response deficit in male mice exposed to water containing a mix of pollutants.
- Mouse that ingested pollutant mix (in the water) did not recognize cat as a potential predator.

GRAPHICAL ABSTRACT



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ABSTRACT

Previous studies have individually confirmed the toxic effects from different pollutants on mammals. However, effects resulting from the exposure of these animals to multi-pollutant mixes have not been studied so far. Thus, the aim of the current study is to assess the effect from the chronic exposure (105 days) of C57Bl/6J mice to a mix of pollutants on their response to potential predators. In order to do so, the following groups were formed: "control", "Mix 1× [compounds from 15 pollutants identified in surface waters at environmentally relevant concentration (ERC)]", "Mix 10×" and "Mix 25×" (concentrations 10 and 25 times higher than the ERC). From the 100th experimental day on, the animals were subjected to tests in order to investigate whether they showed locomotor, visual, olfactory and auditory changes, since these abilities are essential to their anti-predatory behavior. Next, the animals' behavior towards potential predators (*Felis catus* and *Pantherophis guttatus*) was assessed. The herein collected data did not show defensive response from any of the experimental groups to the predatory stimulus provided by *P. guttatus*. However, the control animals, only, presented anti-predatory behavior when *F. catus* was introduced in the apparatus, fact that suggests defensive response deficit

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Complex pollutants
Aquatic pollution

resulting from the treatments. Thus, the current study is pioneer in showing that the chronic intake of water containing a mix of pollutants (even at low concentrations) leads to behavioral disorders able to affect the survival and population dynamics of mammalian species at ecological level.

1. Introduction

Agricultural practices that adopt traditional soil preparation techniques, phytosanitary control by means of pesticides, and substantial amounts of chemical fertilizers are anthropogenic activities that, despite their high toxicological potential, play a key role in the supply of human population demands, (Godfray et al., 2010; Foley et al., 2011). Nowadays, nitrogen is one of the fertilizers most applied to different crops, since it is essential to plant nutrition, development and yield (Fageria and Baligar, 2005); however, it can be highly harmful to different organisms if it is indiscriminately used (Carpenter et al., 1998; Deng et al., 2017). In addition, the use of agrochemicals has considerably increased in recent years (Ecobichon, 2001; Tilman et al., 2002); glyphosate (Woodburn, 2000; Duke and Powles, 2008) and abamectin (Lasota and Dybas, 1990; Bai and Ogbourne, 2016), which may affect non-target organisms and have harmful effects on biological communities, stand out among the most used agrochemicals in the world. If, on the one hand, these agrochemicals strongly help increasing agricultural production and productivity; on the other hand, the loss of chemical fertilizers and the spread of pesticides due to surface runoff or leaching, for example, is an imminent watercourse-contamination source (Carpenter et al., 1998; Hansen et al., 2000).

Another issue negatively affecting different ecosystems lies on the dumping of untreated or ineffectively treated agro-industrial effluents in waterbodies (Ali et al., 2011). Tannery effluents (TE), which result from bovine skin processing (for leather production) conducted in tannery industries mainly located in South American and Asian countries (Sabumon, 2016), stand out among these effluents. The aforementioned industries have great economic importance in these countries, since the leather they produce is used as raw material by different sectors such as automobile, clothing, footwear, furniture, among others (China and Ndaro, 2015; Sabumon, 2016). However, this activity has great pollutant potential, since the effluents produced at different bovine skin processing stages contain several compounds, which are harmful to the health of distinct species (Estrela et al., 2017; Mendes et al., 2017; Souza et al., 2017a; Montalvão et al., 2017; Rocha and de-Oliveira, 2017; Montalvão et al., 2018).

The inappropriate disposal of domestic sewage in the environment has also caused severe environmental issues in different countries (Noorhosseini et al., 2017). The sewage discharge into rivers, streams and/or lakes leads to the dispersion of several aquatic pollutants such as surfactants, synthetic hormones, organic material, medicinal product residues, among others (Deblonde et al., 2011). Studies have shown that medicinal products have been mainly inserted into the environment through domestic sewage (Kanda et al., 2003; Östman et al., 2017), either through its excretion, personal hygiene or direct disposal into sewage systems (Daughton and Ruhoy, 2009).

Aiming at investigating the impacts from different contaminants found in surface waters on the biota, several studies have shown the negative effects from xenobiotics on different experimental models and helped better understanding how these pollutants affect species' survival and reproduction. Among them, it is worth highlighting the studies by Novelli et al. (2012), Bridi et al. (2017) and de Moura et al. (2017) - comprising agrochemicals; by Mennigen et al. (2017), Cardoso-Vera et al. (2017) and Zhou et al. (2017) - about residues of pharmaceutical origin; by Huang et al. (2003) - involving petroleum-derived pollutants; by Hecnar (1995), Yousef et al. (2017) and Abdelfattah et al. (2017) - comprising fertilizers; and by Adeel et al. (2017) and Balmuri et al. (2017), which investigated synthetic hormones and surfactants, respectively.

Although the aforementioned studies play a key role in the identification and characterization of harmful effects from pollutants on different organisms, it is undeniable that they may not represent, in a more realistic way, what happens in the environment. Many of these investigations have assessed the effect from specific contaminants by adopting concentrations or doses much higher than those found in the environment. Moreover, many studies define the contaminant concentrations/doses to be assessed, based on acute toxicity tests (e.g., median lethal concentrations (LC_{50}) or median lethal doses (LD_{50})), which are recommended for very specific cases, only. Thus, the prioritization of studies that do not represent real situations (i.e.: poorly applied), and/or that investigate the effects from a single pollutant on the biota, leads to the neglect of the fact that a great diversity of pollutants derived from different pollution sources is found in the environment. In addition, it is worth taking into consideration that certain pollutant combinations may interact and produce different toxic effects from those attributed to a specific contaminant type.

Therefore, the aim of the present study was to assess the impact from the chronic intake of water containing a mix of pollutants on the behavior of male C57Bl/6J mice exposed to potential predators; it was done in order to improve the knowledge about the effects from complex contaminant combinations on the biota. We herein assume that the intake of water containing several contaminants (at environmentally relevant concentrations) may lead these animals to develop defensive behavior disorders, and it allows better understanding the magnitude of the impacts these pollutants have on natural populations.

2. Materials and methods

2.1. Animals and experimental design

Male C57Bl/6J mice (nulliparous), belonging to the age group 21–32 days, were used in the present study; they were kept in the Animal House of the Biological Research Laboratory of Goiano Federal Institute – Urutaí Campus (GO, Brazil), according to conventional animal housing sanitation standards, at 22–24 °C, under 12 h light cycle, and 58% ± 3% controlled humidity. The animals were placed in polypropylene boxes for mice (41 cm long × 34 cm wide × 16 cm tall) covered with galvanized grille treated with antioxidant. Water (with or without treatment) and standard rodent food ad libitum were supplied to the animals.

Initially, 44 mice were counter-balanced according to co-variables "age" and "biomass", so that their ages and body masses were statistically equal at the beginning of the experiment. Subsequently, the animals were distributed in the following experimental groups:

1) Control ($n = 11$) - consisted of animals that received pollutant-free drinking water;
2) Mix 1× (n = 11) - composed of animals that received water containing a mix of pollutants at concentrations previously found in surface waters (i.e., environmentally relevant concentrations);
3) Mix 10× ($n = 11$) - comprised animals that received water containing the mix of pollutants mentioned above, but at concentrations 10 times higher than the environmentally relevant ones;
4) Mix 25× ($n = 11$) - composed of animals that received water containing the aforementioned mix at concentrations 25 times higher than those set in the Mix 1× group.