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Precopulatory sexual behavior of male mice is changed by the exposure to tannery effluent

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HIGHLIGHTS

- Mice exposed to tannery effluent (TE) show altered sexual behavior.
- Exposure to TE can have a negative impact on the reproduction of mice.
- TE causes behavioral disorders related to sexual motivation and preference in male mice.

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ABSTRACT

Although the toxic potential of tannery effluents (TE) is acknowledged, the impacts these residues have on mammals who intake water contaminated with this pollutant are not completely known. Thus, in order to broaden the knowledge about how these contaminants affect the biota, the aim of the current study is to assess different behavioral categories (e.g.: sexual odor preference, opposite-sex attraction, and sexual discrimination) related to the sexual motivation and pre-copulation of male Swiss mice subjected to TE intake for 30 days, at concentrations 0.8% and 22%. The animals were subjected to locomotor performance evaluation through the Basso Mouse Scale (BMS), as well as to the open field (OF), odor preference (OPT), sexual orientation (SOT) and to scent marking tests (SMT) one week before the experiment ended. Our results evidenced that the treatments did not affect the animals' locomotor activity (in OF and BMS) or caused changes compatible to anxiogenic or anxiolytic behavior (in OF). However, mice exposed to TE (at both concentrations) presented discriminatory capacity deficit in the OPT test at the time to distinguish conspecific odors from the same sex, and from the opposite sex. They randomly explored (without preference) males and females, did not responded to stimuli in the SOT test, as well as did not appear capable of detecting female odor (in estrus phase) during the SMT. Thus, the current study was pioneer in evidencing that TE can influence the reproduction and the population dynamics of small rodents who intake water contaminated with the pollutant.

1. Introduction

It has been consensus that human population growth, followed by the accelerated urbanization and industrialization processes, has been causing, as consequence, the pollution of natural resources (Ma et al., 2009; Benitez et al., 2014). One of the highly polluting residues that has been generated in large scale, mainly in developing countries, concerns those resulting from the tannery industry - which processes bovine skin to produce leather (Sabumon, 2016). Tannery effluents (TE) are generated in different leather manufacturing phases and are constituted by a whole diversity of chemical components (Guimarães et al., 2016) capable of causing negative impact on organisms when they are discarded in the environment without treatment, or after being ineffectively treated (Souza et al., 2016a). However, aquatic pollution resulting from these residues has been observed in different locations, mostly in

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Asian and South American countries (Hu et al., 2011; China and Ndaro, 2015; Sabumon, 2016) that concentrate large numbers of rudimentary small-sized tannery industries.

Accordingly, TE discharge becomes an environmental issue with undesired consequences not just in aquatic organisms living in receptor water courses, but also in those ingesting water from these resources (Souza et al., 2016a). Recent studies have been pointing towards different implications from the exposure of representatives belonging to subphylum Craniata to these contaminants [(i.e.: fish: Aich et al. (2015) and Rocha and Oliveira (2017); amphibians: Montalvão et al. (2017a, b), birds: Souza et al. (2017a) and mammals: Souza et al. (2017b) and Guimarães et al. (2017)]. Overall, such investigations have provided clear evidences about the TE toxicological potential, even at small concentrations and for short exposure periods, thus showing the capacity these pollutants have to cause important biological changes. Among the biological impacts observed, there are histological, biochemical, cytological, behavioral changes (Souza et al., 2016a).

With regard to mammals, previous studies have been pointing towards the negative impacts, mainly on these animals' behaviors (Siqueira et al., 2011; Almeida et al., 2016; Rabelo et al., 2016, 2017; Silva et al., 2016; Guimarães et al., 2016, 2017; Souza et al., 2017b), and evidencing the neurotoxic effect of TE, although some studies also show biochemical (Moysés et al., 2014, 2017) and histopathological (Souza et al., 2016b) changes. Although the aforementioned studies play a key role in the identification and characterization of harmful effects from TE on different rodents, 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. In addition, little attention is given to assessments concerning the impacts from these contaminants on aspects related to the biology of animals living in TE receptor water courses, or who live in areas close to contaminant discharge points and intake contaminated water.

A so far unexplored field refers to the assessment of possible impacts the intake of this water may have on the animals' sexual behavior, which is considered part of a series of standard behaviors capable of having beneficial consequences to the individual or to its species (Agmo, 1997, 1999). According to Mattews & Abdelbaky (2005), such behaviors are preceded by behavioral categories associated with the animals' sexual motivation, which refers to their will on putting their sexual activity in practice (Mattews and Abdelbaky, 2005). The sexual behavior of male rodents, for example, is preceded by stimuli or by hedonically positive events sent by the female; therefore, the motivation triggered by such stimuli is called sexual motivation (Agmo, 1999).

Thus, if one takes into consideration the previous studies about the negative influence of mammal experimental models' exposure to different contaminants on sexual motivation/preference behaviors (Farabollini et al., 2002; Porrini et al., 2005; Cummings et al., 2008; Romano et al., 2012), it is possible questioning whether the intake of water containing TE may also change behaviors of such nature. So far, only the study by Kumar et al. (2008) assessed some relationship between "the reproductive aspects of mammals versus their exposure to TE", in the literature. They orally exposed male Wistar rats to TE for 20 consecutive days and recorded hyperplasia development in the testis seminiferous tubules, as well as an increased of the daily sperm production. Moreover, they found changes in sex accessory tissues followed by changes in the expression pattern of some steroidogenic enzymes in the adrenal and testicular region. Therefore, nothing is known about the sexual motivation/behavior of animals who intake water containing TE.

Assumingly, sexual motivation is essential for the establishment of sexual behaviors; thus, the identification of behavioral abnormalities in individuals exposed to contaminants such as TE allows dimensioning, in a broader sense, how these residues may influence the individuals and the dynamics of their populations. Therefore, the aim of our study was to assess different behavioral categories related to sexual and pre-copulation motivation in male Swiss mice subjected to the intake of TE diluted in water. We start from the hypothesis that individuals exposed to TE, even for a short period-of-time at low pollutant concentrations, present behavior abnormalities linked to their sexual motivations and preferences, by taking into account the chemical complexity of these contaminants, as well as their neurotoxic potential, as previously reported.

2. Materials and methods

2.1. Animals and experimental design

In order to assess the effects of water intake containing TE on behaviors linked to sexual motivation, we used 45 male Swiss males (age group 30–35 days), who were provided by and kept in the Biological Research Laboratory of Goiano Federal Institute (IF Goiano) – Urutaí Campus (Urutaí, GO, Brazil). All the animals were housed in collective standard polypropylene boxes for mice throughout the experimental period (30 cm × 20 cm x 13 cm). The boxes were covered by galvanized wire grills treated with antioxidant and maintained under conventional animal house sanitation, and controlled temperature (from 22 °C to 24 °C) and luminosity (12h light cycle). The animals' diet consisted of standard rodent feed (Nuvilab CR 1) and water, which were provided *ad libitum*.

The following experimental groups were set after the animals' body mass and age were counter-balanced (n = 15/group): i) control group, composed of animals who were treated with drinking water with no contaminants; ii) TE0.8 group, composed of animals kept under the same conditions of the previous group, but who were treated with water contaminated with 0.8% TE and; iii) TE22 group, whose TE concentration in water rated 22%. After the groups were defined, all animals were monitored for 30 days, time that corresponded to approximately one complete spermatogenic cycle in Swiss mice (Oakberg, 1956).

It is worth highlighting that in order to perform the open field test we added a positive control and a baseline group to the experimental design. The positive control group was composed of mice (n = 15) who were administered with intraperitoneal injection of clonazepam (0.5 mg kg^{-1}), 30 min before the test, according to Guimarães et al. (2017). The baseline group (n = 15) was composed of animals who did not receive any treatment; they were minimally handled by the researchers throughout the experimental period and during the 30 days preceding the experiment. These groups were used to assess the sensitivity of the open field test, as well as to show that animals in the control group did not present anxiogenic or anxiolytic behavior.

2.2. Tannery effluent and the determination of the used concentrations

The TE used in the present study was the same one used in the study by Rabelo et al. (2017) and Estrela et al. (2017). It was provided by a tannery industry from Inhumas County (GO, Brazil). The TE was directly collected from a possible illegal discharge point in a brook close to the company. The analysis applied to the organic compounds found in the TE was performed in a desorption electrospray ionization device coupled to a high-resolution mass spectrometer (HRMS), as described by Guimarães et al. (2016). The physiochemical and chemical analyses applied to the raw TE and to