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Testicular damage and farming environments – An integrative ecotoxicological link

Carolina Parelho [a, b, *], Filipe Bernardo [a], Ricardo Camarinho [a, c], Armindo Santos Rodrigues [a, c], Patrícia Garcia [a, b]

[a] Department of Biology, University of the Azores, 9501-801, Ponta Delgada, Portugal
[b] cE3c, Centre for Ecology, Evolution and Environmental Changes, and Azorean Biodiversity Group, University of the Azores, 9501-801, Ponta Delgada, Portugal
[c] CVARG, Center of Volcanology and Geological Risks Assessment, University of the Azores, 9501-801, Ponta Delgada, Portugal

HIGHLIGHTS

- Mice from conventional farming show severe alterations in histological and cellular parameters.
- Mice exposed to conventional farming environments bioaccumulate higher Pb hepatic loads.
- Pb hepatic loads are associated with testicular structural and functional disruption.
- Integrated Biomarker Response revealed that conventional practices entail higher risk to male fertility.

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ABSTRACT

The exposure to agrochemicals during farming activities affects the function of the reproductive system, as revealed by the increasing worldwide evidence of male infertility amongst farmers. The main objective of this study was to untangle the link between agricultural practices and male reproductive impairment due to chronic exposure to xenobiotics (such as agrochemicals) in conventional and organic farming environments. For this purpose, male wild mice (*Mus musculus*) populations from sites representing two distinct farming practices (conventional and organic farming systems) were used as bioindicators for observable effects of testicular damage, namely on a set of histological and cellular parameters: (i) relative volumetric density of different spermatogenic cells and interstitial space; (ii) damage in the seminiferous tubules and (iii) apoptotic cells in the germinal epithelium. Results showed that mice from the conventional farming site bioaccumulated higher Pb hepatic loads, while mice from the organic farming site tend to bioaccumulate higher Cd hepatic loads. In general, for the analyzed testicular damage related parameters, mice from the organic farming site showed a similar performance than mice from the reference site. Mice from the conventional farming site stood out not only by underperforming in most studied parameters, while displaying an association between Pb hepatic loads and the observed testicular structural and functional disruption, but also by the increased stress index (Integrated Biomarker Response value). This study highlights the potential damaging effects of conventional farming practices on testicular structure and function, under natural conditions, raising concern about ensuing fertility risks for farmers.

1. Introduction

Agricultural practices are one of the most significant anthropogenic activities that greatly affect both environment and human health (Horrigan et al., 2002). Human population is expected to grow by one third until 2050, according to FAO estimates (FAO, 2009). How to meet the resulting food demand by sustainable

* Corresponding author. Address: Department of Biology, University of the Azores, Apartado 1422, 9501-801, Ponta Delgada, Azores, Portugal.
E-mail addresses: carolina.pf.parelho@uac.pt (C. Parelho), filipebernardo90@outlook.pt (F. Bernardo), camarinho@uac.pt (R. Camarinho), armindo.s.rodrigues@uac.pt (A.S. Rodrigues), patricia.v.garcia@uac.pt (P. Garcia).

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means, within acceptable environmental impacts, remains a great challenge, since so far prevailing production methods have relied in the intensive use of agrochemicals to enhance soil fertility and crop productivity (Gomiero et al., 2011). On the downside, long-term and extensive land use for agricultural purposes frequently results in the incorporation of several types of pollutants into soil ecosystem, such as pesticides residues and metals, even if they are applied in small amounts (Krami et al., 2013). Present-day agricultural volcanic soils have a unique metallic footprint mostly derived from the volcanic parent rock, modulated by the metal inputs of long-term agricultural practices (Parelho et al., 2014). As a consequence, some of these pollutants slowly accumulate in the ecosystem, contributing to a higher ecotoxicological risk under these environments.

The effects of agrochemical exposures on male reproduction are a topic of considerable concern in environmental, occupational and reproductive toxicology (Mehrpour et al., 2014), as the testicle is considered one of the most vulnerable organs to agrochemicals that often act as endocrine disruptors (Mohapatra et al., 2013). Occupational exposure to agrochemicals in farms can occur by directly dealing through mixing, loading and spraying activities (Shadnia et al., 2005) or simply by frequent attendance of such environments, whose soils can act as a sink for several pollutants (Saaltink et al., 2013). Since many potential pollutants exist in these environments, occupational exposure is not easy to be proved. However, the decreased fertility rate observed in men occupationally exposed to agrochemicals is significantly above the expected amongst the general population (Ashiru and Odusanya, 2009).

Most of the well-known adverse effects of agrochemicals upon the testicle are reported in animals under laboratory conditions, following the administration of a single element in high-dose or short-term exposure (e.g. Rajawat et al., 2015). These effects include genotoxicity (e.g. Urióstegui-Acosta et al., 2014), tissue necrosis (e.g. Cavalli et al., 2013), inflammation and structural seminiferous tubular damage (e.g. Li et al., 2013), ultimately entailing a decrease of male reproductive health. However, the cumulative effects of multiple compounds in low-levels, akin to an occupational exposure in a farm, are still not well established (Mantovani et al., 2008) and available data is not enough to determine whether long-term exposure to agrochemicals entails a significant risk to male fertility under field conditions.

Data collected from vertebrates living in/exposed to contaminated areas are of utmost importance since they can bring pertinent information to be used in human risk assessment (Pereira et al., 2006). Furthermore, field studies using animal models provide crucial ecotoxicological data given that, under laboratory conditions, it is difficult to mimic the complexity of field conditions (Pereira et al., 2006; Sánchez-Chardi et al., 2009). Among these vertebrates, small mammals, such as mice, are preferentially selected, particularly for studies regarding terrestrial pollution (Shore and Douben, 1994).

The assessment of physiological effects of chronic exposure to agrochemicals using integrative multi-biomarker approaches is essential to evaluate testicular damage in natural populations under farming environments. The Integrated Biomarker Response index (Beliaeff and Burgeot, 2002) has been developed as effect-based monitoring approach and may be used to integrate a set of early warning responses into a more simple and realistic stress model, a valuable tool for ecological risk assessment.

According to Mehrpour et al. (2014) meta-analysis, the majority of pesticides affect the male reproductive system by mechanisms such as reduction of sperm density and motility, inhibition of spermatogenesis, reduction of testes weights, reduction of sperm counts, motility, viability and density, inducing sperm DNA damage and increasing abnormal sperm morphology. These authors also refer other possible effects, such as: epididymis, seminiferous tubule, seminal vesicle and ventral prostate degeneration; changes in plasma levels of testosterone, follicle-stimulating hormone, and luteinizing hormone; decreases in the level and activity of the antioxidant enzymes in testes, and inhibition of testicular steroidogenesis.

Hence, given the worldwide increasing evidence of male infertility amongst farmers, the main objective of this study is to assess if chronic exposure to agrochemicals in distinct farming environments, whose volcanic soils are naturally enriched with metals and anthropogenically molded by agricultural practices, entails elevated risks to male reproductive health. For this purpose, three groups of male wild mice *Mus musculus* from sites representing two distinct farming systems (conventional and organic) and from a site without records of farming activity (reference group), were used as bioindicators for the observable histological and cellular alterations associated with testicular damage. All the information is intended to provide new insights, under field conditions, into the link between agricultural practices, occupational exposure to agrochemicals and male testicular damage.

2. Materials and methods

2.1. Study sites

The fieldwork was conducted in S. Miguel Island (Azores, Portugal), where agricultural practices have gone through significant changes during the last decades, with the transformation of the majority of traditional smallholdings into farms for commercial purposes and where intensive agriculture is practiced following the EU directives, either with the use of synthetic agrochemicals (conventional farming) or not (organic farming).

The selected study sites (Supplementary material – Fig. 1) correspond to two representative farms with different agricultural practices [conventional (CF) and organic (OF)]. In the local context, CF refers to agricultural practices in which the use of synthetic agrochemicals (both pesticides and fertilizers) is legally framed by European and national guidelines. OF systems are certified by the European Commission, therefore the use of synthetic agrochemicals is prohibited and soil amendments are confined to organic fertilizers (compost and manure). The selected farms have been explored under the same farming system for at least 10 years. A reference site (RF) was also included in the study. RF corresponds to a forest reserve of centennial Japanese cedar (*Cryptomeria japonica*), with no historical records or evidence of farming activity. All study sites are located in the same geological complex (Picos Fissural Volcanic System), ensuring the same bedrock and pedological conditions, being differentiated only by the type or absence of agricultural soil management. Supplementary material – Table 1 shows the characterization of each studied farm and reference site, regarding the years of exploitation under the corresponding farming system and use of agrochemicals.

2.2. Mice sampling and preparation of samples for histology

Mus musculus fulfill the criteria of selection as bioindicator species, namely: high abundance, wide life expectancy, enough to estimate possible long-term effects (Marcheselli et al., 2010) and relatively small home range [in average 145 m^2 (Lidicker, 1966)], which is significantly smaller than the surface areas of the chosen study sites (the farming areas have, in average, 5000 m^2).

Three separate sets of 12 adult male *M. musculus* (one set per site) were captured using live-catch traps, housing the mice for no longer than 24 hours before euthanization. The relative age of each