

The Environmental and Biological Risks of Extreme Low Doses of Chlorobenzenes

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Abstract

Several environmental chemicals and pesticides have been found to alter neuroendocrine communication and behaviour in exposed biological systems. The aim of this study was to demonstrate the effects of the ubiquitous chlorobenzenes on the behavioural elements of Wistar rats. For this reason rats (n=50 in each group) were treated with a mixture of 0.1 and 1.0 µg/kg each of hexachlorobenzene and 1,2,4-trichlorobenzene via a gastric tube for 30, 60 and 90 days. At the endpoints of the experiment the behavioural elements of rats were detected and tissue samples were taken for chlorobenzene analysis in gas-chromatography. It was found that the anxiety-related behavioural elements were altered and chlorobenzenes were present in different tissues.

Introduction

Persistent interaction evolves among living creatures and their environment, this connection maintains the equilibrium of the biotic and abiotic systems. Results were born with the activity of mankind, which have altered the local environments of the territories of Earth. The procedure was induced by the applied chemicals and the purposes of their use, basic biological and ecological problems of chemical application in agriculture and horticulture, namely by the chemisation. These chemicals are xenobiotics. One group of special interest is the persistent organic pollutants (POPs). One group of the heterogeneous POPs can interfere with the endocrine communication that called endocrine disruptor agents (EDCs). EDCs are proved to have an effect on homeostasis and/or its regulation [1] [2]. The toxicity of POPs results their hazardous nature, in combination with high chemical and biological stability, and a high degree of lipophilicity. Various benzene derivatives such as alkylbenzenes and chlorobenzenes, however, continue to be used as chemical intermediates, solvents, pesticides in spite of incomplete knowledge of their chronic toxicity. Several of the chlorinated benzenes are known to be pro-mutagenic, carcinogenic, mutagenic in animals and humans, in rats anxiety, aggression [3] [4] and in fish predator avoidance and social behaviours are developed [5] by EDCs.

EDCs can affect sexual and reproductive behaviours. For example exposed female mosquitofish displayed male reproductive behaviour and males showed more aggressive courtship behaviour [6] [7]. Sexual and reproductive behaviour of birds and mammals were also altered by the exposition of POPs. Porter et al. [8] indicated that adult male mice exposed to a mixture of aldicarb, atrazine showed significantly more aggressive behaviour compared to the control groups.

Our aim was to present contemporary research in connection with the complex environmental strains and their effects on the equilibrium process of living organisms. We also wanted to demonstrate the effects of chronic exposure to extremely low (legally often negligible) doses

of a chlorobenzene mixture on the behavior of male Wistar rats and to investigate the organic accumulation of chlorobenzenes in rats.

Experimental

Adult Wistar male rats were (weighing 120-250 g, aged 4-6 weeks at the beginning of the research, n=50) exposed daily with a mixture of 0.1 (D1) and 1.0 (D2) $\mu\text{g}/\text{kg}$ each of hexachlorobenzene and 1,2,4-trichlorobenzene via a gastric tube for 30, 60 and 90 days. After CIB treatment, aggression/anxiety-related behavioral elements were detected in open field and elevated plus maze tests. The animal care and research protocols were in full accordance with the guidelines of University of Szeged, Hungary. During the research period, rats were kept under controlled parameters. Control groups were set up: stress control (n=50, gastrostomy tube insertion group) and absolute control (n=50, untreated group). To compare the means of treatment doses (0.1 and 1.0 $\mu\text{g}/\text{b.w. kg}$) to the controls during 30, 60 and 90 days long treatments two-way ANOVA were run.

At the end of the experiment certain tissues (endocrine, immune, neuronal and reproductive) were removed for chlorobenzene analysis. After the specific extraction the CIB levels were measured by gas-chromatography (Hewlett-Packard 5890 Series II. gas-chromatograph).

Results and discussion

Our experiments revealed statistical differences between treated and untreated groups. As shown in Figure 1. after CIB treatment the anxiety related-behavioural elements altered significantly depending on the dose and the duration of exposure. In Figure 2 anxiety-related elements, the total distance moved and the mean velocity of the movement were decreased in open-field tests following 30, 60 or 90-day CIB treatment.

Figure 1. Anxiety related behaviour measured in elevated plus maze test after CIB treatment (means \pm S.E.M., A: $p < 0.001$, n=50)

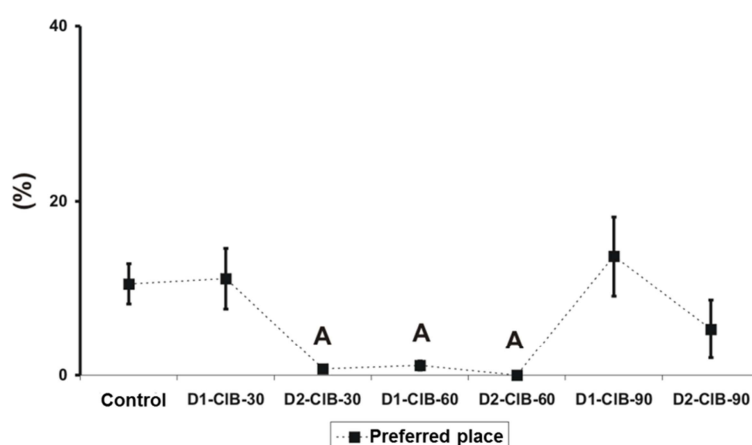
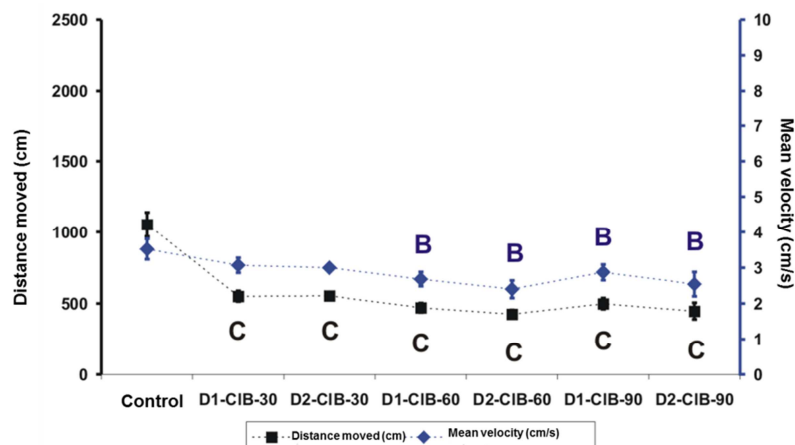


Figure 2. The locomotive behaviour after CIB treatment measured in open field test (means ± S.E.M., B, C: p<0.001, n=50)



In Table 1., it is showed that the CIB is present in the different types (endocrine, immune and neuro) of tissue both in the treated and groups.

Table 1. Determination of CIB content in different tissue types after 0.1 µg/ b.w.kg doses of CIB exposition (pg/g tissue; n=50)

Tissue type	Control	30 days	60 days	90 days
Adipose tissue of abdomen	<0.001	0.048	0.113	0.321
Liver	<0.001	0.011	0.087	0.101
Brain	<0.001	<0.001	0.015	0.027
Bone marrow	<0.001	0.025	0.063	0.131
Adrenal cortex	<0.001	0.031	0.087	0.151

It is generally accepted that POP/EDCs may alter a wide variety of behavior, including sexual and reproductive behavior, communication, dominance, aggression and cognitive elements such as attention, learning and memory. The mechanisms are still often unclear, or the attempts to explain them involve known target hormones (usually steroids and/or thyroids) at the level of synthesis, storage, release, transport, clearance, receptor, or receptor recognition) within the relevant brain areas.

Conclusion

In conclusion, the subtoxic and chronic exposures to extremely low doses of endocrine disruptor chlorobenzenes affect the anxiety-related behavioural elements of Wistar rats. The ratio of accumulated CIB depends on the modulation of the homeostatic status and the reserved CIB volume. Since the living organisms are threatened by several types of environmental loads it was essential to use combined types of CIBs. According to our findings obtained in an experimental model, the ubiquitous POPs and EDCs exposure agents can generate systematic alterations in the homeostasis of biological systems even in extremely low doses, when acting on the long term.

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