# Effects of a Br-containing oxaphosphole derivative on the body weight and the fertility of male mice

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***Abstract***

*The biological activity of oxaphosphole derivatives is not well explored. In this study the effects of Br-containing oxaphosphole derivative (Br-oxph) on body weight and fertility of male mice was evaluated. The mice were treated for 5 days with $2.82x10^{3}$ μg/kg, 2.82 μg/kg and $2.82x10^{-3}$ μg/kg. The body weight change served as an index of the general physiological status. Br-oxph stimulated the growth at 2.82 μg/kg and $2.82x10^{-3}$ μg/kg. After treatment with $2.82x10^{3}$ μg/kg negative influence was observed. The effect of Br-oxph on fertility of male mice was assessed by breeding the treated males with untreated female mice. At all doses tested Br-oxph increased the number of pregnant females, but this effect decreased considerably with increasing the dose.*

***Keywords*****:** *Br-containing oxaphosphole derivative, body weight, fertility, male mice.*

## INTRODUCTION

Phosphorus-containing organic compounds could be successfully applied to agriculture and medicine. Because of that the chemistry of organophosphorus compounds is a subject of increasing interest. A great number of new compounds with different structures and respectively, with different properties have been synthesised [7]. It is established that some allenic phosphororganic compounds stimulate sugar beet root growth, while others have growth-inhibiting effect [14]**.** Phosphorylated allenes served as precursors of the synthesis of new heterocyclic compounds, containing phosphorus and oxygen atoms in the ring - oxaphospholes. Oxaphospholes possess biological activity, which is not well studied. Oxaphospholes revealed a specific biological effect in plant test-systems depending on the structure of the compound, inclusion of different substitutes and dose of treatment [10,11]. Chemical compounds which posses biological activity might influence organisms in different ways and caused disturbances of the physiological functions, as delayed polyneuropathy, immunotoxicity, carcinogenesis and endocrine, developmental and reproductive toxicity [6, 17, 19, 1, 20,]. The reproductive organs have been shown to be among the most vulnerable organs to many chemical compounds [5, 18, 8, 12].

The objective of this investigation was to determine whether the *4-bromo-N,N-diethyl-5,5-dimethyl-2,5-dihydro-1,2-oxaphosphol-2-amine 2-oxide* (Br-oxph) might influence the general physiological status and fertility of male mice.

## MATERIALS AND METHODS

The compound tested *4-bromo-N,N-diethyl-5,5-dimethyl-2,5-dihydro-1,2-oxaphosphol-2-amine 2-oxide* (Br-oxph) was synthesised in the Laboratory of Organic Chemistry of the University of Shumen. The structure of the compound was determined with spectral and elemental analysis [2]**.**

ICR mice were obtained from the Base for Experimental Animals, BAS, Slivnitza. All the experiments were performed under permission and supervision of the Faculty of Natural Sciences of University of Shumen (permission no. 153/02). The animals were kept at temperature of 24°±2°C; 12/12 hours of light/dark cycle. Water and food were supplied *ad libitum*.

Fifteen mature male mice were divided into three groups, treated with $2.82x10^{3}$ μg/kg, 2.82 μg/kg and $2.82x10^{-3}$ μg/kg body weight of Br-oxph. These solutions were administered intraperitonealy with a volume of 1ml per 100 g body weight. Six control

males received injections with 0.9% NaCl instead of Br-oxph. The animals were treated once daily for a period of 5 days.

Body weight change of the treated male mice was used as an index of Br-oxph influence on the general physiological status. Body weight was measured once daily from the first day of the treatment, over a 14-day period.

Fertility test was conducted following the model described by other authors [9]. After the exposure period each male was placed in an individual cage with two virgin untreated females of the same strain and *ad libitum* access to food and tap water. They were left together for ten days during which two estrus cycles should have elapsed. The males were then removed, and 10 days later the females were killed by cervical dislocation under light ether anesthesia, and the following measurements were recorded: numbers of pregnant females, numbers of implantations per pregnant female and numbers of viable fetuses.

Statistical analysis of the experimental data were processed by Student's t-test.

**RESULTS**

The results about the effects of treatment with Br-oxph for 5 days on the body weight and the fertility of male mice are summarized in Figure 1 and Table 1.

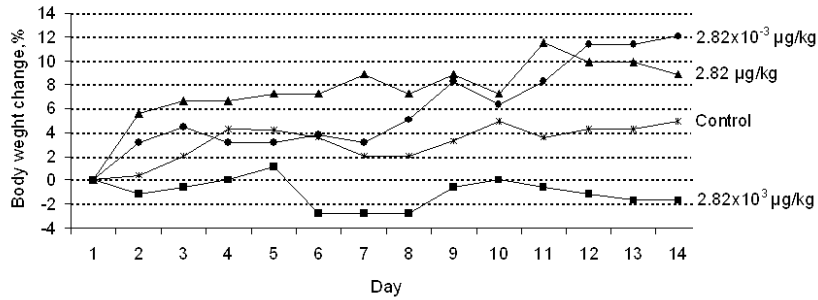


Fig. 1. Effects of 5 days treatment with Br-oxph on the body weight change of male mice.

From Figure 1 it can be seen that the body weight of the animals from the control group increased during the first days, and after that remained constant till the 14-th day. The highest Br-oxph dose of $2.82x10^3$ µg/kg had negative influence on the body weight: in the end of 14-day period, it was with 1.65% lower compared to the initial weight. At the other two doses tested Br-oxph had a positive effect.

Table 1.
Effects of 5 day treatment with Br-oxph on the fertility of male mice

| Dose (µg/kg) | No. of males | Pregnant Females | No. of implantations | No. of viable fetuses |
|---|---|---|---|---|
| Control | 6 | 0.42 ± 0.52 | 9.8 ± 1.48 | 9 ± 2.45 |
| $2.82x10^{-3}$ | 5 | 0.90 ± 0.32* | 9.78 ± 3.27 | 8.22 ± 1.56 |
| 2.82 | 4 | 0.88 ± 0.35* | 8.57 ± 4.5 | 6.43 ± 4.96** |
| $2.82x10^3$ | 5 | 0.70 ± 0.48 | 12.14 ± 4.18*** | 12 ± 4.24*** |

*Note.* Each value is expressed as mean ± SD; *P≤0.05, **P≤0.01, ***P≤0.001.

The number of the pregnant females, the number of implantations per pregnant female, and the number of viable fetuses were used to assess the fertility of the treated animals (Table 1). At all doses tested Br-oxph increased the number of the pregnant females compared to the controls, but the effect considerably decreased with increasing the dose. Noticeable variation of the average number of implantations per pregnant female and of the average number of viable implantations was observed in all experimental groups. Significant increasing of the average number of fetuses was established only after treatment with the highest Br-oxph dose. The same was true about the number of viable fetuses, but significant opposite effect was observed at $2.82x10^{-6}$ μg/kg.

**DISCUSSION**

In the present study the body weight change served as an index of Br-oxph influence on the general physiological status of male mice. After treatment with Br-oxph was observed linear dose-effect dependence. The stimulation of the body weight of male mice at doses of 2.82 μg/kg and $2.82x10^{-3}$ μg/kg revealed positive influence on the physiological processes. This effect decreased with increasing the dose, and at the highest dose of $2.82x10^{3}$ μg/kg even the opposite effect was observed.

The effect of Br-oxph on fertility of male mice was assessed by breeding the treated males with untreated female mice. The analysis of the results showed that Br-oxph at all doses tested increased the number of pregnant females. We observed similar linear dose-effect dependence - this effect decreased with increasing the dose, and at $2.82x10^{3}$ μg/kg turned to a trend.

The increased fertility of males might be consequence of different factors – alterations in specific enzyme activities as well as structural and metabolic alterations in sperm [4, 15, 16]. *In vitro* experiments demonstrated that changes in the sperms functions might be a result of alterations of androgen-dependent enzymes activity [21]. So, the effects of Br-oxph might be connected with its impact on the hormone-dependant reactions. This could as well be an explanation for the positive influence on the body weight of males because of the anabolic effect of androgens [13, 3]. This influence was established at doses of 2.82 μg/kg and $2.82x10^{-3}$ μg/kg. It requires further clarification, as the increased percentage of pregnant females does not corresponded to the number of the viable embryos.

In summary, our study demonstrated positive influence of *4-bromo-N,N-diethyl-5,5-dimethyl-2,5-dihydro-1,2-oxaphosphol-2-amine 2-oxide* (at doses below $2.82x10^{3}$ μg/kg) on the general physiological status and fertility of male mice.

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**Докладът е рецензиран.**