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*Full Length Research Paper*

# Activity of boric acid on German cockroaches: Analysis of residues and effects on reproduction

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**In order to complete previous studies, boric acid (an inorganic compound) was evaluated on reproduction during the first gonadotrophic cycle (0, 2, 4 and 6 days) of the German cockroach, *Blattella germanica* (Insecta, Dictyoptera). The compound was incorporated into the diet and orally administrated at two concentrations 8.2 and 49.6% corresponding to LC50 and LC90, respectively. The observed effects were correlated with the amount of boric acid detected into the body. Data showed that boric acid reduced the number of oocytes per paired ovaries and the size of basal oocytes. Furthermore, the compound was examined on ovarian contents of proteins, lipids and carbohydrates. Results from biochemical analyses revealed a significant reduction of ovarian constituents with the two tested doses. Finally, the quantity of boric acid really incorporated into the body was determined for the two tested doses (LC50 and LC90) during the first six days of the adult life. The amounts of residues incorporated into the body increased as a function the doses and the period of treatment.**

**Key words:** German cockroach, boric acid, reproduction, Ovary, residues.

## INTRODUCTION

Neurotoxic insecticides such as organophosphates, carbamates and pyrethroids were successful in controling insect pests during the past decades. Unfortunately, many of these chemicals are harmful to man and beneficial organisms and cause ecological disturbances (Ishaaya and Horowitz, 1998). Furthermore a rapid development of resistances was noted in many, species (Georghiou and Lagunes-Tejeda, 1991) especially in the most prevalent cockroach, the German cockroach *Blattella germanica* (Scott et al., 1990; Dong et al., 1998). This species is known for its high reproductive performance and its economic and medical importance (Gordon, 1996; Grandcolas, 1998).

Thus, many conventional pesticides have been replaced by newer biorational or low risk insecticides with novel mode of action like insect growth regulators and biopesticides (Horowitz and Ishaaya, 2002; Dhadialla et al., 2005). Moreover, the most recent technological advances in cockroach control consist in use of bait formulations (Appel, 2004). Additionally, interest has again centred on lesser-used compounds such as boric acid (Gore and Schal, 2004; Gore et al., 2004). However, the mode of action of boric acid on insects has not been well established and several hypotheses have been proposed, including abrasive effect on the cuticle followed by slow drying (Ebeling et al., 1975) or destruction of foregut cells causing death by starvation (Cochran, 1995). In a previous study, we have shown that boric acid affected the cuticular profile of *B. germanica* (Kilani-Morakchi et al., 2005). More recently, we have demonstrated that ingested boric acid caused structural alterations in the mid-gut of German cockroach. Moreover, this compound exhibited a neurotoxic action as evidenced by symptoms of poisoning and a reduction in acetyl cholinesterase activity (Habes et al., 2006). In order to extend these previous findings and to obtain more information on the mode of action of boric acid, we examined the effects of this compound administrated by ingestion at two doses (LC50 and LC90) on reproduction

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**Table 1.** Amounts of boric acid residues (ng/mg of tissue) in insect body as function the dose (8.2 and 49.6%) and the duration of treatment (days) following oral application on newly emerged adult females of *B. germanica* (mean ± SEM; n =3-5). For each exposure time, mean values followed by different letters are significantly different (*** P≤0.001).

| Exposure time (days) | Control | LC50 (8.2%) | LC90 (49.6%) |
|---|---|---|---|
| 0 | 0 ± 0 | 0 ± 0 | 0 ± 0 |
| 2 | 0 ± 0 a | 3159.4 ± 40.70b | 4942.33 ± 183.86c |
| 4 | 0 ± 0 a | 5090.16 ± 54.33b | 5568 ± 114.05c |
| 6 | 0 ± 0 a | 6810.35 ± 233.02b | 8321 ± 98.98c |

of *B. germanica* and measured the quantity really incorporated into the body.

## MATERIALS AND METHODS

### Insects

Colonies of *B. germanica* were reared in plastic boxes (30 x 30 x 30 cm) and maintained at 27 ± 1°C, 80% relative humidity under a 12:12 dark-light photoperiod. The cockroaches were provided *ad libitum* with water and dry dog food as previously described (Habes et al., 2006).

### Chemical and treatments

Boric acid (Merck, France) was incorporated into the diet (weight/weight) and orally administrated to newly emerged adults females (< 3 h old) at two doses, 8.2 and 49.6% corresponding respectively to LC50 and LC90 after 6 days of treatment (Habes et al., 2006). Control cockroaches were provided with untreated diet.

### Analysis of residues

The amount of boric acid incorporated into insect body was determined as a function the doses (LC50 and LC90) and the duration of treatment (0, 2, 4 and 6 days). Insect's bodies were sonicated in ethanol 90°C and centrifuged (4,000 g for 15 min). The supernatants were removed and boric acid content in each samples was quantified in an aliquot (100 µl) according to Rodier (1975) using 300 µl of curcumin solution (12.5 mg curcumin, 10 ml acetic acid), 300 µl acid reagent (acetic acid/sulfuric acid: v/v) and 1 ml of ammonium acetate buffer. Absorbances were measured at 555 nm using a boric acid standard solution (40 mg/l) of serial dilution. Boric acid amounts were expressed as ng per mg of fresh tissues.

### Morphometric measurements

Each treated female was immediately paired with males, in individual plastic box (9.5 x 6.5 x 2 cm) containing food and water. Adult females from control and treated series were sampled at 0, 2, 4 and 6 days of adult life, during the first gonadotrophic cycle (Pascual et al., 1992), and their ovaries dissected out. After removal of circumovarian fat body, the number of oocytes in each paired ovaries was recorded. The volume of basal oocytes was calculated according to Lambreas et al. (1991) using the following formula:

$$\text{Volume} = 4\pi/3 \text{ (length / 2) (width / 2)}^2$$

### Determination of ovarian constituents

The extraction of the different constituents was made following the procedure of Shibko et al. (1966). Paired ovaries were collected at various times from control and treated adult females and homogenized individually in 1 ml of aqueous trichloroacetic acid (20%). The amount of proteins was measured in an aliquot (100 µl) using Coomassie blue method (Bradford, 1976) with bovine serum albumin (Sigma) as standard. Ovarian lipids were quantited according to Goldsworthy et al. (1972) using the vanillin method. Carbohydrates content was determined by the anthrone method (Duchateau and Florkin, 1959) as previously described (Soltani and Soltani-Mazouni, 1992). Data on ovarian constituents were expressed as µg per paired ovaries.

### Statistics

Results are presented as the mean ± SEM. The age and the number of animals tested per series are given with the results. The significance difference was estimated using Sudent's test. Data were subjected to a two-way analysis of variance (ANOVA). Homogeneity of variances was controlled by the Levene method (Dagnelie, 1998). All data were statistically analyzed by the MINITAB Software (version 13.31, PA State College, USA) and the significant level was p<0.05.

## RESULTS

### Dosage of residues

Boric acid was applied by ingestion on new adult females. Residues were determined by a colorimetrical method in whole insect bodies during the first six day following adult emergence. The results presented in Table 1 showed that the amount of residues increased progressively as a function the dose and the duration of treatment (p≤0.001). The mean amount detected was 6810.35 ± 233.02 and 8321.24 ± 98.98 ng of boric acid per mg of tissue after 6 days, respectively, with the LC50 and LC90.

### Effects on morphometric measurements of ovaries

In controls, the number of oocytes per paired ovaries increased at 2 days and decrease thereafter (p<0.001). Boric acid orally administrated to newly emerged females

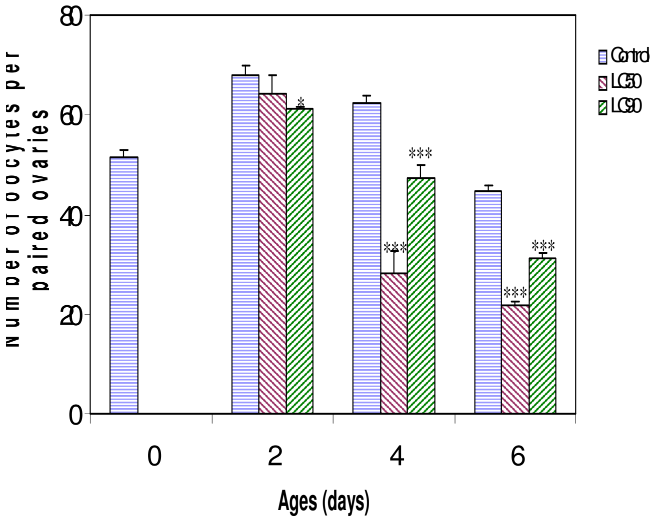


**Figure 1.** Effect of ingested boric acid (8.2 and 49.6%) on the number of oocytes per paired ovaries in adult females of *B. germanica* during the first 6 days following adult emergence (mean ± SEM; n = 6 - 10; asterisks indicate a significant difference between control and treated series of the same age; *p<0.05; **p<0.01; ***p<0.001).

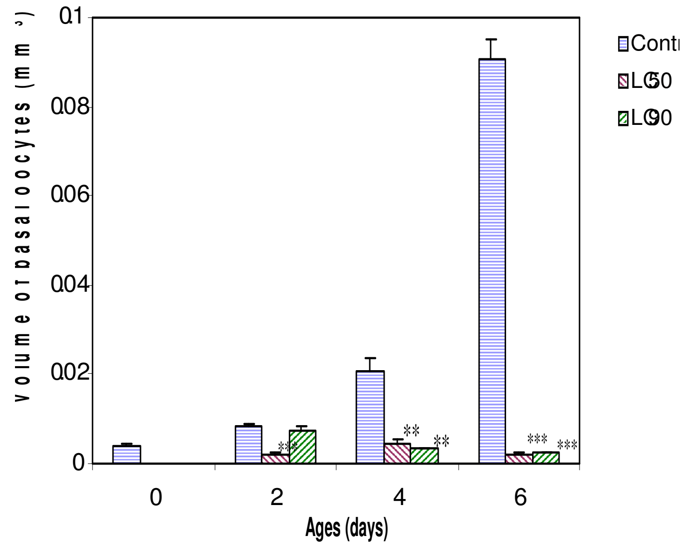


**Figure 2.** Effect of ingested boric acid (8.2 and 49.6%) on the volume of basal oocytes in adult females of *B. germanica* during the first 6 days following adult emergence (mean ± SEM; n = 6 - 10; asterisks indicate a significant difference between control and treated series of the same age; *p<0.05; **p<0.01; ***p<0.001).

of *B. germanica*, significantly reduced the number of oocytes recorded at days 4 and 6 ($p \leq 0.001$) for the LD50 and at all tested ages during the experimental period for the LD90 ($p \leq 0.001$) as compared to controls (Figure 1).

ANOVA revealed a significant effect of the compound on number of oocytes ($p \leq 0.001$) as function of the dose and the duration of treatment during the first days of the adult life. Treatment affected the number of oocytes per paired ovaries with a dose-response relationship. The volume of basal oocytes also increased during this period from 0.004 ± 0.0003 at 0 day to 0.09 ± 0.004 $mm^3$ at 6 days in control series. Boric acid caused a significant decrease ($p<0.01$) in the volume of basal oocytes respectively at all ages with the LC50 and at 4 and 6 days with the LC90 (Figure 2). The ANOVA ($p<0.001$) indicated a significant effect of treatment, age and interaction treatment-age.

### Effects on biochemical composition of ovaries

In control, the principal constituents (proteins, lipids and carbohydrates) of ovaries showed a peak at day 4 after adult emergence (Figure 3). Boric acid reduced significantly the ovarian protein content with a dose-response relationship at day 4 ($p \leq 0.01$) with the LD50 and at days 2 ($p=0.007$) and 4 ($p<0.001$) with the LD90 as compared to controls (Figure 3A). The ovarian lipid content was also reduced in treated series at 2 ($p<0.001$), 4 ($p=0.011$) and 6 days ($p<0.001$) with the low dose and at days 4 and 6 ($p \leq 0.001$) with the high dose (Figure 3B), respectively. Finally, the compound at the two tested doses also reduced significantly ($p \leq 0.01$) the carbohydrate contents in ovaries at all tested ages (Figure 3C). These effects were dose-dependant. ANOVA showed a significant effect of the compound ($p<0.001$) as function of the dose and the duration of treatment for all ovarian constituents.

## DISCUSSION

In insects, reproduction comprises a succession of interdependent steps, from sex determination to oviposition, all of which are regulated by certain hormonal factors, including ecdysteroids and juvenile hormone, but also neurohormones with gonadotropic and antigonadotropic effects (Bellés, 1995; Gäde and Hoffmann, 2005). In *B. germanica,* as in all cockroaches studied to date, vitellogenesis and cyclic maturation of oocytes depends upon juvenile hormone III synthesis by the corpora allata (CA). The relative activity of the CA in adult female is dependent upon and modulated by intrinsic signals which may originate from the brain and ovary and which may be influenced by nutrient status of the female (Schal et al., 1997).

In the present study we orally applied an inorganic compound boric acid to newly emerged females of *B. germanica*. Boric acid residues were accumulated in insect body with a dose-response effect. However, despite the significant differences observed between the amounts of residues incorporated with the two doses at all tested

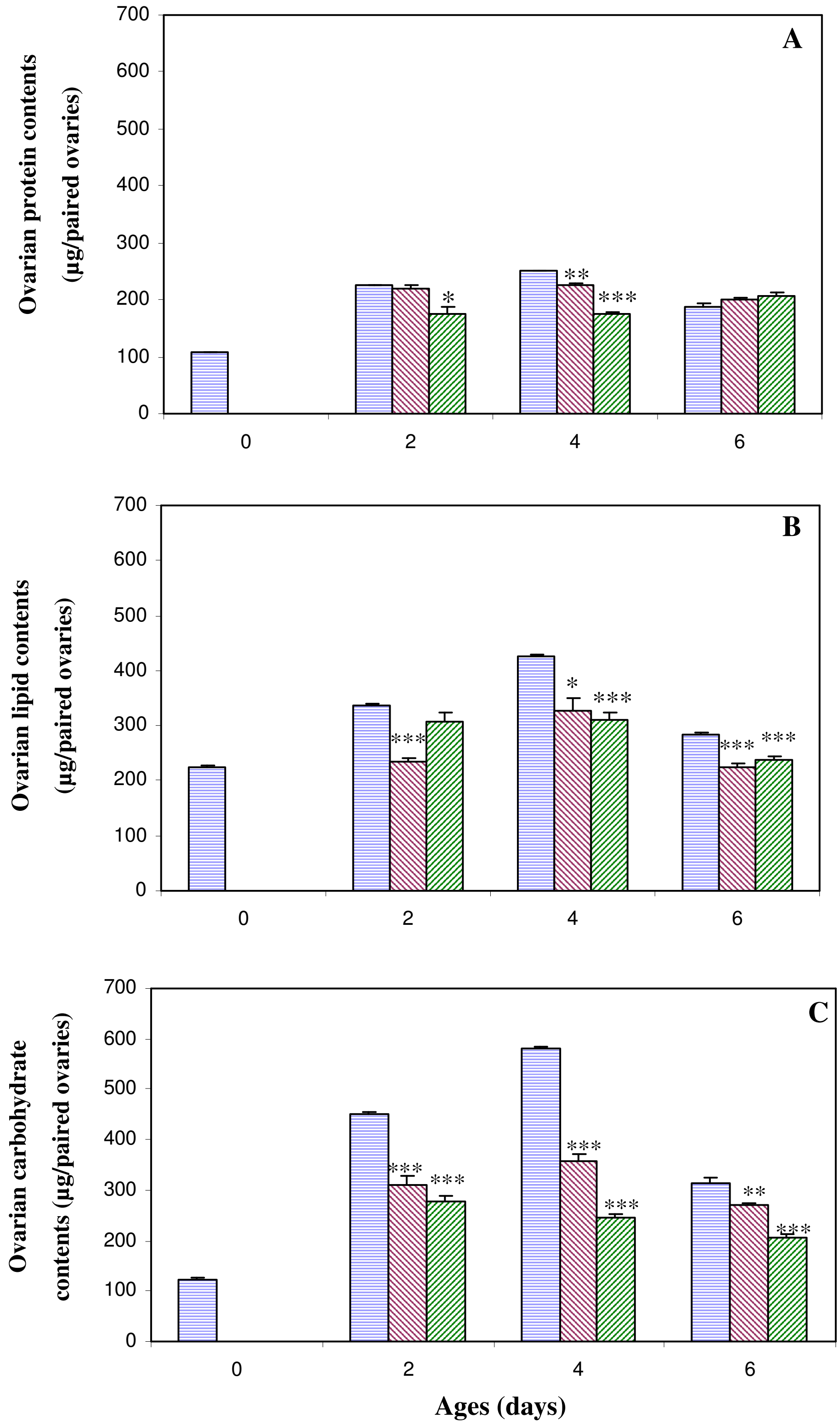


**Figure 3.** Effect of ingested boric acid (8.2 and 49.6%) on the ovarian contents (µg/paired ovaries) of proteins (A), lipids (B) and carbohydrates (C) in adult females of *B. germanica* during the first 6 days following adult emergence (mean ± SEM; n = 6 - 8; asterisks indicate a significant difference between control and treated series of the same age; *p<0.05; **p<0.01; ***p<0.001).

days, the incorporation of residues in females treated with the LC90 was at most of 1.5 fold than the amounts of residues incorporated with the LC50.

In the second series of experiments, boric acid was evaluated on ovarian morphometry. In controls, the number of oocytes per paired ovaries decreases at day 4; this reduction coincides with the beginning of ovulation (Schal et al., 1997). Boric acid was found to reduce the number of oocytes per paired ovaries and the volume of basal oocytes as compared to controls with a marked effect of LC50. This is probably due to a repulsive effect of the compound at the highest dose. The neurotoxic effect of this compound (Habes et al., 2006) might act negatively on neuropeptides with myotropic properties regulating ovulation. Our previous studies reported similar effects in ovarian morphometry of *B. germanica* treated with benfuracarb, a neurotoxic compound, and halofenozide, an ecdysteroid agonist (Maiza et al., 2004). In contrast, methoprene, a juvenile hormone analogue, reduced the number of oocytes per paired ovaries but increased the volume of basal oocytes (Maiza et al., 2004). Similary, diflubenzuron, a benzoylphenylurea derivative, topically applied to *Cydia pomonella* reduced the number of oocytes and the size of basal oocytes (Soltani and Soltani-Mazouni, 1992). Smagghe and Degheele (1992, 1994a) also reported that RH-5849 and tebufenozide (RH5992) reduced fecundity and/or fertility of several lepidopteran pest species by interfering with ovulation and oviposition.

Finally, boric acid was evaluated on ovarian constituents. Our results showed a significant decrease in ovarian protein, lipid and carbohydrate contents suggesting an interference with the vitellogenesis process. The amount of protein content at 6 days is probably due to the end of vitellogenesis and the preparation of the ovary to new gonadotrophic cycle; whereas, the reduction in lipids and carbohydrates correspond to the energy consumption required to vitellogenesis. Boric acid was found to alter the structure of the midgut in German cockroaches (Habes et al., 2006). It is well known that in this species an intimate relationship between feeding and vitellogenesis exist (Schal et al., 1993). The quantity of ingested food and its quality profoundly influence corpora allata activity and hence reproductive rate in the female (Schal et al., 1993; Schal et al., 1997). Reduction on nutriments absorption caused by midgut alteration can explain the negative effect of boric acid on vitellogenesis. Thus, the neurotoxicity of the compound might interfere with hormones and neurohormones that regulate vitellogenesis and caused inhibition in vitellogenin synthesis and uptake by perturbation of nutrition. The reduction of proteins content was also observed in *B. germanica* treated with benfuracarb a carbamates (Maiza et al., 2004). Halofenozide caused a significant decrease in ovarian proteins in *B. germanica* (Maiza et al., 2004). Diflubenzuron decreased the protein content per ovary in the codling moth *C. pomonela* (Soltani and Soltani-Mazouni, 1992). In contrast, methoprene a juvenile hormone analogue increased the ovarian protein content in *B. germanica* (Maiza et al., 2004).

The differences between the amounts of residue incurporated into body of females treated with the two tested doses can explain the slight differences effect observed between the two treated series on morphometric and biochemical composition of ovaries.

In conclusion, our results reveal that ingested boric acid affects reproduction in *B. germanica.* Further studies with other modes of application, such as injection in order will be conducted to obtain more information on the effect of boric acid action in this insect species.

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