A Review of Boron Toxicity in Insects With an Emphasis on Termites[1]

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ABSTRACT Managing urban pests with "environmentally-friendly" compounds has gained importance in recent years with the global deregistration of persistent organochlorine insecticides. Boron compounds, especially boric acid, zinc borate, and disodium octaborate tetrahydrate, are considered excellent options for preserving lumber from insect attack as well as decay by fungi and bacteria. Remedial borate applications are used primarily to treat cockroach and flea infestations but have also achieved recent popularity as wood treatments to protect timber from colonization by subterranean termites. The goal of this review is to provide a synthesis of the research on the toxicity of boron in insects with a specific focus on termites and urban pests.

KEY WORDS Isoptera, Rhinotermitidae, boric acid, wood preservation

Boron, a ubiquitous element, is an essential nutrient but can be toxic at greater concentrations. Found naturally in rocks, soil, and water, boron is present in the soil at an average concentration of 10–20 ppm and always as a boron–oxygen complex. These compounds, which are mined in arid areas that previously experienced volcanic or hydrothermal processes, have been used by humans for a variety of purposes. Around 4000 years ago, the Babylonians imported tincal (mineral borax, $Na_2B_4O_7 \cdot 10H_2O$) for goldsmithing. Boron has also been used in mummification rituals in ancient Egypt, in medicines, and for metalworking (Woods 1994). In modern history, borates have been useful as fire retardants in glass and ceramics, as a component of detergents, as a timber treatment to preserve lumber from wood decay, and as insecticides.

Boric acid is a stable and biologically benign compound at levels found in nature (Hall 2005). Boranes, which comprise chemical compounds of boron and hydrogen, are able to complex with a variety of substrates, especially those with adjacent hydroxyl groups, and are mild Lewis acids. Most small boronic acids are amphiphilic, containing both lipophilic and hydrophilic moieties.

Boron, which is present as a trace element in many foods, has been shown to be necessary for metabolism (Lloyd et al. 1990, Rainey et al. 1999). The most important documented role of boron is in stimulatory and inhibitory enzyme function. Without boron, plants and animals demonstrate decreased metabolic efficiency and sometimes exhibit severe symptoms of cellular level "starvation"

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(Lloyd et al. 1990, Woods 1994, Rainey et al. 1999). Lloyd et al. (1990) have suggested that borates in biological systems yield competitive inhibition as the result of a substrate-inhibitor complex, rather than the more commonly described enzyme–inhibitor complex. In addition to interfering with enzyme activity, research has suggested that boron deficiency in plants interferes with the integrity of root cells, in turn reducing the ability of cells to absorb ions and terminating root growth (Woods 1994).

Boron Compounds as Wood Preservatives

When used appropriately, borates are cost-effective biocides with low toxicity to vertebrates and to the environment. Toxicity is low in mammals and in vertebrates in general because of rapid excretion of excess boron by the kidneys (Lloyd et al. 1990). In rodents, the lethal dose of boron is comparable with that of table salt, or approximately 4000 mg/kg for sodium tetraborate decahydrate; during development in rats, the no-observed-adverse-effect level (NOAEL) was reported as 0.075% boric acid (55 mg/kg/day) during gestation and 0.1% boric acid (74 mg/kg/day) shortly after birth (Price et al. 1996). Boron treatments are effective at protecting wood from termite attack and colonization by harmful fungi, and also act as fire retardants at concentrations greater than 8% by weight (LeVan & Tran 1990, Barrett 1995). The diffusible nature of borates is an advantage in penetrating refractory timber species, such as spruce or Douglas fir. However, diffusion can also be a disadvantage: the main problem with the use of borate-treated lumber for construction is leaching. When placed directly in contact with damp soil, where a moisture gradient can be created, or if exposed to running water, the retention of boron in the treated wood is reduced over time (Barrett 1995).

Different boron formulations have been reported to produce similar behavioral and physiological responses in *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), although anhydrous boric acid acted most rapidly (Gentz & Grace 2007). Although borates do not act as feeding repellents foraging termites have demonstrated a delayed avoidance of borate-treated wood after one week in field tests (Grace & Campora 2005, Campora & Grace 2007). In laboratory choice tests, termites fed less on borate-treated pieces than untreated samples (Ahmed et al. 2004, Kartal et al. 2004, Kartal & Ayrilmis 2005). In field tests, similar results have been reported, with borate-treated lumber fed on less than untreated wood (Jones 1991, Tokoro & Su 1993a, Grace et al. 1995, Grace et al. 2006, Tsunoda et al. 2006).

The use of borates for termite prevention and control has been previously reviewed (Grace 1997). Disodium octaborate tetrahydrate (DOT), in particular, has proven to be a successful commercial treatment of structural lumber (Grace 2002). A variety of formulations for superficial application and lumber impregnation have been developed, and the solvent used may affect toxicity. Ethylene glycol, a solvent used with DOT, may act as a synergist and decrease the amount of boric acid needed for the delivery of a lethal dose (Tokoro & Su 1993b), although other work indicated that the greatest benefit of ethylene glycol was as an efficient solvent for DOT rather than as a synergist (Grace & Yamamoto 1994). The mechanism of this possible synergy is still unknown and is contrary to what might be expected, namely that the presence of an ethylene glycol/boric acid complex would mean the boron would be bound and thus rendered less likely to bind in vivo and become biologically active.

Many efforts have been made to curtail leaching, the major setback to the widespread use of borates in unprotected exterior applications as wood treatments for the prevention of both insect and fungal damage (Manning et al. 1996). Kartal et al. (2004) successfully used chemical modifications of DOT with copolymerizations of allyl glycidyl ether and methyl methacrylate to limit boron leaching in laboratory experiments; even after severe leaching cycles, the wood specimens suffered reduced attack by *C. formosanus*. Working with blockboard veneer made of untreated fir (*Abies bornmulleriana* M.) strips between borate-treated or untreated veneers of Ekaba (*Tetraberlinia bifoliolata* Harms.), decreased damage was demonstrated by *C. formosanus* as well as by both brown-rot (*Fomitopsis palustris*) and white-rot fungus (*Trametes versicolor* (L. ex. Fr.) Quel. (Kartal & Ayrilmis 2005). In laboratory experiments with wood superficially treated with DOT in water alone or water plus ethylene glycol boron has been reported to accumulate in the wood immediately adjacent to termite galleries—perhaps as a result of termite activity (e.g., gallery construction) and/or moisture accumulation in these areas (Tokoro & Su 1993a).

Biological Role and Toxicity in Living Organisms

The common occurrence of boron in living organisms makes the mechanism of toxicity difficult to determine. While investigating whether boron facilitates wound healing in humans directly or indirectly, Nzietchueng et al. (2002) demonstrated that extracellular matrix regeneration and protein phosphorylation is increased in vivo in borate-treated human fibroblasts. In addition, they described how boron treatment, most likely through a cellular mediator, enhanced trypsin-like, collagenase, and cathepsin D activities. One of these cellular mediators was determined to be a tumor necrosis factor-α, a cytokine that is also present in the insect immune system (Franchini et al. 1996).

Although the mechanism of borate toxicity has not been fully elucidated, their toxic nature at high concentrations has been well-established. Previous research has shown that termite mortality is caused at both high and low borate concentrations more rapidly than defaunation and subsequent starvation (Khoo & Sherman 1979) would account for alone (Ahmed et al. 2004, Kartal & Ayrilmis 2005). The in vivo biochemistry of borates is complicated, particularly because the tetrahydroxyborate ion [$B(OH)_4^-$] can complex with any molecule with two adjacent hydroxyl groups (Kim et al. 2004). In vitro, high concentrations of borates are toxic to all cells, and the toxicity appears to be a result of rapid esterification of borates with molecules of biological significance, although those molecules have not been clearly defined. The cells seem to be "starved," pointing to a biostatic, rather than biocidal, toxicity (Lloyd et al. 1990). Borates have been reported to interact with molecules ranging from riboflavin to vitamin B6, coenzyme A, vitamin B-12, and nicotinamide adenine dinucleotide (NAD^+) (Lloyd et al. 1990, Williams et al. 1990, Woods 1994). Kim et al. (2004) have shown that boron-nucleotide complexes are affected by the pH of the solution and that at pH 7.4 only borate-NAD^+ complexes are detected. Additionally, borates can also act as purely ionic inhibitors; this may affect membrane stability and the polyols within them (Lloyd et al. 1990).

Recent research by Habes et al. (2006) suggests that boric acid induced glutathione S-transferase activity and inhibited acetylcholinesterase activity in the German cockroach, *Blattella germanica* (L.) (Blattaria: Blattellidae). Zurek et al. (2002), also working with *B. germanica*, found that the use of an entomopathogenic fungus, *Metarhizium anisopliae* (Metchnikoff) Sorokin (Deuteromycota: Hyphomycetes) in concert with topical applications of boric acid dust (12% w/w) or aqueous formulations of boric acid (0.1% w/v) as drinking water increased cockroach mortality in comparison with the use of either alone, suggesting a synergistic interaction between the two management strategies.

Organismal respiration, which can be an indicator of overall condition, has been shown to be affected in termites exposed to boron or boron-treated lumber. Nunes & Dickinson (1995) found an increase in respiration of *Reticulitermes lucifugus* (Isoptera: Rhinotermitidae) at certain boric acid concentrations (0.04, 0.64% boric acid equivalent [BAE] in paper). Toyoshima et al. (1997) reported an overall decrease in respiration in termites immediately after feeding on borate-treated timber across concentrations of boric acid (5, 10, and 20 kg/m^3), although the 5 kg/m^3 treatment caused respiration at 15 and 30 min to slightly increase over the initial rate. It is unlikely that a decrease in organismal respiration is the primary mechanism of action of boron compounds, because this would be expected to cause death more rapidly than has been reported from exposure to borates.

Toxicity parameters for boron have been well-established in the literature, and there is a clear dose-dependent mortality relationship. Because of the difficulty in measuring ingestion of boron by individual termites, the lethal dose of 50% of a test population (LD_{50}), as well as more general data on mortality, is commonly reported. Toyoshima et al. (1997) reported the LD_{99} at 16 days as 930 μg/g in *C. formosanus* worker termites, and the lethal cumulative dose as 136 μg/g.

Other work with termites reports more general effects of boron on survival, for example Grace (1990) observed mortality >90% in *Reticulitermes flavipes* within 15 days when exposed to substrates containing concentrations of ≥30,000 ppm (≥30,000 μg/g) barium metaborate monohydrate, and mortality of >90% at ≥1000 ppm (≥1000 μg/g) within 30 days. Grace et al. (1992) found mortality of *C. formosanus* >90% after 16 days of exposure to DOT-impregnated filter paper at a solution concentration of 1.5 g/L (1500 μg/g), and 100% by day 12 with a 12.0 g/L (12,000 μg/g) DOT solution or 7 days with a 120.0 g/L (120,000 μg/g) solution. Su et al. (1994) determined the LD_{50} of boric acid as 721.29 μg/g in *C. formosanus* workers and 264.02 μg/g in *R. flavipes* (Kollar). Mortality through the exchange of different boron dusts has also been reported, with boric acid powder-treated termites experiencing mortality more rapidly than DOT dusted ones in laboratory experiments when proportions as low as 10% of the experimental population were treated; the dust may be transferred via the close interactions between these social insects, e.g., mutual feeding or grooming (Grace et al. 1992). In laboratory tunneling assays, *R. flavipes* exposed to DOT (1.20 BAE) in sand showed greater mortality and less tunneling activity than those exposed to zinc borate (0.86 BAE), but the reverse pattern was found with *C. formosanus*, possibly because of differences in the tunneling behavior of these two species (Grace 1991).

Summary

Boron is an element crucial for survival, but toxic at concentrations above those necessary for physiological function. Boron, especially in its complexed forms as DOT, zinc borate, and anhydrous boric acid, is used as a wood preservative to protect from attack by decay fungi and some insects, including termites and wood-boring beetles. Boron is also used in insecticide formulations against urban insects like cockroaches and fleas. Despite the benefits of boron as an insecticide and commercial use for over 200 years, the mechanism for toxicity caused by boron is not well understood. Recent research has focused on the effects of boron in termites and cockroaches, both physiological and behavioral, and has provided an excellent basis for future work on the mechanism of action. As a crucial component of biological function, boron serves a role in plants, insects, and animals, and work focused on understanding both beneficial and toxic mechanisms will surely be complex and exciting.

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