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# Toxicity of Hydramethylnon to the Leaf-cutting Ant *Atta sexdens rubropilosa* Forel (Hymenoptera: Formicidae)

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**Abstract**

Since 2009, when sulfluramid was listed in annex B of the Stockholm Convention's Persistent Organic Pollutants, effort has been made to search for other active ingredients to use in baits for controlling leaf-cutting ants in Brazil. Considering that active ingredients that inhibit insect cellular respiration have been shown to be effective in controlling ants, the current work aimed at assessing the toxicity of hydramethylnon to *Atta sexdens rubropilosa* workers. Hydramethylnon was dissolved in acetone and in a solution of acetone + soy oil then incorporated in artificial diet at concentrations of 1 µg/mL, 5 µg/mL, 10 µg/mL, 100 µg/mL, 200 µg/mL and 1000 µg/mL. The treatments where ants were daily fed on the diet containing hydramethylnon at 100 µg/mL, 200 µg/mL and 1000 µg/mL, especially those dissolved in soy oil, exhibited high mortality in comparison to the controls. The data presented here confirms the insecticidal activity of hydramethylnon and highlights the importance of employing soy oil in the formulation of baits to control leaf-cutting ants because it enhances hydramethylnon efficiency.

## Introduction

Chemicals used to control pests of cultivated plants, which include leaf-cutting ants, have been always one of the main ecological concerns because of their harmful effects on the environment, human health and other animals (Williams, 1990). As a consequence, the number of studies has greatly increased aiming to replace traditional pesticides for those of rapid degradation, high specificity and less noxious to the environment (Morini et al., 2005).

Most of the strategies of chemical control are based on killing leaf-cutting ants by contact, but it is usually not enough to control populations in a certain area. Efficient control involves exterminating the whole colony, not only some individuals. Currently, the most appropriate method for controlling leaf-cutting ants is the use of toxic baits because they are incorporated into the colony feeding cycle and the insecticide acts through ingestion (Loeck & Nakano, 1984).

Since dodecachlor (organochlorine pesticide - Mirex) was prohibited in 1993, the chemical sulfluramid became the most used active ingredient in toxic baits in Brazil. Nevertheless, this compound was included in annex B of the Stockholm Convention's Persistent Organic Pollutants in 2009 with restrictions of only being used for controlling leaf-cutting ants in Brazil until a novel compound is found to replace it (Stockholm Convention, 2009).

To develop efficient and economically viable toxic baits for ant control, it is essential that the active ingredient acts slowly, so that workers live long enough to spread the chemical among other ants, is toxic by ingestion, does not repel workers, is lethal at low concentrations and environmentally acceptable (Etheridge & Phillips, 1976; Forti et al., 1993; Bueno & Campos-Farinha, 1999). Recent toxicological analysis of several active ingredients used for pest control reveal that in general inhibitors of cellular respiration meet the requirements for use in baits to control leaf-cutting ants (Nagamoto et al., 2004; Decio et al., 2013).

Hydramethylnon acts on insect cellular respiration by inhibiting electron transport system and consequently blocking ATP production and decreasing mitochondrial oxygen consumption. Metabolism disruption and subsequent decrease in ATP result in delayed mortality by this active ingredient (Bloomquist, 2010; Irac, 2010).

In view of this, the aim of the current work was to assess the toxicity of hydramethylnon to workers of *Atta sexdens rubropilosa* Forel.

## Material and Methods

The *A. sexdens rubropilosa* workers used in the assays, whose body mass was about 20-25 mg, were randomly picked from a laboratory nest kept at Centro de Estudos de Insetos Sociais (Instituto de Biociências, UNESP – Univ. Estadual Paulista, Campus de Rio Claro, SP) and some specimens were deposited in the Coleção Entomológica Adolph Hempel (Instituto Biológico, São Paulo – SP, Brazil). Before the assays, nests were daily supplied with leaves of *Eucalyptus* sp., oat seeds and occasionally with leaves of other plants such as *Hibiscus* sp., *Ligustrum* sp. or rose petals.

Fifty ants were put into five Petri dishes (ten ants per dish) for each treatment. During the assays the ants were maintained on an artificial diet prepared with glucose (50 g/L), bacto-peptone (10 g/L), yeast extract (1.0 g/L) and agar (15 g/L) in distilled water (0.1 L) (Bueno et al. 1997). The diet (0.5 g per dish) with hydramethylnon (experimental) or without (control) was offered daily on a small plastic cap.

Hydramethylnon dissolved either in acetone (HA) or in acetone and soy oil (9 mL of acetone per 1 mL of oil) (HAO) was added to the artificial diet at concentrations of 1 µg/mL, 5 µg/mL, 10 µg/mL, 100 µg/mL, 200 µg/mL and 1000 µg/mL. Three controls were established to verify that the hydramethylnon toxicity results were not biased by the chosen solvent: one group received the artificial diet ('diet control'), another group received the artificial diet with acetone ('acetone control'), and a third group received the artificial diet with acetone and soy oil ('acetone + oil control'). Acetone or the combination of acetone and soy oil were added at the same proportions as those used for the hydramethylnon-treated groups.

During the assays, ants were maintained in an incubator at temperature of 25 ± 1ºC and relative humidity ranging between 70-80% for maximum length of 25 d and the number of dead was registered daily. The survival average 50% ($S_{50}$) was calculated and survival curves were compared by the computer-assisted software Graph-Pad ™ using the log-rank test (Elandt-Johnson & Johnson 1980).

## Results and Discussion

Mortality rates of both controls 'acetone' and 'acetone + oil' did not significantly differ comparing to the group that was only fed on artificial diet, no solvent added (diet control), indicating that solvents did not affect the mortality of *A. sexdens rubropilosa* workers (Tables 1 and 2).

Hydramethylnon dissolved in acetone resulted in decreased worker survival which was more drastic at concentrations of 200 µg/mL and 1000 µg/mL. Ant survival median time was reduced from 14 d (acetone control) to 10 d (200 µg/mL) and 9 d (1000 µg/mL). All ants were dead on the 19$^{th}$ day for the concentration of 100 µg/mL, 16$^{th}$ for 200 µg/mL and 15$^{th}$ for 1000 µg/mL (Table 1).

Hydramethylnon dissolved in acetone + soy oil also resulted in decreased worker survival being more drastic at 100 µg/mL, 200 µg/mL and 1000 µg/mL. Ant survival median was reduced from 13 d (acetone + oil control) to only 6 d (100 µg/mL, 200 µg/mL and 1000 µg/mL). Mortality of all ants occurred on the 13$^{th}$ day for concentration 100 µg/mL and on the 9$^{th}$ day for concentrations of 200 µg/mL and 1000 µg/µL (Table 2).

Soy oil in the diet made the toxicity of hydramethylnon to leaf-cutting ants more potent, a fact that was shown by the higher ant mortality rate in the treatment with hydramethylnon dissolved in acetone + oil in comparison with the ants treated with hydramethylnon dissolved only in acetone. Few studies have revealed the mode of action of oils in insects. Hewlett (1947) suggested that intoxication by oil was due to the mechanical action, interfering with breathing by blocking the spiracles. On the other hand, there are authors who believe that toxicity of oils is attributed to chemical action (Singh et al. 1978) or may act both physically and chemically in the insect (Obeng-Ofori 1995). For Taverner et al. (2001), oils can act in the insect nervous system by increasing the permeability of neuron membrane and thus affecting ion change and stressing excitability of neuron cells. However, in leaf-cutting ants, the oil allows an alternative via for the ingestion of oil-soluble active ingredients due to the feeding behavior of workers (Bueno et al., 2008; Decio et al., 2013).

Adult ants feed primarily on liquid. The ingested food moves into the infrabuccal cavity of workers, wich remain for 24h. Solid parts of the food are retained in the cuticle folds and spines present on infrabuccal cavity and are subsequently discared in the trash (Fowler et al., 1991; Moreira et al., 2011). Nevertheless, the liquid portion of the food passes after the opening post-pharyngeal gland, where occurs the separation of water-soluble compounds and oil-soluble compounds. The water-soluble compounds moving into the crop and oil-soluble compound enter the ducts of post-pharyngeal gland, where they are absorbed and transferred to the hemolymph and subsequently to the whole body (Bueno et al., 2008). Thus, recently Decio et al. (2013) observed major part of soy oil from the diet is likely allocated in the lumen of post-pharyngeal glands where it will be metabolized and suggested that ant post-pharyngeal glands are involved in the metabolism of lipids.

The current work demonstrated that hydramethylnon has a great potential as active ingredient to be incorporated in baits for leaf-cutting ant control especially because of its slow mode of action. This characteristic is fundamental for

**Table 1.** Mortality (%) of *A. sexdens rubropilosa* workers fed on hydramethylnon dissolved in acetone * $S_{50}$: Survival median 50%. Different letters after $S_{50}$ values show a significant difference according to the log-rank test (P < 0.05).

| Treatment | % Daily accumulated mortality | | | | | | | | | | $S_{50}$ * |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 6 | 8 | 10 | 14 | 17 | 21 | 25 | |
| Diet control | 0 | 0 | 0 | 8 | 16 | 30 | 54 | 84 | 100 | — | 13 *a* |
| Acetone control | 0 | 0 | 0 | 10 | 18 | 30 | 58 | 84 | 100 | — | 14 *a* |
| Hydramethylnon 1 µg/ml | 0 | 0 | 0 | 8 | 16 | 30 | 54 | 72 | 92 | 98 | 13 *a* |
| Hydramethylnon 5 µg/ml | 0 | 0 | 0 | 10 | 28 | 38 | 56 | 78 | 100 | — | 13 *a* |
| Hydramethylnon 10 µg/ml | 0 | 0 | 0 | 14 | 32 | 48 | 82 | 96 | 100 | — | 11 *b* |
| Hydramethylnon 100 µg/ml | 0 | 0 | 0 | 6 | 18 | 48 | 92 | 98 | 100 | — | 10 *b* |
| Hydramethylnon 200 µg/ml | 0 | 0 | 0 | 10 | 22 | 54 | 90 | 100 | — | — | 10 *b* |
| Hydramethylnon 1000 µg/ml | 0 | 1 | 2 | 20 | 38 | 62 | 94 | 100 | — | — | 9 *b* |

**Table 2.** Mortality (%) of *A. sexdens rubropilosa* workers fed on hydramethylnon dissolved in acetone and soy oil* $S_{50}$: Survival median 50%. Different letters after $S_{50}$ values show a significant difference according to the log-rank test (P < 0.05).

| Treatment | % Daily accumulated mortality | | | | | | | | | | $S_{50}$ * |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 6 | 8 | 10 | 14 | 17 | 21 | 25 | |
| Diet control | 0 | 0 | 0 | 6 | 16 | 30 | 54 | 74 | 82 | 94 | 13 *a* |
| Acetone + oil control | 0 | 0 | 0 | 6 | 18 | 32 | 56 | 76 | 86 | 94 | 13 *a* |
| Hydramethylnon 1 µg/mL | 0 | 0 | 0 | 10 | 20 | 36 | 62 | 88 | 96 | 100 | 13 *a* |
| Hydramethylnon 5 µg/mL | 0 | 0 | 2 | 10 | 28 | 46 | 90 | 100 | — | — | 11 *b* |
| Hydramethylnon 10 µg/mL | 0 | 0 | 0 | 4 | 6 | 60 | 96 | 100 | — | — | 10 *b* |
| Hydramethylnon 100 µg/mL | 0 | 0 | 2 | 56 | 90 | 92 | 100 | — | — | — | 6 *b* |
| Hydramethylnon 200 µg/mL | 0 | 0 | 4 | 52 | 88 | 100 | — | — | — | — | 6 *b* |
| Hydramethylnon 1000 µg/mL | 0 | 0 | 2 | 50 | 98 | 100 | — | — | — | — | 6 *b* |

obtaining ant-insecticidal baits of high efficiency since it allows that the workers live long enough to spread the active ingredient inside the colony. The concept of slow toxicity was firstly used to select active ingredients for controlling fire ants (Stringer et al., 1964; Williams, 1983; Vander Meer et al., 1985) and more recently it was adapted by Nagamoto et al. (2004) for leaf-cutting ants. The selection based on this concept favour compounds that cause low mortality on the first 24 h after application (lower than 15%), but high mortality at the end of the experiment (above 90%).


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