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PRELIMINARY COMMUNICATION

INHIBITION OF A MIXED FUNCTION OXIDASE SYSTEM AND CONSEQUENT INCREASE IN POTENCY OF CARBARYL BY BUTYLATED HYDROXYANISOLE IN THE HOUSEFLY

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The potency of many insecticides may be enhanced by simultaneous administration of synergists which act by inhibiting the mixed function oxidases (MFO) of insects and hence the metabolism and inactivation of these insecticides (1). Many of the commercial synergists have been found to also interfere with the detoxification of xenobiotics in mammals, such as the insecticides themselves as well as industrial pollutants and drugs (1-6). The rising demand for insecticides, non-persistent in and less harmful to mammals, and the increasing resistance of insects to these insecticides have produced a need for new synergists (2-4,7,8). Butylated hydroxyanisole (BHA) appears to be of low toxicity in mammals (1,8,9) and inhibits rodent (10-12) but induces primate (13) MFO. In this communication, we present data to indicate that this common food additive is an inhibitor of an O-demethylase of the common housefly (Musca domestica) and synergizes in this insect with the insecticide, carbaryl (1-napthyl-N-methylcarbamate).

All studies were performed on a non-resistant strain of a 4- to 8-day-old female housefly obtained from and reared by methods and supplies donated by J. Roger Ramsay of Rohm & Haas Co., Springhouse, PA 19477. The adult flies were supplied ad lib. with water, a 50-50% by weight mixture of Instant Carnation Dried Milk mix and 10 X Jack Frost Sugar. The room temperature was kept at 26 ± 3° and a photoperiod of 16 hr light and 8 hr dark was used. A standard bait-toxicant test (14) was used to ascertain the toxicity in vivo of BHA and its synergism with carbaryl,

a suitable insecticide for testing synergists. All tests in vitro were performed by standard procedures in the cold (14,15).

The results in Table 1 are illustrative of a number of experiments whereby BHA inhibited fly p-nitroanisole demethylase both in vitro and in vivo. When 0.3% BHA is added to the incubation media, it approximates 50 percent inhibition level, while 4.8% BHA in the sucrose-bait decreased the activity of the enzyme 50 per cent in mobile flies. Usually older fl are more susceptible to toxic agents. More immobility was observed in 7- than in 4-day flies, but as our first priority was in BHA as a synergi rather than as an insecticide, we assayed mobile flies. The p-nitroaniso O-demethylase in the insect has been shown previously to be a MFO system (15) and, as in our hands, to be also dependent on NADP; thus, it was lik that BHA would act as a synergist in vivo of insecticides metabolized by the MFO (2,4,14), such as carbaryl. This insecticide is one of the stand insecticides used in the sucrose-bait toxicant test for synergists and, as it is metabolized by a demethylase, its use here corresponds well with the MFO system in vitro, p-nitroanisole (pNA) demethylase.

Table 1. Inhibition of fly p-nitroanisole demethylase by addition of BHA in vitro or by dosing BHA in vivo

Fly age (days)	Treatment	p-Nitroanisole demethylase activity Control	Treated	% of Contro
7	In vitro - 0.3% BHA in incubation of media	36	16	45
7	In vivo - 4.8% BHA in sucrose offered for 4 hr+	30	16	53
4	In vivo - 4.8% BHA in sucrose offered for 2 hr‡	39	21	54

*Activity was expressed as nmoles p-nitrophenol formed by 20 female fly abdomens/ 30-min incubation at 37°. The abdomens were placed immediately after sacrifice in a cold 1.0 M, pH 7.4, $NaHKPO_4$ solution (20/ml), homogenized in a glass homogenizer with a Teflon pestle and centrifuged at 9000 g. The incubation mixture contained 1.0 ml enzyme, 0.1 mM $MgCl_2$, 1 mM NAD, 1 mM NADP, 4.6 mM glucose 6-phosphate, 3 mM pNA and buffer to 3.0 ml. The reaction was stopped and protein precipitated with a basic acetone-water solution, centrifuged and the extinction was determined at 412 nm on a Beckman DU-2.

+No deaths, but 60 per cent immobilization of flies. Only mobile flies were used for the enzyme activity assay.

‡No deaths; no immobilization occurred.

The data in Table 2 indicate that BHA does indeed increase the lethal of carbaryl in the housefly even at very low lethal levels of BHA.

Table 2. Toxicity of Carbaryl and BHA alone and in combination in the 5-day-old female housefly*

% Concentration (w/w) in sucrose		% Mortality within 24 hr
BHA	Carbaryl	
0.00	0.00	0.00
0.00	0.06	23.0
1.20	0.00	3.3
1.20	0.06	100.0

*Percentage mortality was obtained from three to nine containers of 20 flies each. The toxicants were dissolved in acetone and mixed with sucrose. After evaporation of the acetone for 24 hr, the bait-sucrose was pulverized. The bait-toxicant media was then offered to the flies along with water. After a period of 24 hr, the total mortality was recorded.

Although acute and subacute mammalian toxicity of synergists registered for commerical use in insecticides appears to be low (2), repor that at high concentrations some of these compounds have been shown to possess tumorigenic properties in mice and rats are disturbing. As the route of administration by which humans are most frequently exposed appear to be by inhalation of aerosol formulations, the rapidly rising incidence of lung cancer should be taken into consideration. Also possible toxic effects of plant residues should also be considered.

This extremely low toxicity of BHA (9) and the apparent induction primate MFO (13) but inhibition of insect MFO (9) point to the possible utilization of BHA analogues and other antioxidants such as ethoxyquin (16 as synergists in insecticide preparations, especially in those which come in contact with man.

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