Formamidines are a relatively new class of agricultural chemicals, and within this group there exist diverse types of pesticidal activity. For example, compounds active as herbicides, fungicides, nematocides, bactericides, insecticides, and acaricides have been discovered. The insecticide and acaricide N''-(4-chloro-o-tolyl)-N,N-dimethylformamidine or chlordimeform has received the most attention from a toxicological standpoint since it has been used extensively for insect and acarine control (Knowles, 1976).

The known biochemical effects of chlordimeform in mammals were recently reviewed (Hollingworth, 1976; Knowles, 1976; Matsumura and Beeman, 1976). We have been most interested in the interaction of chlordimeform with biogenic amine regulatory mechanisms (Knowles, 1976). Our approach has been to study the biochemical effects of known mammalian metabolites of chlordimeform as well as those of the parent compound. This paper describes the toxicity and symptomology of several formamidines to mice and rats as well as their interaction with certain components of the biogenic amine system. Emphasis is given to the neurotoxic effects of chlordimeform and its metabolites.

MATERIALS AND METHODS

Animals and Compounds

Male Swiss-Webster strain mice weighing about 20 g each were obtained from National Lab., O'Fallon, Mo., and male Sprague Dawley rats weighing about 150 g each were obtained from Charles River
The sources and properties of the formamidines and related compounds listed in Table 1 were reported by Chang and Knowles (1977).

Toxicity and Symptomology

The toxicity to mice of the compounds in Table 1 was investigated. Depending upon its solubility properties, the compound was dissolved in distilled water, 0.01N HCl, acetone, or corn oil to yield a concentration of 20 mg/ml. Three mice were injected intraperitoneally with each compound at a dosage of 100 mg/kg, and mortality was recorded at 24 hr.

Rats were injected intraperitoneally, subcutaneously, and intraventricularly with various doses of chlordimeform and its known metabolites to determine toxicity and symptomology. For intraperitoneal and subcutaneous treatment the hydrochloride salts of the formamidines and 4'-chloro-o-toluidine were dissolved in distilled water; 4'-chloro-o-formotoluidide was dissolved in dimethyl sulfoxide. For intraventricular injection the technique of Noble et al. (1967) was used. Rats were anesthetized with ether, and the formamidines (base forms) and 4'-chloro-o-formotoluidide in dimethyl sulfoxide solution were injected into the lateral ventricle of the brain at a maximum rate of 1 μl/second. The total volume for injection never exceeded 30 μl. Following injection the rats were administered oxygen for about 5 min.

Symptoms of poisoning were observed, and the time interval between treatment and death was recorded.

MAO Inhibition In Vitro

Mouse brains were removed and homogenized in 1.15% KCl to yield a concentration of 50 mg of tissue/ml. The homogenate was centrifuged at 1200 g for 5 min, and the supernatant was used as the monoamine oxidase (MAO) source.

The radiochemical technique of Wurtman and Axelrod (1963) as described by Nagatsu (1973) was used to assay for MAO. The standard 0.3-ml incubation mixture contained 0.1 ml of MAO preparation, 0.1 ml of phosphate buffer (0.5M, pH 7.4), 0.05 ml of radiolabeled substrate (tryptamine-14C) and 0.05 ml of nonradioactive tryptamine. The final substrate concentration was 7.5 x 10^{-5}Μ. Incubation was carried out for 30 min with shaking at 37°C, and the reaction was stopped with 2N HCl. Toluene was added, and the mixture was shaken vigorously to extract the deaminated metabolites. Following a brief centrifugation an aliquot of the toluene extract was radioassayed by liquid scintillation spectrometry.