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Stability of Matacil® in Aqueous Media as Measured by Changes in Anticholinesterase Potency

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The carbamic acid ester insecticide aminocarb (4-dimethylamino-3-methylphenyl N-methylcarbamate, MATACIL^R) has been used extensively by several provincial governments in Canada for the control of a lepidopterous forest pest, the spruce budworm (Choristoneura fumiferana (Clemens)). In the 1978 spraying program in New Brunswick, some 94,000 kg of this agent were formulated and sprayed over some 700,000 hectares of forest at an application rate of 52.5 g active ingredient/hectare (0.75 oz/acre) (PEARCE et al. 1979). Concern has been raised about the stability of residues which might find their way into fresh and salt water. Carbamate insecticides are known to be unstable in alkaline aqueous media, the variability in the rate of breakdown being modified by the physicochemical properties of the particular ester and photolytic degradation, as well as the pH and temperature of the water (ALY and EL-DIB 1971; EICHELBERGER and LICHTENBERG 1971). In eastern Canadian mixed forest, fresh water is acidic, the pH being 5.0-6.0, whereas salt water is alkaline with a pH of 8.0 (ECOBICHON 1979). This report presents results conducted in a laboratory, examining the chemical stability of aminocarb in buffered solutions at different pH by measuring the change with time in the anticholinesterase activity of measured aliquots of solutions.

MATERIALS AND METHODS

Aqueous solutions of aminocarb (MATACIL^R, 97.4% purity, obtained from Mobay Chemical Corporation, Kansas City, Missouri) at initial concentrations of 10^{-3}M were prepared in 0.2M sodium acetate buffer at pH 5.0, in 0.9% sodium chloride at pH 6.5 and in 0.1M phosphate buffer at pH 8.0. The solutions were incubated at 25°C with periodic shaking and were kept in a dark cupboard to eliminate photolytic degradation. At suitable intervals, aliquots (0.1 ml) of the insecticide solution were removed for the in vitro analysis of anticholinesterase activity, adding the aliquot to 0.1 ml of rat plasma in 3.2 ml of 0.067M phosphate buffer at pH 7.4 and incubating the mixture for 5 min with shaking at 37°C. The residual rat plasma pseudocholinesterase (PChE) was measured spectrophotometrically using 1 x 10^{-3}M propionylthiocholine iodide as the optimal substrate for the enzyme and measuring the change in the yellow-coloured sulphydryl-dye complex continuously for 1-2 min at 412 nm at 37°C (ECOBICHON and COMEAU 1973). The I_{50} (concentration of agent producing a

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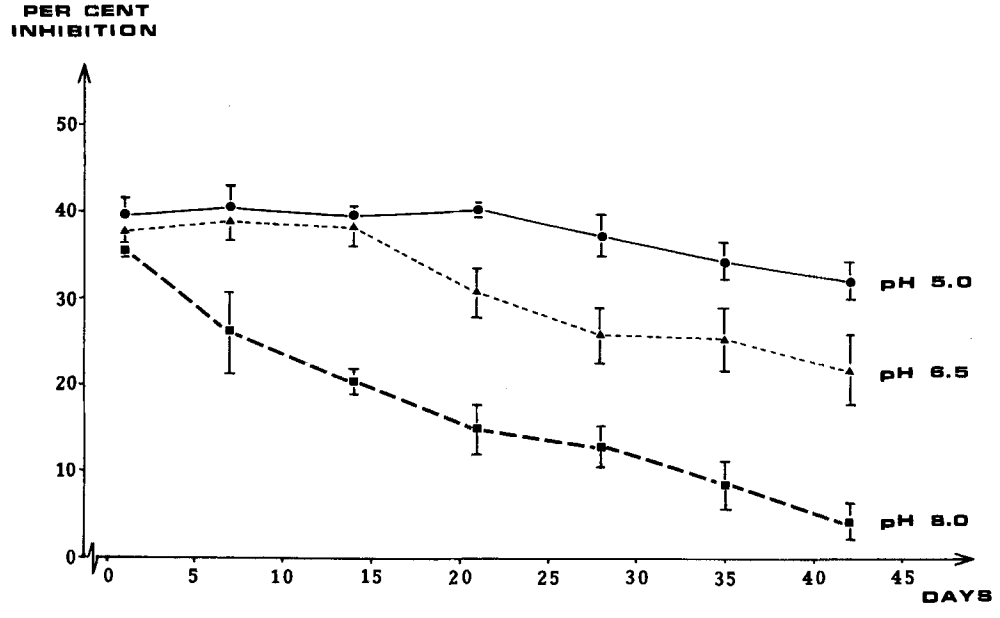

Figure 1. The change in anticholinesterase potency of aminocarb with time and incubation at 25 C in aqueous buffers at pH 5.0, 6.5 and 8.0.

50% inhibition of a standard amount of enzyme) for aminocarb has been determined under similar incubation conditions to be 2.9 x 10^{-5}M for rat plasma PChE (VASSILIEFF and ECOBICHON 1982). A single sample of rat plasma was used throughout the study, with the percent inhibition of the enzymatic activity being calculated from control and inhibited assays.

RESULTS AND DISCUSSION

Aliquots of the incubating buffered solutions of 10^{-3}M aminocarb were analyzed at 1, 7, 14, 21, 28, 35 and 42 days for residual anticholinesterase activity. The inhibition of rat plasma PChE is shown in Fig. 1 plotted against the duration of incubation and shows a pH-dependent loss of potency. At an acidic pH of 5.0 or 6.5, little change in anticholinesterase activity was observed for 21 and 14 days respectively, indicating that aminocarb was relatively stable at these pH values. After 42 days of incubation at pH 5.0, the loss of anticholinesterase activity did not exceed 25% of the original value. At pH 6.5, a light pink colour developed in the solution during the first week of incubation and changed slowly to a rosé colour between 21 and 42 days of incubation. While the initial colour developed, the carbamate still appeared to retain its biological activity and lost its anticholinesterase property only as the colour became darker. Approximately 50% of the anticholinesterase activity disappeared in the 42 day period. In contrast, a significant loss of inhibitory potency was observed after 7 days of incubation of aminocarb at pH 8.0, only negligible (4%) inhibition being observed at day 42. The loss of biological activity was accompanied by the formation of a rosé colour which rapidly changed to a wine-red colour within 21 days. Similar results were obtained with aminocarb incubated in fresh stream water (pH 5.2) and in seawater (pH 7.8).

In speculating on the molecular structure of the coloured components of the solutions at different pH values, the first assumption was that, with time, hydrolysis of the carbamate ester would occur in the aqueous medium with release of the phenolic moiety. As is shown in Fig. 2, the dimethylamino group, having a pKa = 5.85, would be protonated at the acidic pH, this form being responsible for the pink colour. Near neutrality, either a zwitterionic form with partial ionization of both the dimethylamino and the hydroxy group or the completely unionized molecule might be present in the solution. At an alkaline pH, the dark red colour could possibly be due to the large proportion of the phenoxide ion, this group having a pKa = 11.4. The observed colour changes were confirmed by preparing solutions of 1.0 mg of 4-dimethylamino-3-methylphenol in the buffers at pH 5.0, 6.5 and 8.0. These distinct colours developed within 6 hr of preparation and were reversible following the adjustment of the pH.

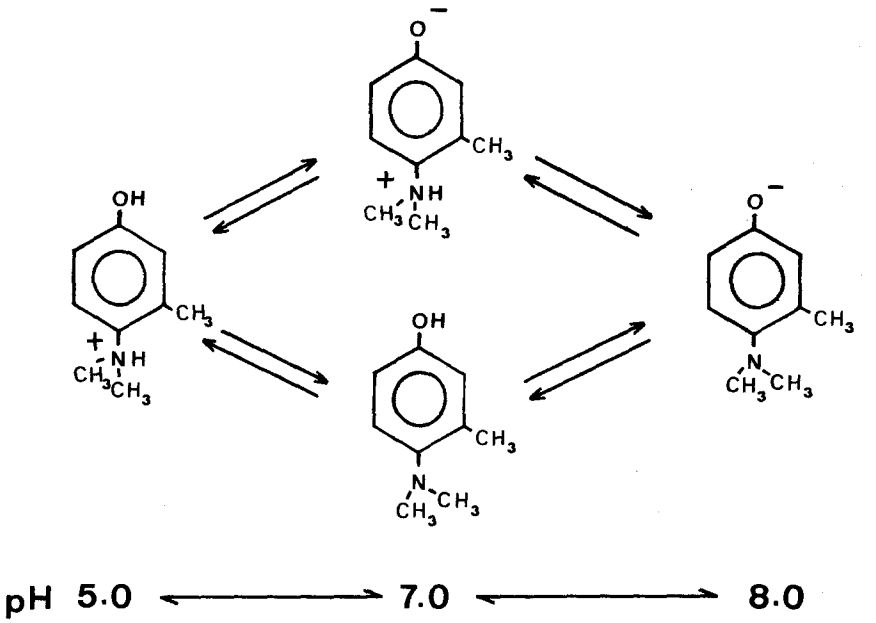

Figure 2. The ionization of 4-dimethylamino-3-methylphenol at acidic and alkaline pH values following the hydrolytic degradation of aminocarb.

The pH of natural fresh waters generally lie in the range of 5.0-8.0 (ALY and EL-DIB 1971). The stability of a number of carbamate esters in water has been investigated, N-disubstituted carbamates such as dimetilan (2-dimethyl carbomyl-5-pyrazolyl-N,N-dimethylcarbamate) being extremely resistant to hydrolytic degradation even at pH 10.0 (ALY and EL-DIB 1971). N-monosubstituted esters were more easily degraded, the biological half-life of propoxur (0-isopropoxyphenyl-N-methylcarbamate) was 10.5 days at pH 7.0. Carbaryl and propoxur were stable to hydrolysis at all pH values below 6.0 (ALY and EL-DIB 1971). Mexacarbate (4-dimethylamino-3,5-xylyl N-methylcarbamate) was found to have a half-life of 14 days at pH 7.4 and 2 days at pH 9.5 but was extremely stable at acidic pH (HOSLER 1974). A study of a number of carbamates in water from the Little Miami River (pH 7.3-8.0) revealed a rapid degradation of carbaryl and mexacarbate but a persistence of both propoxur and aminocarb with 50% and 60% of the original residues present after 7 days of incubation and 30% and 10% of the residues present after 14 days (EICHELBERGER and LICHTENBERG 1971).

Under conditions in which photolytic degradation would not occur, and using the anticholinesterase activity as an index (Fig. 1), the biological half-life of aminocarb was in excess of 90 days at pH 5.0 and was 47 and 25 days at pH 6.5 and 8.0 respectively. These values, of course, were determined in the absence of microbial breakdown, uptake and metabolism by plants and aquatic organisms, adsorption to stream bed detritus and soil and volatilization, all factors which could contribute to the "disappearance" from ponds and streams. SUNDARAM et al. (1976) found the half-life of aminocarb to be 4.4 days in pond water (pH 5.5) and 8.7 days in stream water (pH 7.1). The greater apparent persistence of the agent in the stream may have been due to the addition of foliar washings by rain and runoff water.

The present results have demonstrated that, in acidic fresh water characteristic of lakes and streams of heavily forested eastern Canada, aminocarb would be extremely stable and would persist long enough to be absorbed intact into various trophic levels of food chains. The bioaccumulation and/or fate of aminocarb in various species in food chains is beyond the scope of this paper though it has been shown that aminocarb is rapidly degraded into inactive products in mammals such as the laboratory rat (VASSILIEFF and ECOBICHON 1982).

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