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Alkylating Potency of Azamethiphos

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Azamethiphos (S-[(6-chloro-2-oxooxazolo[4,5-*b*]pyridin-2(2*H*)-ylmethyl] O,O-dimethyl phosphorothioate, CA RN 35575-96-3, Worthing 1987) is a relatively obscure organophosphate insecticide. It was introduced over 20 yr ago, but its use has not become very extensive and is described in only a few publications. Since the 'leaving group' moiety of azamethiphos (the oxooxazolopyridinyl methyl structure) is very unusual for organophosphate insecticides, and in view of the mutagenicity of azamethiphos in several *in vitro* tests (Committee for Veterinary Medicinal Products 1999), it was of interest to determine the alkylating potency of this insecticide. As mentioned in the discussion, an elevated alkylating potency may explain the mutagenicity of azamethiphos. Following a report that azamethiphos is effective against insects resistant to other organophosphates (Price 1988), it is now used in formulations against flies under the trade name Insectex® (GLOE Export-Import GmbH), and was used in flybaits during the Gulf war (Anon. 1999). Azamethiphos has been introduced into fish farming (Committee for Veterinary Medicinal Products 1999). Lethal and sublethal effects on non-target species in this application have been described (Abgrall 2000; Burridge et al. 1999, 2000).

MATERIALS AND METHODS

Pure (99%) azamethiphos was purchased from AccuStandard Inc, (New Haven, CT 06511 USA). To determine the alkylating potency, tested compounds, in 2 mL of methanol, were added to an approximately 0.5 M solution 4-(4-nitrobenzyl)pyridine (NBP, 98%, Aldrich) in methanol, in 15-mL glass-stoppered test tubes, maintained in a water bath at 25°C. Aliquots (0.5 mL) were withdrawn at intervals, one drop of cyclohexyl amine was added, the volume was adjusted, according to the intensity of the colour, to 3-15 mL, and the spectrum was scanned from 400 to 700 nm against a reagent blank, by a Hewlett-Packard Diode Array spectrophotometer Model 8452A, with a 2 nm resolution. Absorbance at 550 nm, A_{550}, was plotted against time. The slope Sl (ΔA_{550}/(mole$_n$*mole$_p$*hour)

where (ΔA_{550} is the increase of absorbance, and $mole_n$, $mole_p$ are molar concentrations of NBP and compound, respectively)), of the resulting straight line was calculated by linear regression.

The tested compounds included, in addition to azamethiphos, methyl iodide as an example of a strong electrophile, dichlorvos (DDVP, 2,2-dichloroethenyl dimethyl phosphate, CA RN 62-73-7), as an electrophilic organophosphorus insecticide, and the organophosphorus insecticides dicapthon (O-(2-chloro-4-nitrophenyl)O,O-dimethyl phosphorothioate, CA RN 2463-84-5), fenchlorphos (O,O-dimethyl O-2,4,5-trichlorophenyl phoshorothioate CA RN 299-84-3), fenitrothion (O,O-dimethyl O-(3-methyl-4-nitrophenyl) phosphorothioate, CA RN122-14-5), and parathion (O,O-diethyl O-4-nitrophenyl phosphorothioate, CA RN56-38-2). The organophosporus insecticides were from the Pesticide Kit (Chem Service, Inc, West Chester, PA 19381-3108, USA).

RESULTS AND DISCUSSION

The plots of A_{550} *vs* time were linear, indicating that the concentrations of NBP and of the tested compounds were in the range that allowed the approximation of the bimolecular kinetic equation by A_{550} = Sl * $mole_n$*$mole_p$*hour. The alkylating potency of azamethiphos is about two-thirds that of dichlorvos, and both are considerably higher than the potencies of other tested organophosphorus pesticides (Table 1).

Table 1. Alkylating potency

Compound	Slope	Standard deviation
Methyl iodide	53.3	8.9
Dichlorvos	31.3	0.5
Azamethiphos	22.3	1.9
Dicapthon	8.1	0.6
Fenchlorphos	6.9	0.6
Fenitrothion	5.8	Nd
Parathion	0	Nd

Slope = $\Delta A_{550}/(mole_n*mole_p*hour)$,
ΔA_{550} is the increase in absorbance at 550 nm, in 1 cm cell
$mole_n$, $mole_p$ are molar concentrations of NBP and tested compound, respectively.

The primary mechanism of action of organophosphorus insecticides is based on phosphorylation, but some organophosphate insecticides may also act by alkylation. The alkylating potency is usually measured by the alkylation of NBP (see, for example, Lohs et al. 1976; Wooder and Wright 1981; Hermens et al. 1986; Schüürmann 1990). The NBP alkylating

potency may be related to the potency of some organophosphates to inhibit carboxyesterases (Imamura and Talcott 1985). Dichlorvos is one of the more potent organophosphate alkylators and is carcinogenic in rats and mice (Chan et al. 1991) but, in general, the correlation between the alkylating potency and toxic effects, such as carcinogenicity and mutagenicity, is poor (Bartsch et al. 1983). However, the fairly high alkylating potency of azamethiphos may explain its observed mutagenicity (Committee for Veterinary Medicinal Products 1999) and is an indication that the possibility of delayed effects on the non-target biota should be examined.

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