Anticholinesterase Activity and Enzymatic Degradation of Phosphamidon and γ-Chlorophosphamidon A Comparative Study

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Technical phosphamidon, O,O-dimethyl-O-(1-methyl-2-chloro-2-diethyl-carbamyl-vinyl) phosphate, is known to contain an impurity, γ-chlorophosphamidon, which represents one to two percent of the total product. Chemically this by-product is O,O-dimethyl-O-(1-chloromethyl-2-chloro-2-diethyl-carbamyl-vinyl) phosphate. The contaminant inhibits bovine erythrocyte acetylcholinesterase (AChE) about ten times, and human plasma cholinesterase (ChE) about twenty times more than pure phosphamidon (Table 1). Yet to mice and rats it is approximately ten times less toxic than the parent compound (1). As it is theoretically possible that the higher anticholinesterase activity of γ-chlorophosphamidon might present a hazard during field use of technical phosphamidon, the in vivo anticholinesterase activity of the technical product was compared with pure phosphamidon. In addition the rates of degradation in liver homogenates of various animal species were determined for both compounds.

Material and Methods

Animals. Animals used were mice (24-34 g), guinea-pigs (about 800 g), rats (200-300 g), and beagle-dogs (6 to 10 months old), all obtained from the Tierfarm AG., Sisseln, Switzerland. Rabbits (about 3 kg) and White Leghorn chickens (about 2 kg) were obtained locally.

Cholinesterase studies in vivo. Eight female rabbits, divided into two groups of four, were used. One group was fed a diet containing 200 ppm analytically pure phosphamidon, and the second group was fed a diet containing the same concentration of technical phosphamidon with a particularly high content of 4.3 percent of γ-chlorophosphamidon. Feeding was continued for ten days.

Blood samples were taken every other day and plasma cholinesterase activity determined by the method of

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Voss and Schuler (2).

Determination of I_{50}-values. These were determined by using the automated colorimetric method of Voss and Geissbühler (3). The enzyme sources were purified bovine erythrocyte acetylcholinesterase (AChE) from Serva Entwicklungslabor, Heidelberg, Germany, and outdated human blood plasma obtained from the local blood bank.

Preparation of liver homogenates. Dogs were killed with an intravenous injection of pentobarbital and all other animals by a blow on the neck. Livers were removed immediately after death, and homogenates prepared by the method of Donninger et al. (4).

Degradation experiments. Each compound was dissolved in liver homogenate to yield a final concentration of 100 µg/ml. Treated homogenates were then incubated, using a shaking bath and a temperature of 37°C. Following incubation, 0,5 ml of the γ-chlorophosphamidon containing homogenate was transferred into 9.5 ml ethanol. This solution was filtered and further diluted with water to a concentraction range appropriate for cholinesterase inhibition assay. For phosphamidon treated homogenates, 0.5 ml was added to 9.5 ml of chloroform. The mixture was shaken and the chloroform phase exchanged for n-hexane, from this solvent the insecticide was re-extracted with water (3).

The Final aqueous extracts were analyzed for cholinesterase inhibiting substances by the method of Voss and Geissbühler (3). This procedure does not distinguish between the parent compounds and any possible metabolites with anticholinesterase activity. From the toxicological aspect, however, it was considered more important to measure the total amount of all inhibiting compounds present.

Results and Discussion

It is evident from the results presented in Table 1 that γ-chlorophosphamidon is a much more potent cholinesterase inhibitor when compared with phosphamidon. This is true for both types of cholinesterases tested.

Feeding the rabbits with treated diet for a period of ten days produced a significant in vivo inhibition of their plasma cholinesterase ($P = 0.0005$). Inhibition was 40 ± 9 % (mean ± SEM) in the animals fed pure phosphamidon, and 42 ± 9 % in the group treated with technical phosphamidon. The difference between the two groups was not significant ($P = 0.4$).

TABLE 1

I_{50}-values (final molar concentration during preinhibition) of phosphamidon and γ-chlorophosphamidon, determined for two different cholinesterases by means an automated procedure.

Compound	AChE	ChE
Phosphamidon	6.6×10^{-5}	2.2×10^{-6}
γ-chlorophosphamidon	2.8×10^{-6}	1.6×10^{-8}

Ten days after returning the animals of both groups to a normal diet, their plasma cholinesterase activities had become normal.

This finding clearly demonstrates that 4.3 % γ-chlorophosphamidon in technical phosphamidon contributes nothing to the cholinesterase inhibition in vivo.

The reason for this lack of inhibition potency of γ-chlorophosphamidon in vivo became apparent, when its rate of degradation in liver homogenates was compared with that of phosphamidon (Table 2).

TABLE 2

Degradation of phosphamidon and γ-chlorophosphamidon by liver homogenates of various animal species. Results are expressed as mean ± SEM microgram of compound degraded by one gram of liver in ten minutes.

Species	Sex	Number	Phosphamidon	γ-chlorophosphamidon
Mice	female	10	66 ± 1	1690 ± 32
Dogs	both	4	75 ± 7	1114 ± 123
Rats	male	4	121 ± 10	1544 ± 162
Rats	female	4	95 ± 2	1702 ± 178
Chickens	female	4	122 ± 10	280 ± 66
Rabbits	female	4	132 ± 14	1914 ± 48
Guinea pigs	male	6	180 ± 26	1800 ± 52

Both compounds were readily broken down by liver homogenates of all species tested, however, γ-chlorophosphamidon was degraded at a much faster rate than phosphamidon. Liver homogenates of the chickens degraded

γ-chlorophosphamidon at a slower rate than observed in the mammals, although its rate of breakdown was still more than twice that of phosphamidon.

It can be concluded from the results described above, that the inverse relationship between I_{50}-values of both compounds on one hand and their toxicities on the other hand is due to rapid enzymatic degradation of γ-chlorophosphamidon. The presence of the contaminant in technical phosphamidon, therefore, contributes little or nothing to the toxicity of the technical material.

References

(1) SACHSSE K. and VOSS G., Residue Reviews (in press).
(2) VOSS G. and SCHULER J., Bull. Environm. Contam. Toxicol. 2, 357 (1967).
(3) VOSS G. and GEISSBUEHLER H., Mededelingen Rijksfaculteit Landbouwwetenschappen Gent 32, 877 (1967).
(4) DONNINGER C., HUTSON D.H. and PICKERING B.A., Biochem. J. 102, 26p (1966).