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MALATHION A AND B ESTERASES OF MOUSE LIVER—III: *IN VIVO* EFFECT OF PARATHION AND RELATED PNP-CONTAINING INSECTICIDES ON ESTERASE INHIBITION AND POTENTIATION OF MALATHION TOXICITY

NEMANI RAMAKRISHNA and BHIKSHANDER V. RAMACHANDRAN
Indian Drug Research Laboratory, Poona 411005, India

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Abstract—The reported failure of parathion to potentiate the toxicity of malathion and the high degree of potentiation effected by the closely related EPN have been re-investigated using certain new techniques. Parathion, EPN, paraoxon, fenitrothion and methyl parathion were injected to mice in sub-multiples and multiples of their LD_{50}'s, in the latter case after protecting the animals with atropine and toxogonin. After an interval of 18 hr the malathion B esterase and the phenyl acetate esterase activities of the liver and the acetylcholinesterase activity of the brain were determined and the extent of inhibition calculated. In batches of mice similarly treated with the synergists the LD_{50} of malathion was determined to assess the degree of potentiation. Contrary to available knowledge parathion is found to be a powerful potentiator of malathion, almost equal in potency to EPN. Fenitrothion which is an insecticide of low mammalian toxicity is also a strong potentiator at sub-lethal doses. An attempt is made to correlate the extent of inhibition of the esterases with the degree of potentiation of malathion toxicity.

Malathion* is a highly selective insecticide, its low mammalian toxicity being attributable to the easy hydrolysability of the α-carboxyethyl ester group by the non-specific CE (EC 3.1.1.1) of the liver[1–5]. Since all OP insecticides are potentially CE inhibitors *in vivo* after activative metabolism in the liver to their oxygen analogues, all of them should in theory, potentiate the toxicity of malathion by decreasing its rate of degradation. This postulate explains the high degree of potentiation effected by EPN and TOTP[3, 6, 7] but fails in cases where there is just an additive or less than additive effect[8–10]. A puzzling case is the disparate behaviour of EPN and parathion which are closely related in chemical structure. While EPN is the most powerful potentiator of malathion known, parathion not only fails to potentiate but has a negative effect. A mixture containing 0.5 LD_{50} each of parathion and malathion when injected to rats effected 10 per cent mortality against the expected 50 per cent[9]. The biochemical basis for these differences has not been worked out, the difficulty in such studies being the lack of an ester substrate whose hydrolysis and inhibition rates would parallel those of malathion[11, 12]. Malathion itself which should be the primary choice was found to be unsuitable in manometric studies[13, 14].

In the present study the problem of the differential effects of EPN and parathion on the potentiation of malathion has been re-investigated using certain new techniques and approaches. The recently developed methods[15–17] for the estimation of malathion A and B esterases have been employed. The synergist and the challenger have been injected sequentially instead of simultaneously to avoid competition between the two for the microsomal oxidase system to activate them. The mice used in these studies were protected with atropine and a quaternary pyridinium oxime (toxogonin) so that a clear picture of the synergistic mechanism would emerge due only to the inhibition of CE. It has been shown by Ramachandran [18–20] that these antidotes have no influence on the CE system. The extent of inhibition of brain AChE due to OP synergists has been determined to assess its contribution to the overall toxicity other than via CE inhibition. Though the ultimate cause of death due to OP compounds is respiratory failure due both to peripheral and central effects[21] it is not clear whether the oxime antidotes penetrate and protect the brain AChE[20]. Though the study is primarily to solve the anomalous behaviour of parathion and EPN as malathion potentiators, 3 other compounds containing a PNP-moiety as the leaving group have been included for comparison, viz.,

*Abbreviations and trivial names: AChE—acetylcholinesterase; CE—carboxylesterase; DFP—diisopropyl phosphorofluoridate; EPN—0-ethyl 0-*p*-nitrophenyl phenylphosphorothionate; Fenitrothion—0,0-dimethyl 0-*p*-nitro-*m*-cresylphosphorothioate; Malathion—*S*-(1,2-dicarbethoxyethyl) 0,0-dimethyl phosphorodithioate; Methyl parathion-0,0-dimethyl 0-*p*-nitrophenylphosphorothioate; Parathion—0,0-diethyl 0-*p*-nitrophenylphosphorothioate; Paraoxon—0,0-diethyl 0-*p*-nitrophenylphosphate; PNP—*p*-nitrophenol or *p*-nitro-*m*-cresol; TOTP-tri-0-tolylphosphate; Toxogonin-bis-(4-hydroxyiminomethyl pyridinium-1-methyl) ether dichloride.

methyl parathion, fenitrothion and paraoxon. As a result of these studies it is found that contrary to present available knowledge, parathion is a powerful potentiator of malathion provided the two compounds are injected sequentially.

MATERIALS AND METHODS

The materials used together with their sources are as follows: Malathion secondary standard 99% (American Cynamid Co., Princeton, NJ); Parathion 98%, fenitrothion 95% and methyl parathion 80% (through Bayer India Ltd., Bombay); Atropine sulphate, acetylcholine iodide, DFP and paraoxon (Sigma Chemical Co., St. Louis, MO); Toxogonin (Merck, Darmstadt) and EPN analytical grade (E.I. duPont de Nemours, Wilmington, Delaware). The reagents for malathion B esterase and phenylacetate esterase determinations were as already given [15–17].

For injections, atropine 17.4 mg (50 μmoles) and toxogonin 35 mg (100 μmoles)/kg were weighed together and dissolved in 4 ml of physiological saline and injected 15 min before the synergists [19]. For organophosphates the appropriate quantity to be injected/kg was weighed and dissolved in 0.4 to 0.8 ml of ethanol and diluted to 4 ml with propylene glycol. Trial experiments showed that this amount (10–20 per cent) of ethanol in the medium neither had *per se* any effect nor affected the LD_{50}'s. For DFP and paraoxon the required amount of a 10% stock solution in propylene glycol was weighed and diluted with the appropriate medium. Unless otherwise stated all injections were by the i.p. route in a vol. of 4 ml/kg. Antidotes were used for protection where the dose of the synergist was at or above one LD_{50}. Graded doses of the PNP-insecticides in sub-multiples and multiples of the LD_{50} were injected to groups of 3–6 mice, and 18 hr thereafter the malathion B esterase and phenylacetate esterase activities of the liver were determined at 37° and 20° respectively according to the methods described [16, 17]. AChE activity was determined in the brain tissue according to Hestrin [22]. The organ was removed immediately after sacrifice and homogenised in a Potter–Elvehjem type of homogeniser with 9 times (w/v) the quantity of ice-cold 0.067 M phosphate buffer of pH 7.2. The homogenate was used immediately for assay [15–30 min from sacrifice] to minimise the spontaneous release especially of dimethoxyphosphoryl moiety. A typical digest contained phosphate buffer 0.067 M of pH 7.2, AChI 0.004 M and 1 to 3 ml of homogenate in a total vol. of 25 ml at 37°. One ml aliquots were withdrawn at 3 min intervals and added to 2 ml of a cold 1 + 1 mixture of 3.5 N sodium hydroxide and 2 M hydroxylamine and the ferric hydroxamate colour developed as described [15, 22]. The esterase activities were calculated from an 8 point graph and the average per cent inhibition from standard esterase values for untreated mice. It was separately established that neither the vehicles used nor the antidotes had any statistically significant effect on the normal esterase values or the LD_{50} of malathion after an interval of 18 hr. To groups of 20 mice the same dose of synergists were injected as in esterase experiments and the LD_{50} of malathion determined by a 4 point assay of 5 animals each according to the methods of Thompson [23] and Weil [24]. The average LD_{50} was calculated from at least 2 such series.

As a preliminary to these studies the LD_{50}'s of the samples of OP compounds used in this work were determined. The values are given in Table 1. Fenitrothion was sparingly soluble in ethanol–propylene glycol at higher doses. Refined groundnut oil (arachis oil) was therefore used in this case. For use as synergist which was never above 200 mg/kg, ethanol–propylene glycol was the medium for fenitrothion as well as other compounds. In one set of experiments paraoxon was injected by the s.c. route. Ethanol–prppylene glycol was found unsuitable in this case as the liquid was apt to ooze out from the site of injection. Physiological saline was used as the medium. It is seen that the LD_{50} of paraoxon by the s.c. route is 0.63 mg/kg which is almost exactly one half of that by the i.p. route.

In a number of cases the effect of injecting PNP-insecticides to mice on malathion A esterase was studied, but as in the work of Bhagwat and Ramachandran [17] the influence was either nil or only marginal. These values are therefore not reported.

RESULTS

Effect on CE inhibition

The consolidated results are given in Table 2. With regard to CE inhibition the thiono compounds seem to fall in two groups. Parathion and EPN belong to one class effecting a high degree of CE inhibition at low doses (below 10 mg/kg). Fenitrothion and methyl parathion belong to a second category needing larger amounts to effect the same degree of inhibition. A possible explanation is that in the case of the dimethyl compounds glutathione-*S*-alkyl transferase operates to a greater extent than in the case of OP compounds with longer side chains [25]. Thus lesser quantities of fenitrothion and methyl parathion may be available for CE inhibition. It is

Table 1. LD_{50} of OP compounds to mice

Compound	Medium	LD_{50} (mg/kg)	Range
Malathion	E + PG	1223	994–1505
Fenitrothion	GN oil	980	871–1096
Methyl parathion	E + PG	21	15.6–28.1
Parathion	E + PG	5.6	4.6–6.6
Paraoxon	E + PG	1.3	1.1–1.6
Paraoxon	saline	1.3	1.1–1.6
Paraoxon (s.c.)	saline	0.63	0.56–0.69
EPN	E + PG	8.4	6.6–10.7

LD_{50}'s were determined according to Thompson [23] and Weil [24]. The volumes were 4 ml/kg in all cases except at high doses of malathion when it was 6 ml/kg. All injections were by the i.p. route except in one case indicated. E + PG = ethanol 20 per cent and propylene glycol 80 per cent; GN oil = refined groundnut oil, Arachis oil.

Table 2. Effect of various doses of PNP-containing insecticides on esterases and malathion toxicity

OP compound	Dose injected In LD_{50}	Dose injected In mg/kg	Percent inhibition of esterase with SD and no. of trials MT	PA	ACh	LD_{50} of malathion with range mg/kg
Parathion	0.1	0.56	32 ± 8(3)	36 ± 3(3)	9 ± 5(4)	1122 (891–1413)
	0.25	1.4	51 ± 8(8)	44 ± 10(8)	12 ± 11(8)	251 (182–347)
	0.5	2.8	53 ± 6(8)	53 ± 7(7)	51 ± 4(4)	150 (117–191)
	1.0	5.6	100	58 ± 5(14)	67 ± 12(3)	91 (68–123)
	2.0	11.2	100	72 ± 5(8)	83 ± 8(4)	91 (83–109)
	3.0	16.8	—	72 ± 2(4)	93 ± 6(4)	—
EPN	0.15	1.25	36 ± 7(4)	48 ± 5(4)	8 ± 4(3)	630 (555–718)
	0.3	2.5	64 ± 6(5)	52 ± 2(3)	3 ± 5(6)	200 (154–258)
	0.6	5.0	62 ± 4(5)	71 ± 11(5)	52 ± 5(6)	89 (63–127)
	0.9	7.5	65 ± 7(3)	72 ± 6(3)	74 ± 2(3)	50 (39–65)
	1.2	10.0	67 ± 2(3)	75 ± 3(3)	75 ± 8(5)	25 (18–35)
Fenitrothion	0.0125	12.5	25 ± 15(4)	39 ± 6(8)	3 ± 2(3)	—
	0.025	25	41 ± 18(6)	51 ± 6(6)	2 ± 4(3)	355 (251–501)
	0.05	50	68 ± 3(6)	63 ± 1(6)	4 ± 8(4)	288 (190–441)
	0.1	100	72 ± 2(6)	74 ± 4(8)	24 ± 2(3)	136 (103–176)
	0.2	200	72 ± 3(3)	74 ± 2(5)	36 ± 6(4)	80 (63–126)
Methyl parathion	0.25	5.3	45 ± 9(7)	48 ± 8(8)	3 ± 4(3)	—
	0.5	10.5	54 ± 11(9)	50 ± 2(4)	24 ± 3(6)	631 (433–919)
	1.0	21	60 ± 5(4)	58 ± 9(4)	41 ± 8(5)	447 (315–634)
	2.0	42	65 ± 9(8)	71 ± 10(8)	54 ± 8(3)	141 (100–200)
	3.0	63	72 ± 9(5)	68 ± 7(4)	62 ± 4(4)	—
Paraoxon	0.25	0.33	30 ± 4(4)	26 ± 6(4)	15 ± 4(3)	~1260
	0.5	0.65	26 ± 5(4)	35 ± 6(4)	32 ± 3(4)	794 (515–1225)
	1.0	1.3	31 ± 7(4)	48 ± 4(4)	59 ± 7(3)	501 (325–773)
	2.5	3.3	52 ± 8(3)	50 ± 2(4)	64 ± 2(3)	447 (315–504)
	7.5	9.8	52 ± 7(4)	52 ± 7(4)	71 ± 1(3)	316 (244–409)
Paraoxon s.c.	0.5	0.32	15 ± 2(3)	4 ± 4(2)	55 ± 3(3)	~800
	1.0	0.63	32 ± 7(3)	20 ± 7(3)	65 ± 5(3)	316 (182–550)
	2.0	1.26	40 ± 9(4)	45 ± 11(4)	66 ± 2(4)	282 (223–355)
	5.0	3.2	45 ± 6(4)	48 ± 7(6)	71 ± 2(6)	251 (174–363)
	15.0	9.5	58 ± 2(4)	69 ± 6(6)	75 ± 4(6)	159 (110–230)

All injections were by the i.p. route except in one case indicated. At and above one LD_{50} mice were protected with atropine and toxogonin given 15 min earlier. All analyses including LD_{50} determinations of malathion were carried out 18 hr after the synergist. Esterase values for control mice were carried out with no treatment in one case and with atropine and toxogonin given 18 hr earlier in another. The 2 sets of values with S.D. and no. of trials in parenthesis are as follows: malathion B esterase 111 ± 3(4) and 110 ± 8(4) μmoles of MT/g liver/hr; phenylacetate esterase 8597 ± 389(15) and 8506 ± 540(12) μmoles PA/g liver/hr; and AChE 516 ± 55(8) and 524 ± 33(4) μmoles AChI/g brain/hr respectively. The differences were not significant (P values 0.15 to 0.3). All esterase inhibition values given above were calculated in terms of the average of these values. The LD_{50} of malathion control was also unaffected by injecting atropine and toxogonin 18 hr earlier. MT = malathion; PA = phenylacetate; AChI = acetylcholine iodide; S.D. = standard deviation.

seen that among all the compounds parathion is the only insecticide which effects 100 per cent inhibition of malathion B esterase. The inhibition of phenylacetate esterase to the extent of 72 per cent may also be considered as maximal since the un-inhibited part cannot be further inhibited by treating the homogenate *in vitro* with 10^{-5} M DFP or paraoxon. This part is obviously of the A type in the classification of Aldridge [26]. It is also observed that in all cases where the inhibition of malathion B esterase is partial, the un-inhibited portion can be completely inhibited *in vitro* by DFP or paraoxon. The reason for the higher activity of parathion on CE may be that it penetrates the lipoid cell wall better than the other insecticides. This is confirmed by the finding that paraoxon which is the oxygen analogue of parathion and which is quite effective *in vitro* as CE inhibitor effects only 52–58 per cent inhibition at the highest doses administered. In cases where the phenylacetate esterase inhibition is less than about 72 per cent, e.g. with paraoxon, it can be further inhibited to this level by treatment *in vitro* with DFP or paraoxon.

Effect on AChE

As in the case of CE, parathion behaves in a fashion different from others. The extent of inhibition is as high as 93 per cent at 16.8 mg/kg while it is much less with all other compounds. In contrast with CE in which the inhibition level seems to reach a stable maximum, the inhibition of brain AChE increases steadily with the dose without a tendency to stabilise. Ramachandran

[20] observed a similar effect with DF^{32}P. It is seen that with all thiono compounds, especially fenitrothion and EPN, there is an initial lag in AChE inhibition. With fenitrothion even at a dose of 50 mg/kg, when the CE inhibition is 63–68 per cent, there is hardly any inhibition of AChE. It has been pointed out by Miyamoto[27] that fenitrothion penetrates the brain tissue poorly, a possible reason adduced by him for the low mammalian toxicity of this compound as compared to methyl parathion.

Paraoxon is *per se* an esterase inhibitor and such direct acting compounds are known to react chemically with quaternary pyridinium oximes [28, 29]. In this study since most of the experiments with paraoxon were at doses above the LD_{50} and animals had to be protected with atropine and toxogonin, there is a possibility of the oxime and paraoxon interacting with each other if they are injected by the same i.p. route. This would result in less paraoxon being available for esterase inhibition. Therefore in one series of experiments paraoxon was administered by the s.c. route after the antidotes by the i.p. route. It can be seen from the figures for paraoxon in Table 2 that CE inhibition by paraoxon is approximately the same for a particular dose by weight given by either route. However, the degree of AChE inhibition differs under the two conditions. At 0.3 mg/kg, for example, the AChE inhibition values are 15 and 55 per cent respectively by the i.p. and s.c. routes. The corresponding values at 0.6 mg/kg level are 32 and 65 per cent.

Effect of potentiation of malathion toxicity

The most dramatic effect is in the case of parathion. At 0.56 mg/kg there is only a marginal reduction in the LD_{50} of malathion, but at 1.4 mg/kg there is a steep fall, the LD_{50} of malathion being reduced to 251 mg/kg which is about a fifth of the normal value. This is further reduced to 150 mg/kg when the pre-treatment with parathion is with 2.8 mg/kg. Thus parathion which is considered a non-potentiator is in fact a powerful synergist of malathion at sub-lethal doses. EPN is a known potentiator of malathion and the findings in this work are in consonance with those of others. It can be seen however that approximately 5 mg/kg of either EPN or parathion the extent of malathion potentiation is the same, the LD_{50} of the latter being 89–91 mg/kg, a thirteen-fold increase of toxicity from the normal. At higher doses EPN behaves differently from parathion. Whereas with parathion the lowest LD_{50} achieved for malathion is 91 mg/kg, values of 50 and 25 mg/kg are obtained when the EPN doses are 7.5 and 10 mg/kg respectively. It is also seen that the increase in the inhibition of CE between these doses of EPN is negligible. It is likely that the potentiation by EPN at these doses is mediated by a mechanism other than CE inhibition [30].

Fenitrothion and methyl parathion belong to a class different from parathion and EPN in that larger quantities of these are needed on a weight basis to effect the same degree of malathion potentiation. In terms of LD_{50} however, fenitrothion is a powerful synergist. At 0.025 and 0.05 LD_{50}s, it brings down the LD_{50} of malathion to 355 and 288 mg/kg respectively. The AChE inhibition at these levels is negligible but the CE inhibition is very high. Potentiation in this case is clearly due to CE inhibition and unrelated to inhibition of AChE of the brain. Synergism by paraoxon is predominent only at levels above the LD_{50}. Weight for weight the synergistic effect of paraoxon is more when it is given by the s.c. route than the i.p. route.

DISCUSSION

An oft-quoted anomaly in the mechanism underlying the potentiation of malathion toxicity by other OP compounds is the behaviour of parathion which is reported to have a less-than-additive effect [8, 9]. This puzzle has been solved in this work wherein it is shown that parathion is also a powerful potentiator being as potent as EPN the classical synergist of malathion. The failure of previous workers to demonstrate parathion–malathion synergism may be due to their having administered the two compounds together. DuBois [9] himself had predicted that since the time taken for different thiono compounds to be activated may be different, in cases of non-potentiation, an additive or synergistic effect might be demonstrable if the administration of the 2 compounds were so spaced as to obtain optimal effects. In this work parathion and malathion were injected sequentially with an interval of 18 hr in between.

Fenitrothion which like malathion is an insecticide of low mammalian toxicity and hence used extensively on food crops, is a potentiator of malathion at sub-lethal doses. This finding may be of significance from the public health point of view.

In Table 3 the dosages needed to effect 50 per cent inhibition of malathion B esterase, phenylacetate esterase and AChE are given together with the LD_{50} of malathion at these levels of esterase inhibition. The values are mostly from Table 2 and in some cases from dose-response graphs. It is seen that the values for CE determined either with malathion or phenylacetate as substrate are more or less the same though the phenylacetate esterase activity consists of two components, one of which is sensitive to organophosphates and another which is not, while the malathion esterase consists entirely of the OP sensitive type. It is seen that the dosage needed for 50 per cent inhibition of CE differs from compound to compound both in terms of weight as well as in LD_{50}. As pointed out earlier parathion and EPN belong to one class which inhibit at comparatively lower doses on a weight basis. Larger amounts of methyl parathion and fenitrothion are needed for the same effect. The same is the case with regard to the inhibition of AChE. Paraoxon belongs to a third category. In this case the amount needed for 50 per cent inhibition of CE is always greater than for 50 per cent AChE inhibition. With thiono compounds the reverse is the case, smaller doses being needed for CE than for AChE inhibition. These differences in the dosages required to effect

Table 3. Approximate dosages of PNP-insecticides needed to effect 50 per cent inhibition of esterases together with the LD_{50} of malathion at these levels

OP compound	Esterase	Dose needed for 50 per cent inhibition In LD_{50}	In mg/kg	LD_{50} of malathion at this level mg/kg
Parathion	MTE	0.25	1.4	251
	PAE	0.25–0.5	1.4–2.8	150–251
	AChE	0.5	2.8	150
EPN	MTE	0.15–0.3	1.25–2.5	~200
	PAE	0.15–0.3	1.25–2.5	~200
	AChE	0.6	5.0	89
Fenitrothion	MTE	0.025–0.05	25–50	288–355
	PAE	0.025	25	355
	AChE	>0.2	>200	<80
Methyl parathion	MTE	0.25–0.5	5.3–10.5	>631
	PAE	0.5	10.5	631
	AChE	2.0	42.0	141
Paraoxon	MTE	2.5	3.0	447
	PAE	2.5	3.0	447
	AChE	0.5–1.0	0.6–1.2	501–794
Paraoxon s.c.	MTE	5.0–15.0	3.2–9.5	159–251
	PAE	5.0–15.0	3.2–9.5	159–251
	AChE	<0.5	<0.32	>800

Except in one case indicated all injections were by the i.p. route. MTE = malathion B esterase; PAE = phenylacetate esterase; AChE = acetylcholinesterase.

a particular degree of esterase inhibition in the intact animal are understandable since the rate of activative and degradative metabolism as also the degree of penetrability must be different for different OP compounds. But what may be more reasonable to expect is that at a particular level of esterase inhibition, e.g. 50 per cent the degree of potentiation of malathion toxicity should be approximately the same. From results in Table 3 it can be seen that this postulate is broadly correct. At 50 per cent CE level (malathion B or phenylacetate esterase), the LD_{50} of malathion is in the range of 150–355 mg/kg in all cases. The apparent exceptions are methyl parathion and paraoxon (i.p.). On a closer examination it can be seen that even these are marginal cases. In Table 2 it is seen that for methyl parathion there is an abrupt fall in the LD_{50} of malathion from 447 to 141 mg/kg in the CE inhibition level of 58–60 per cent. With paraoxon i.p., the rate of CE inhibition is very slow at higher doses, the level of inhibition being static at 50–52 per cent in spite of a 3-fold increase in the dosage. The LD_{50} of malathion at the 52 per cent inhibition level of CE is 316 mg/kg which is within the 150–355 mg/kg mentioned earlier.

With regard to AChE, at 50 per cent of its normal value, the potentiation of malathion is quite high in the case of thiono compounds. The LD_{50} of malathion is in the range of 80–150 mg/kg. But this effect is not surprising since the dose needed for 50 per cent inhibition of AChE is always greater than that for CE inhibition in all thiono compounds. With paraoxon in which the inhibition of AChE occurs at doses less than for CE, it is seen that at 50 per cent AChE level, the potentiation of malathion is low, the LD_{50} being between 501 and 800 mg/kg.

A number of instances can be cited from Table 2 in which, when there is hardly any inhibition of AChE, there is considerable malathion potentiation. Examples are: 1.4 mg/kg of parathion, 1.25 and 2.5 mg/kg of EPN and 25 and 50 mg/kg of fenitrothion. On the other hand, there is no instance of malathion potentiation without concomitant inhibition of CE. It may therefore be concluded that the basic mechanism of malathion potentiation is by the inhibition of CE, though the actual LD_{50} values of malathion may be slightly modified by the intrinsic anti-AChE activity of the synergists which may contribute additively to the overall toxicity.

A finding of interest is that there are qualitative differences in the CEs. It has been shown that parathion inhibits malathion B esterase completely while EPN inhibits only to the extent of 62–67 per cent. Yet, EPN is as good as or perhaps even better than parathion as a malathion potentiator. To explain this one may have to invoke the available knowledge on the class of enzymes mentioned by the general term of non-specific carboxylesterases (EC 3.1.1.1). It is known that these enzymes consist of several components with different substrate specificities [31] and widely differing sensitivities to OP compounds [32]. It is likely that EPN *via* its oxygen analogue selectively inhibits only those CE components which are important in malathion hydrolysis and potentiation while parathion is less selective. Compounds like methyl parathion probably act less effectively on esterase components concerned in the potentiation of malathion.

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