Original Article

Combined Toxicity of Malathion and Carbamate Insecticides in Mice and Rats

Hiroaki TAKAHASHI, Teiji MIYAOKA, Shuji TSUDA and Yasuhiko SHIRASU

*Toxicology Division, Institute of Environmental Toxicology,
Suzuki-cho, Kodaira 187, Japan*

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The acute oral toxicity (LD_{50}) of equi-toxic doses of malathion plus a carbamate insecticide was determined in mice and rats. The observed LD_{50} of a "mixture" was compared with the expected LD_{50} calculated from each LD_{50} and LD_{50} (expected)/ LD_{50} (observed) was used as a ratio of combined effect. Of 5 combinations studied in male mice, malathion plus BPMC was the only combination which exhibited a striking synergism (the ratio, 5.4). On the other hand, clear synergism was not observed with the combination of malathion plus one of the other carbamate insecticides, MTMC, NAC, XMC or MPMC. When malathion or BPMC was administered alone, the toxicity in female mice or male rats was not different significantly from that in male mice. In female mice, the synergism of malathion plus BPMC was similar to that in male mice (the ratio, 5.2). However, relatively less potent synergism was noted with the combination in male rats (the ratio, 2.0). The marked synergism of malathion plus BPMC could not be explained on the basis of an inhibition of the acetylcholinesterase (AChE) by malaoxon plus BPMC, or an inhibition of malathion's detoxication by BPMC.

INTRODUCTION

The two most important classes of synthetic insecticide are carbamate and organophosphorus compounds, these have been used extensively in large quantities and simultaneously in rice fields. There have been a number of studies on the combined acute toxicity of organophosphorus compounds by simultaneous administration.¹⁻³⁾ However, we have no information in which marked synergism was shown with the combination of an organophosphorus compound and another class of compounds administered simultaneously.

In this paper, we report the combined toxicity of the combination of malathion and a carbamate insecticide, the combination of which has been actually used in the field.

MATERIALS AND METHODS

1. Animals

SPF animals of male (30–37 g) and female (20–25 g) ICR mice and male Fischer 344 rats

(150–180 g) were used at 7 weeks of age. The animals were purchased from Charles River Japan, Inc. at 6 weeks and housed 10 per cage in a room controlled under a cycle of 14 hours light–10 hours dark, constant temperature ($24\pm 2^\circ\text{C}$) and humidity ($55\pm 5\%$). Food and water were allowed *ad libitum*.

2. Chemicals

Technical grades of malathion (S-1,2-bis-(ethoxycarbonyl)ethyl *O,O*-dimethyl phosphorodithioate, 97.1%), BPMC (2-*sec*-butylphenyl methylcarbamate, 98.9%), MTMC (*m*-tolyl methylcarbamate, 97.4%) and MPMC (3,4-xylyl methylcarbamate, 97.2%) were supplied by Sumitomo Chemical Co., Ltd. Technical grades of NAC (1-naphthyl methylcarbamate, 99.9%) and XMC (3,5-xylyl methylcarbamate, 99.9%) were provided by The Agricultural Chemicals Inspection Station of the Ministry of Agriculture, Forestry and Fisheries (Tokyo). Analytical grade of malaoxon was obtained from Nanogen.

Suspensions of insecticide were freshly prepared by Kinematica Polytron with a mixture of 1% tween 80 and 1% sodium carboxymethyl cellulose solutions (1 : 1) before each experiment. The prepared suspension was given orally (less than 30 ml/kg). At least 5 dose levels and 10 animals per level were used for the determination of LD₅₀ value. Animals were observed at least 7 days following administration and times of death were recorded.

3. Acute Toxicity

LD₅₀ and parallelism of two dosage-mortality lines were estimated by the method of Litchfield *et al.*⁴⁾ When slopes of two dosage-mortality lines were not significantly different, the combination of equi-toxic doses (based on the LD₅₀) was administered. Combined effect was estimated by comparing the observed LD₅₀ value, which was determined experimentally, with the expected LD₅₀ value calculated by Finney's equation⁵⁾:

$$\frac{100(\%)}{\text{Expected LD}_{50} \text{ of mixture}} = \frac{A(\%) \text{ in mixture}}{\text{LD}_{50} (A)} + \frac{B(\%) \text{ in mixture}}{\text{LD}_{50} (B)}$$

The ratio of combined effect was defined as LD₅₀ (expected)/LD₅₀ (observed).

4. Estimation of AChE Activity

Mice were killed by decapitation and exanguinated. Brains were homogenized in 4 volumes of 0.5 mM Tris/HCl buffer (pH 7.4). The homogenates were centrifuged at 15,000 *g* for 10 min and the supernates were used as the enzyme source. As inhibitors, malaoxon, BPMT and a mixture of equi-effective concentrations (based on the IC₅₀) dissolved in ethanol were used. After preincubation at 37°C for 10 min, the residual activity was measured by the automated method of Humiston *et al.*⁶⁾ with the use of acetylthiocholine iodide as a substrate.

The estimation of combined effect on the AChE was the same as that for combined toxicity. Fifty percent inhibition concentration (IC₅₀) value was determined by linear regression analysis of the data (log concentration in nM; percentage inactivation of AChE in probits).

5. Estimation of Carboxylesterase (C.E.) Activity

Mice were killed by decapitation after oral administration of carbamate insecticide. The residual activity of C.E. in the liver homogenates was measured by the method of Bhagwat *et al.*^{7,8)} with the use of malathion as a substrate.

RESULTS

1. Combined Toxicity in Male Mice

Prior to the tests of combination, the acute toxicity of each insecticide in male mice was measured (Table 1). The parallelism was observed between the dosage-mortality line of malathion and that of a carbamate insecticide.

Of all combinations studied, malathion plus BPMT was the only one which exhibited a striking synergism; the ratio was 5.4. On the other hand, the mixture of malathion plus the other carbamate insecticides, MTMT, NAC, XMT or MPMT did not show significant potentiation. The ratio ranged from 0.7 to 1.6.

2. Combined Toxicity of Malathion and BPMT in Female Mice and Male Rats

The combination of malathion plus BPMT was also tested in female mice and male rats (Table 2). When malathion or BPMT was administered alone, the potency of toxicity in them was not significantly different from that in male mice. The parallelism of dosage-mortality lines was noted between malathion and BPMT in both animals. The degree of synergism in female mice (the ratio, 5.2) was similar to that in male mice, indicating that there is no difference in the degree of synergism between the two sexes. In rats, however, the synergism (the ratio, 2.0) was relatively less potent than that in mice.

3. Symptoms, and Times of Deaths

In mice and rats, the symptoms observed for each compound and for the combinations were similar and peculiar to those of anticholinesterase compounds: muscle fasciculation, increased salivation, urination, convulsion and dyspnea.

The times elapsed from administration to death were studied after the administration of malathion or BPMT alone. Mice usually died

Table 1 Combined toxicity of malathion plus a carbamate insecticide in male mice.

Combination	LD ₅₀ of individual		LD ₅₀ of mixture		Ratio ^{a)}
	Malathion	Carbamate	Expected	Observed	
Malathion+BPMC	2700	320	1500	280	5.4
Malathion+MTMC	2700	210	1500	930	1.6
Malathion+NAC	2700	290	1500	1000	1.5
Malathion+XMC	2700	220	1400	1300	1.1
Malathion+MPMC	2700	79	1400	1900	0.7

Each figure of LD₅₀ is expressed as mg/kg.

^{a)} LD₅₀ (expected)/LD₅₀ (observed).

Table 2 Combined toxicity of malathion plus BPMC in female mice and male rats.

	LD ₅₀ of individual		LD ₅₀ of mixture		Ratio ^{a)}
	Malathion	BPMC	Expected	Observed	
Female mice	2500	380	1400	270	5.2
Male rats	3200	350	1800	910	2.0

Each figure of LD₅₀ is expressed as mg/kg.

^{a)} LD₅₀ (expected)/LD₅₀ (observed).

Fig. 1 Carboxylesterase activity after administration of BPMC, XMC or MPMC in male mice.

BPMC (30 mg/kg), XMC (98 mg/kg) or MPMC (53 mg/kg) was orally administered. Values of 12 non treatments were 77.8 ± 9.3 (S.D.) μ moles malathion metabolized/20 min/g of liver. Vertical bars were S.E. of 4 mice.

Significantly different from non treatment: * $p < 0.05$, *** $p < 0.001$.

10 min to 2 hours after the administration of malathion, 5 to 90 min after the dose of BPMC. In rats, deaths after the dose of BPMC were observed between 20 and 80 min, which roughly corresponded to those in mice. However, the

occurrence of deaths by malathion in rats, which was usually observed after 3 hours, was much later than that in mice.

4. Combined Effect of Malaoxon and BPMC on AChE

The combined effect of malaoxon and BPMC on brain AChE activity was studied *in vitro*. IC₅₀'s of malaoxon and BPMC alone were 170 nM and 870 nM, respectively. The observed IC₅₀ of equi-effective mixture based on each IC₅₀ was 510 nM. On the other hand, the expected IC₅₀, which was calculated from each IC₅₀, was 520 nM. Since the observed IC₅₀ was similar to the expected IC₅₀, an additive effect was noted.

5. C.E. Activity after the Administration of Carbamate Insecticide

To investigate the inhibition of malathion's detoxication by a carbamate insecticide, the activity of C.E. was studied after oral dose of carbamate insecticide in male mice (Fig. 1). BPMC, XMC or MPMC was administered with the dose of carbamate fraction in each LD₅₀ (observed). Inhibition was observed after the dose of MPMC or XMC. However, no inhibi-

tion was shown after the administration of BPMC.

DISCUSSION

The acute toxicity of malathion or a carbamate insecticide is due to the inhibition of AChE activity of nerve tissues. Malathion was metabolized *in vivo* through the oxidative desulfuration to the oxygen product, malaoxon, which was a potent inhibitor of the AChE.⁹⁾ To investigate whether the marked synergism of malathion plus BPMC can be explained by an inhibition of AChE resulting from direct interaction, the combined effect of malaoxon plus BPMC on the AChE was studied *in vitro*. We did not, however, obtain evidence to support this possibility (Table 3).

In studies of the toxicological interactions of two chemicals which had significantly different times of onset and of maximum effect, the importance of temporal factors, i.e., the time interval between the first and second dose, was reported.^{10,11)} When the elapsed times from BPMC administration to death were studied, no time difference was observed in the occurrence of deaths between rats and mice. In rats, however, the deaths occurred much later than those in mice after a dose of malathion. The observation of lesser synergism in rats compared with that in mice might be explained by temporal factors.

The synergism of malathion plus EPN, TOCP or IBP has been extensively studied and explained by the inhibition of C.E. which hydrolytically detoxifies malathion and malaoxon.^{3,12-14)} However, the marked synergism of malathion plus BPMC could not be explained by the inhibition of malathion's detoxication by BPMC, since the activity of C.E. was not inhibited after the administration of BPMC (Fig. 1).

Carbamate insecticides were mainly metabolized through the oxidation of parent molecules to less or non toxic products by microsomal drug-metabolizing enzymes,¹⁵⁾ the activity of which was inhibited by malathion.¹⁶⁾ However, the acute toxicity of BPMC was not potentiated markedly by the treatment of SKF 525A, which was a potent inhibitor of the enzymes (in preparation). These results suggest a possibility that some other mechanism is

operating in malathion-BPMC interaction.

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要 約

マウス、ラットにおけるマラチオンとカーバメート殺虫剤の相互作用

高橋宏明, 宮岡貞次, 津田修治, 白須泰彦
マウス、ラットを用い、マラチオンとカーバメート殺

虫剤の等効力混合剤の急性毒性 (LD_{50}) を調べた。混合剤の LD_{50} (実験値)と単剤の LD_{50} から計算した LD_{50} (期待値)を比較し、相互作用を示す比率として、 LD_{50} (期待値)/ LD_{50} (実験値)を用いた。雄マウスで検討した5種類の組合せのなかで、マラチオンと BPMC の混合剤は著明な協力作用を示した(比率, 5.4)。しかしマラチオンと他のカーバメート殺虫剤(MTMC, NAC, XMC, MPMC)の混合剤では明確な協力作用は認められず、0.7 から 1.6 倍の比率がみられた。

雌マウス、雄ラットに対するマラチオン、もしくは

BPMC 単剤の急性毒性は雄マウスに対する毒性と比べ有意な差はなかった。雌マウスで、マラチオンと BPMC の混合剤を検討すると 5.2 倍の協力作用がみられ雄マウスと比べ性差はみられなかった。一方、雄ラットでは、マウスに比べ比較的低い 2.0 倍の協力作用が認められた。

マラチオンと BPMC の著明な協力作用は、マラオキソンと BPMC のアセチルコリンエステラーゼに対する直接作用、もしくは BPMC によるマラチオンの解毒阻害では説明できなかった。