Synergism of Toxicity of *N*,*N*-Diethyl-m-toluamide to German Cockroaches (Orthoptera: Blattellidae) by Hydrolytic Enzyme Inhibitors

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ABSTRACT Various compounds were tested for effects on the toxicity of the insect repellent *N*,*N*-diethl-m-toluamide (DEET) in German cockroaches, *Blattella germanica* (L.). Organophosphate and carbamate acetylcholinesterase inhibitors carbaryl, DEF, eserine (physostigmine), malathion and pyridostigmine bromide synergized DEET toxicity. Of those tested, their toxicity was synergized by DEET. Compounds that synergized DEET toxicity also synergized the toxicity of the formamidine pesticides Amitraz and chlordimeform. Results suggest that DEET may have some toxic actions that are similar to those of formamidine pesticides. DEET synergized the toxicity of some acetylcholinesterase inhibitors but not others. Results further suggest that some mechanism other than acetylcholinesterase inhibition was responsible for the toxic interactions observed between DEET and the acetylcholinesterase inhibitors.

KEY WORDS *Blattella germanica*, DEET, insecticide, carbamate, organophosphate, formamidine

THE INSECT REPELLENT *N*,*N*-diethyl-m-toluamide (DEET), which was developed by USDA in the 1950s (McCabe et al. 1954), is used by ≈30% of Americans every year (Veltri et al. 1994). Some reports indicate that excessive doses of DEET may be toxic to humans (Lipscomb et al. 1992, Schaefer and Peters 1992, Clem et al. 1993) and vertebrates other than humans (Mount et al. 1991, Verschoyle et al. 1992, Schoenig et al. 1993).

It would be helpful to have repellents that are as effective as DEET and less likely to have toxic side effects. The molecular mechanism(s) of the repellent and toxic action of DEET are unknown. Since the mid-1950s, a great deal of effort has been spent to improve the efficacy and duration of action, and to increase the safety margin of insect repellents (Schreck and McGovern 1989, Magnon et al. 1991, Kuthiala et al. 1992, Robert et al. 1992). However, DEET remains the most effective, commonly used insect repellent. An understanding of the toxic mode of action of DEET will assist the design of insect repellents that are less likely to have toxic effects, provided the mode of action for repellency and toxicity are distinct.

This research was done to characterize the toxicity of DEET in German cockroaches, *Blattella germanica* (L.), with the eventual goal of discovering the molecular actions of DEET. This information can be used to test whether cockroaches are a suitable model for DEET toxicity to vertebrates. This approach assumes risks because of differences in responses of insects and vertebrates; however, some classes of insecticides act in a similar way in insects and vertebrates (Matusmura 1985), and the potency of DEET toxicity to German cockroaches (this project) is not significantly different from that of rats on a weight basis (Macko and Weeks 1980). Once the information on the toxicity of DEET to cockroaches is available, testing of specific mechanistic hypotheses for vertebrate toxicity can proceed rapidly and be confirmed or rejected. Much of the initial trial and error that occurs in such exploratory research can thus be circumvented, with resulting reduced cost and less vertebrate pain and suffering. Such information would also provide a basis for further research to examine whether the repellent and toxic effects of DEET are related.

The structure of DEET superficially resembles that of some formamidines. As part of another investigation, I found that some serine hydrolase inhibitors synergized the toxicity of 2 formamidine pesticides, Amitraz and chlordimeform. Synergistic interactions in insects between formamidines and some carbamates (Fisher 1992) and organophosphates (Fisher 1992) and pyrethroids (Mosupi and Terry 1991, Liu and Plapp 1992) also have been reported. Therefore, I tested whether the activity of DEET was synergized by compounds that synergize formamidine toxicity. If so it might be useful to test whether DEET had some of the specific actions of formamidines.

Table 1. Effect of sublethal doses of various compounds on the toxicity of Amitraz, chlordimeform, and DEET on German cockroaches

Toxicant	Synergist	Dose, μg/g	Synergism ratio[a]	LD_{50}, μg/g[b]	95% FL	P	n	Slope (±SE)
DEET	None	0	1.0	2,711.00	2,172–3,474	0.56	240	3.39 (0.75)
DEET	Lambda-cyhalothrin	0.05	1.6	1,645.00	1,018–2,090	0.21	179	3.42 (0.88)
DEET	Permethrin	1.23	2.0	1,361.00	886–1,704	0.16	169	3.70 (0.97)
DEET	Chlordimeform	205	3.7	725.00	553–888	0.09	210	2.80 (0.47)
DEET	PMSF	2,049	6.2	437.00	278–583	0.05	350	1.62 (0.29)
DEET	Pyridostigmine	2,049	6.7	404.00	101–658	0.93	230	1.35 (0.42)
DEET	Amitraz	205	7.6	357.00	233–471	0.91	231	2.36 (0.39)
DEET	Eserine	102	904.0	3.00	—	—	70	—
DEET[c]	None	0	1.0	15,528.00	10,497–36,699	0.75	146	1.58 (0.41)
DEET[c]	DEF	410	2.9	5,346.00	2,644–9,247	1.00	97	1.76 (0.59)
DEET[c]	DEF	1,025	4.7	3,326.00	667–54,783	0.62	50	0.83 (0.35)
Amitraz	None	0	1.0	1,246.00	883–1,559	0.98	150	3.50 (0.74)
Amitraz	PMSF	2,049	7.7	161.00	42–184	0.05	80	9.99 (4.56)
Amitraz	Eserine	102	1,058.0	1.18	4×10^{-4}–3.47	0.82	90	0.84 (0.35)
Chlordimeform	None	0	1.0	711.00	559–981	0.10	242	2.76 (0.65)
Chlordimeform	DEF	1,025	3.8	187.00	112–243	0.20	110	3.20 (0.77)
Chlordimeform	Eserine	102	107	6.66	2.83–34.8	0.31	240	0.77 (0.30)
Chlorpyrifos	None	0	1.0	2.99	2.52–3.38	0.99	150	4.8 (0.73)
Chlorpyrifos	DEF	1,025	11.0	0.27	0.21–0.32	0.11	210	4.5 (1.07)
Chlorpyrifos	PBO	2,049	0.697	4.29	3.83–4.68	0.64	149	7.79 (1.56)
Chlorpyrifos[d]	None	0	1.0	21.10	18.3–25.2	0.22	210	3.92 (0.66)
Chlorpyrifos[d]	DEF	1,025	14.0	1.51	1.13–1.88	0.14	179	3.86 (0.99)
Chlorpyrifos[d]	PBO	2,049	0.844	25.00	20.5–33.8	0.17	180	3.02 (0.65)
Malathion	None	0	1.0	62.90	41.5–84.1	0.11	480	1.81 (0.37)
Malathion	DEF	1,025	95.2	0.66	0.60–0.82	0.71	160	3.98 (0.93)
Malathion	PBO	2,049	1.09	57.70	44.7–68.8	0.51	180	3.89 (0.62)

[a] LD_{50} toxicant/LD_{50} toxicant + synergist.
[b] LC_{50} s 48 h after treatment.
[c] Village Green strain German cockroaches (Atkinson et al. 1991).
[d] Gallina field-collected German cockroaches.

Materials and Methods

Bioassays. Adult male Orlando Normal German cockroaches, reared at 26°C, 55% RH, and a photoperiod of 12:12 (L:D) h (Koehler and Patterson 1986) were used in the bioassays. The cockroaches were anesthetized with CO_2, then treated topically on the abdomen between the rear coxae with 1.0 μl of a potential technical grade synergist in acetone or with 1.0 μl acetone alone. Rates were chosen that were as high as possible without causing >10% control mortality. Two hours after the putative synergist was applied, these cockroaches were again treated topically on the abdomen with 1.0 μl technical grade insecticide in acetone or with 1.0 μl acetone alone. All tests were repeated at least 3 times. Sample sizes ranged from 120 to 200.

The cockroaches were held in petri dishes (8.5 by 1.5 cm) and held in an incubator at 26°C. Cockroaches that were on their backs or could not right themselves after 48 h were considered dead. Data were analyzed by probit analysis (Raymond 1985). Mortality was adjusted for control mortality with Abbott's (1925) formula. Cockroaches pretreated with a compound (potential synergist or acetone) were used as controls in those tests. Significant differences were determined by failure of the 95% CI to overlap. LD_{50} values were converted to μg/g with an average weight of 48.8 mg (SEM = 0.027, n = 40) per cockroach.

Results and Discussion

DEET toxicity was increased by lambda-cyhalothrin and permethrin, as well as by the carbamate pyridostigmine bromide (Table 1). The synergism by pyridostigmine may be similar to that seen in rats (McCain 1995), although the degree of synergism by pyridostigmine has not been reported. On a weight basis, the LD_{50} for DEET (McCain 1995) was not significantly different than that reported here for cockroaches, so similar toxic mechanisms are plausible. An estimate for DEET synergism by eserine (physostigmine) is based on the lowest dose used which killed 7 of 10 cockroaches because the project was terminated before data collection was completed. I do not know whether a large degree of synergism of DEET by eserine would also be found in rats; this possibility should be investigated because eserine has been reported as a potential prophylactic against organophosphate nerve gas poisoning (Miller et al. 1993) and for the treatment of Alzheimer's disease (Sano et al. 1993).

Phenylmethylsulfonylfluoride (PMSF) synergized the toxicity of Amitraz and DEET and S,S,S, tri-n-butylphosphorotrithioate (DEF) synergized chlordimeform and DEET toxicity (Table 1). PMSF is known to reversibly inhibit neurotoxic esterase (Lotti et al. 1983). DEF, in addition to causing cholinesterase inhibition in hens (Abou-Donia et al. 1986) and catfish (Habig and Giulio 1988),

Table 2. Effect of sublethal doses of DEET on toxicity to German cockroaches by some acetylcholinesterase inhibitors

Toxicant	DEET[a]	Synergism ratio[a,b]	LD_{50}, μg/g[c]	95% FL	*P*	*n*	Slope (±SE)
Bendiocarb	−	1.00	15.1	10.9–19.3	0.73	181	2.57 (0.80)
Bendiocarb	+	0.90	16.7	13.6–18.9	0.30	217	6.09 (1.67)
Chlorpyrifos	−	1.00	3.39	2.78–3.87	0.11	269	4.43 (0.72)
Chlorpyrifos	+	1.01	3.34	2.88–3.72	0.95	180	5.75 (0.99)
Malathion	−	1.00	62.9	41.5–84.1	0.11	480	1.81 (0.37)
Malathion	+	2.36	26.7	17.7–34.7	0.25	180	2.46 (0.42)
Carbaryl	−	1.00	21.1	16.7–26.4	0.46	319	2.34 (0.53)
Carbaryl	+	2.42	8.72	2.28–12.6	0.13	180	2.79 (0.85)
Pyridostigmine	−	1.00	7,003.00	6,246–7,826	0.95	310	3.62 (0.66)
Pyridostigmine	+	3.75	1,868.00	1,494–2,518	0.51	200	2.42 (0.51)

[a] DEET dose used = 1,020 μg/g.
[b] = (LD_{50} toxicant/LD_{50} toxicant + synergist).
[c] LD_{50} s 48 h after treatment.

inhibits plasma butyrylcholinesterase (Abou-Donia et al. 1986) and hen (Lapadula et al. 1984) and mouse (Hur et al. 1992) liver microsomal carboxylesterase, esterases that hydrolyze pesticides in German cockroaches (Dong and Scott 1992) and other insects (Metcalf 1967), neurotoxic esterase in hens (Lapadula et al. 1984) and probably other enzymes. The spectrum of DEF activity is wide; it also causes defoliation of cotton (Lotti et al 1983) by unknown mechanisms.

DEET synergized the toxicity of malathion, carbaryl, and pyridostigmine but not bendiocarb or chlorpyrifos (Table 2). Prevention of hydrolysis by esterase inhibition seems unlikely because DEET is not known to inhibit esterases, and its structure is not consistent with esterase inhibition. Esterase inhibition should increase the toxicity of chlorpyrifos, as occurred when the esterase inhibitor DEF was used with both susceptible and a chlorpyrifos-resistant strain of German cockroaches (Table 1). However, DEET did not synergize chlorpyrifos toxicity (Table 2). DEF caused a drastic increase in malathion toxicity (Table 1) whereas DEET synergism of malathion was relatively weak (Table 2).

Inhibition of oxidases is another common mechanism of insecticide synergism (Matusmura 1985) and is a potential action of DEET. Piperonyl butoxide (PBO), a mixed-function oxygenase inhibitor, has been shown to antagonize chlordimeform toxicity in German cockroaches (Hollingworth and Lund 1982), yet in my experiments DEET and the formamidines interacted in a synergistic manner (Table 1). If acting as an oxidase inhibitor, DEET would decrease the toxicity of chlorpyrifos as did PBO (Table 1), because the activity of this class of organophosphates is considered largely dependent on the replacement of the sulfur by an oxygen by the action of mixed function oxygenases (Metcalf 1967). No protection by DEET occurred in the tests with chlorpyrifos (Table 2).

DEET, which is an oily liquid, has the potential to alter the penetration of other toxicants. Sun and Johnson (1972) found that toxicity of some carbamates to *Musca domestica* L. was increased by solvents; they attributed this to *quasisynergism* in which the solvent increased the penetration rate of the insecticide. In these experiments, the DEET dose was small (0.05 μl DEET in 0.95 μl acetone) when DEET was used as the synergist. Despite this, these experiments do not eliminate the possibility that synergism by DEET was the result or partial result of increased penetration of the companion compound caused by DEET.

A tendency to attribute synergism of insecticides to the classical synergistic mechanisms of blocking degradation or changing of toxicokinetics may, in some instances, divert attention from the possibility of effects by compounds which converge on a particular biochemical target or targets. The term *cosynergism* might be more appropriate in these cases, and such effects yield information about the actions of the cosynergistic compounds. One example is the formulation of a formamidine, which causes cyclic adenosine monophosphate (cAMP) elevation by octopamine receptors, and caffeine, which prevents cAMP hydrolysis by phosphodiesterase inhibition (Nathanson 1990).

If DEET were acting on the same targets or related biochemical pathways as formamidines, CDM or Amitraz should affect DEET toxicity. DEET toxicity was increased 3.7 times by CDM and 7.6 times by Amitraz (Table 1), indicating synergistic effects and suggesting that DEET interacts in some way with a biochemical system that is affected by the formamidines used in these experiments.

Apparently synergism of the acetylcholinesterase inhibitors tested here by DEET is not by cholinergic mechanisms, because synergism occurred between DEET and some acetylcholinesterase inhibitors but not others (Table 2). A parallel effect can be found with formamidines because they are synergized by some organophosphates, as mentioned previously, yet CDM toxicity has been antagonized by parathion (Fisher 1992). Takahashi et al. (1994) found that the toxicity of some carbamates to rabbits was by nonacetylcholinesterase actions and suggested that the mode of lethal action for ace-

tylcholinesterase inhibitors could be from a balance between anticholinesterase activity and some mechanism other than cholinesterase inhibition.

Chlordimeform potentiated carbaryl toxicity in German cockroaches (Fisher 1992), and DEET synergized carbaryl toxicity in my experiments (Table 2). With respect to carbamates, this reinforces some similarities between DEET and the formamidines. Some similarities between DEET and the formamidines also were observed with respect to some pyrethroids. For example, DEET was synergized by lambda-cyhalothrin and permethrin (Table 1). Chlordimeform and Amitraz synergize the toxicity of permethrin (Mosupi and Terry 1991) and Amitraz has an apparent ability to synergize lambda-cyhalothrin toxicity in some Lepidoptera (DuRant 1993). Toxic interactions between pyrethroids and DEET have not previously been documented for insects, however, such an interaction is suggested for vertebrates as indicated by unanticipated illness to dogs and cats caused by a commercial product which contained DEET and a pyrethroid (fenvalerate) (Mount et al. 1991)

If DEET does have actions similar to formamidines, which have adrenergic effects in vertebrates (Wu et al. 1990, Costa et al. 1991), the effect of DEET on vertebrate adrenergic systems and interaction with adrenergic pharmaceuticals in vertebrates merits investigation.

In summary, this research shows similarities in the effects on German cockroaches by synergists on the toxicity of the insect repellent DEET and two formamidine insecticides. A examination of the effects of DEET on insect octopaminergic sensitive systems would clarify the differences and similarities of DEET and formamidine actions. The differences in synergism of cholinesterase inhibitors by DEET suggests that noncholinergic mechanisms may be partly responsible for the cosynergism of DEET and some organophosphates and carbamates. The potent formamidine synergism by eserine suggests this compound may have a target that could be exploited to expand the insecticidal utility of formamidines.

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