

EVALUATION OF PROADIFEN HCL AS A SYNERGIST FOR TEMPORARY IMMOBILIZATION AND TOXICITY OF EIGHT CHEMICALS IN COTURNIX QUAIL

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Abstract—Proadifen HCl (SKF-525A), a hepatic microsomal enzyme inhibitor, was evaluated as a synergist for eight chemicals that produced temporary immobilization (TI) at sublethal levels in birds. Probit analysis showed that Proadifen HCl administered orally and concurrently at 100 mg/kg to quail (*Coturnix coturnix japonica*) with four of the test chemicals (*alpha*-chloralose, chlordiazepoxide, nicotine sulfate and phencyclidine) significantly lowered ($p = 0.05$) the estimated median lethal (LD50) and/or temporary immobilization response (TI50). Proadifen HCl reduced the LD50 dose for *alpha*-chloralose 1.56-fold and the TI50 2.03-fold; the TI50 dose for chlordiazepoxide HCl 2.47-fold; the LD50 dose for nicotine sulfate 4.10-fold and the TI50 2.98-fold; and the LD50 dose for phencyclidine HCl 2.35-fold and the TI50 27.5-fold. The synergism exhibited by Proadifen HCl with metomidate and tribromoethanol was less or negative. The LD50 of metomidate was reduced 1.96-fold, but the TI50 was not affected. The LD50 of tribromoethanol was increased 1.15-fold, but the TI50 was reduced by a factor of 1.48. There was no synergism between Proadifen HCl and methiocarb or pentobarbital. These data show that the LD50 and/or TI50 of temporary immobilizing agents for birds can be affected by the use of a synergist such as Proadifen HCl, and indicate that synergism between bird control chemicals or pesticides and drugs could be used to improve efficacy, lower application rates and reduce environmental contamination.

Keywords—Synergist Immobilization Toxicity Proadifen HCl Quail

INTRODUCTION

Since the early 1950s, personnel at the Denver Wildlife Research Center (DWRC) have been charged with the responsibility of developing methods for mitigating damage caused by wild birds. One important area of research has been the development of chemicals for bird damage control. The DWRC has tested more than 2500 chemicals as potential bird repellents, stupeficients, toxicants or reproductive inhibitors and has developed several chemicals for use when damage is severe and control is practical. The purpose of this study was to determine if the toxicological activity of eight chemicals known to produce temporary immobilization (TI) at sublethal levels in birds could be enhanced by combining them with a synergist. By definition, synergists are chemicals that when combined with another chemical produce a toxicological response greater than the sum of the individual responses to each chemical administered alone. If synergism of existing bird control chem-

icals could be demonstrated, it could provide a new means for reducing the amount of potentially hazardous chemicals introduced into the environment. Also, demonstrated synergism of the toxicological responses to these chemicals would suggest that additional research with other vertebrate pesticides might be warranted.

The literature was searched for data pertaining to chemical synergism with toxicological effects on birds, but none were found. However, the literature described a number of *in vitro* and *in vivo* studies with mammals [1-5]. These studies evaluated the effects of synergists on the stimulation or inhibition of hepatic microsomal enzymes and the resultant changes in the absorption, distribution, metabolism and excretion of drugs and other chemicals. They supported the concept that the duration and intensity of toxicological effects are largely determined by the mode of action of the chemical and its rate of metabolism or degradation in the body. For example, either increased or decreased toxicological responses can occur, dependent upon whether the microsomal enzymes are inhibited or stimulated and whether the metab-

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olites are more or less active than the parent chemical. Proadifen HCl is a potent and relatively nontoxic hepatic microsomal enzyme inhibitor for a wide variety of drugs [6]. We decided to investigate the potential of Proadifen HCl as a synergist for chemicals producing temporary immobilization in birds because of its activity in mammals. It was also determined that the use of Proadifen HCl and the immobilizing chemicals would allow us to evaluate the relationship between potential synergistic effects on two endpoints, one sublethal and one lethal.

MATERIALS AND METHODS

To determine the ability of Proadifen HCl [SKF-525A, Chemical Name: 2-(diethylamino)-*alpha*-phenyl-*alpha*-propyl benzeneacetic acid, ethyl ester, HCl, CAS: 62-68-0, Source: Smith, Kline and French, Purity: 98%] to synergize temporary immobilization or lethality in birds, eight chemicals reported by Schafer and Cunningham to temporarily immobilize birds [7] were selected for testing. These were: *alpha*-chloralose [1,2-*O*-(2,2,2-trichloroethylidene)-*alpha*-*D*-glucofuranose, 15879-93-3, Aldrich, 90%], chlordiazepoxide HCl (7-chloro-*N*-methyl-5-phenyl-3*H*-1,4-benzodiazepin-2-amine-4-oxide HCl, 438-41-5, Hoffmann-La Roche, 99%), methiocarb [3,5-dimethyl-4-(methylthio)phenol methylcarbamate, 2032-65-7, Mobay, 96%], metomidate [1-(1-phenyl-ethyl)-1*H*-imidazole-5-carboxylic acid, methyl ester, 5377-20-8, McNeil, 98%], nicotine sulfate [3-(1-methyl-2-pyrrolidinyl)pyridine sulfate (2:1), 65-30-5, Sigma, 40%], phencyclidine HCl [1-(1-phenylcyclohexyl)piperidine HCl, 956-90-1, Parke-Davis, 98%], phenobarbital [5-ethyl-5-phenyl-2,4,6-(1*H*,3*H*,5*H*)-pyrimidinetrione, 50-06-6, Abbott, 99%], and tribromoethanol (2,2,2-tribromoethanol, 75-80-9, Aldrich, 97%).

Coturnix quail (*Coturnix coturnix japonica*) were used as the test species. Birds weighing 110 to 220 g were chosen at random from mixed sex and age groups of 100 to 200 individuals bred and raised at the DWRC. These quail were produced by crossing two inbred lines (University of California-Davis and University of Georgia) that have been maintained at the DWRC since 1972.

Range-finding tests were conducted for each chemical with three to nine treatment groups (four birds per group). Fasted birds were administered one of the eight chemicals by gavage between 1030 and 1130 h. The chemicals were dissolved in propylene glycol and administered at the rate of 2 ml/kg body weight (0.2%) using one-fourth or

one eighth logarithmic dosage intervals. After treatment, each bird was placed in individual 24 × 18 × 18 cm³ (L × W × H) wire mesh cages and closely observed for 4 h. Time to temporary immobilization (bird was unable to fly or walk to avoid capture) and recovery or mortality were recorded. These data were used to estimate TI50 (median temporary immobilization dose) and LD50 values and their 95% confidence limits using the method of Thompson [8].

Birds in a second set of range-finding tests were dosed as described above except that Proadifen HCl was concurrently administered at a constant dosage of 100 mg/kg with the test chemical at each dosage level. Chemicals were selected for further testing if a synergistic response to Proadifen HCl was indicated. This was determined by applying the following formula:

$$\frac{\text{LD50 or TI50 (chemical)}}{\text{LD50 or TI50 (chemical + Proadifen HCl)}} \geq 1.50$$

The selection of the value 1.50 for indication of synergism excluded those chemicals with weak or nonexistent synergistic effects.

alpha-Chloralose, chlordiazepoxide HCl, nicotine sulfate and phencyclidine HCl were tested for synergism using 12-bird treatment groups to enable statistical definition of the effects caused by Proadifen HCl. These tests were also conducted as described above, except that with nicotine sulfate the Proadifen HCl was dosed at two additional levels, 31.6 and 10.0 mg/kg. LD50s and TI50s were determined by probit analysis [9].

Because we were unable to assume that the relationship between equivalent doses of the chemical and the synergist would remain constant over the entire range of responses (0–100%), estimation of the synergistic activity of Proadifen HCl could require complicated comparisons of LD50 or TI50 values. Thus, we tested for parallel slopes of the probit regression lines (probit value of the response versus log dose) of the chemical with and without Proadifen HCl. If the hypothesis of parallelism was not rejected, then a constant relationship could be assumed and a term designated relative potency (RP) was used to define the summary statistic which described the magnitude of the synergistic response. RP for an LD50 or TI50 response of a given chemical was defined to be the ratio of equally effective log doses of the chemical plus Proadifen HCl versus the chemical alone.

For example, an RP value of 0.25 indicated that if a specific amount of chemical produced a given level of response, then one-quarter of that amount would be needed to produce the same response when 100 mg/kg of Proadifen HCl was added to the chemical being tested. An RP value of 1.00 indicated that Proadifen HCl had no synergistic effect. If the parallelism hypothesis was rejected, the RP value was not constant and depended on the level of response; therefore a single summary statistic could not suffice. In such a case, interpretation of the results could be difficult, and a specific discussion of each experiment would be necessary. Two computer programs developed by Daum [10] were used to perform the probit and RP analyses.

RESULTS

The results of data gathered during the range-finding tests for each chemical are presented in Table 1. The LD50 and TI50 values for four of the chemicals: *alpha*-chloralose, chlordiazepoxide HCl, nicotine sulfate and phencyclidine HCl were enhanced by more than 1.50 when the chemical was combined with 100 mg/kg Proadifen HCl. These four chemicals were selected for further testing. The activities of methiocarb, metomidate, phenobarbital and tribromoethanol were not sufficiently increased or were negatively affected by the concurrent use of Proadifen HCl. Quail dosed with 562 mg/kg Proadifen HCl did not exhibit any

toxic symptoms and thus the effect at 100 mg/kg or less was considered zero.

Probit regression lines were fitted to each data set generated by the final 12-bird tests. In all instances, the fit was sufficient (p greater than 0.05) to permit determination of the LD50 and TI50 and their 95% confidence intervals (Table 2). With the exception of the TI50 for nicotine sulfate, confidence intervals were reasonable, indicating that the number of birds per dose level and the number of dose levels were statistically adequate. The synergistic effect of Proadifen HCl on the LD50s of three chemicals was estimated using the RP statistic, because in each case the parallelism hypothesis for the LD50 response could not be rejected (Figs. 1-3). When *alpha*-chloralose and phencyclidine HCl were evaluated with 100 mg/kg of Proadifen HCl, the amount of chemical necessary to produce a given level of response was reduced by roughly one-half for both chemicals (Table 3).

Three levels of Proadifen HCl were tested with nicotine sulfate, and the results indicated that the rapid decline in the RP value (an increase in the synergistic effect) should have leveled off at a dose of about 50.0 mg/kg Proadifen HCl. However, 31.6 mg/kg Proadifen HCl reduced the amount of nicotine sulfate necessary to produce a given response by 70% (Table 3). The LD50s of all three chemicals (*alpha*-chloralose, phencyclidine HCl and nicotine sulfate) were significantly synergized by Proadifen HCl since none of the confidence

Table 1. LD50 and TI50 values for quail given eight chemicals with and without Proadifen HCl, using Moving Point Interpolation ($n = 4$)

Immobilizing chemical	Proadifen HCl (mg/kg)	LD50 (mg/kg)	95% Confidence interval	TI50 (mg/kg)	95% Confidence interval
<i>alpha</i> -Chloralose	0	205	154-274	20.5	13.2-31.9
	100	105	70.2-158	13.3	8.90-20.0
Chlordiazepoxide HCl	0	>1000	a	100	49.4-202
	100	>1000	a	16.2	9.70-27.0
Methiocarb	0	17.8	12.8-24.8	11.6	a
	100	13.3	11.3-15.8	8.70	a
Metomidate	0	56.2	40.3-78.4	21.6	16.7-27.8
	100	28.7	a	21.5	15.4-30.0
Nicotine sulfate	0	562	403-784	75.0	63.4-88.4
	100	21.0	16.0-28.0	18.0	a
Phencyclidine HCl	0	56.2	40.3-78.4	6.50	4.90-8.90
	100	56.0	35.0-89.6	0.164	0.127-0.211
Phenobarbital	0	>1000	a	48.7	36.5-64.9
	100	>1000	a	42.1	24.5-72.5
Tribromoethanol	0	366	274-487	133	113-158
	100	422	a	86.5	a

^aConfidence intervals could not be calculated.

Table 2. LD50 and TI50 values for quail given eight chemicals with and without Proadifen HCl, using Probit Analysis ($n = 12$)

Immobilizing chemical	Proadifen HCl (mg/kg)	LD50 (mg/kg)	95% Confidence interval	TI50 (mg/kg)	95% Confidence interval
<i>alpha</i> -Chloralose	0	145	128-166	23.3	19.5-25.7
	100	92.4	80.3-106	11.5	9.50-13.8
Chlordiazepoxide HCl	0	>1000	a	40.0	24.5-66.3
	100	>1000	a	16.2	12.8-20.5
Nicotine sulfate	0	313	264-372	210	83.0-309
	10.0	189	169-215	125	89.3-151
	31.6	96.4	81.2-133	80.0	68.3-97.2
	100	76.3	65.5-92.7	70.5	61.0-82.7
Phencyclidine HCl	0	51.3	40.3-66.2	5.50	3.50-7.30
	100	21.8	15.9-29.1	0.201	0.187-0.221

^aConfidence interval could not be calculated.

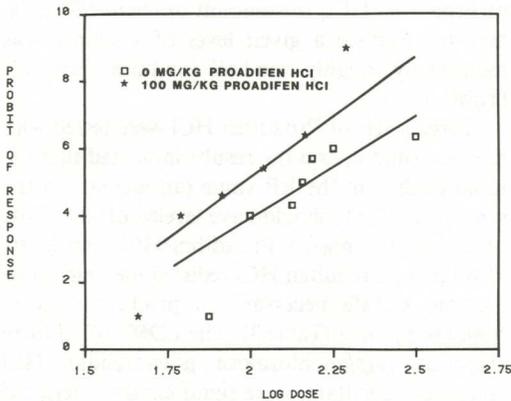


Fig. 1. Probit regression lines for the LD50 response to *alpha*-chloralose.

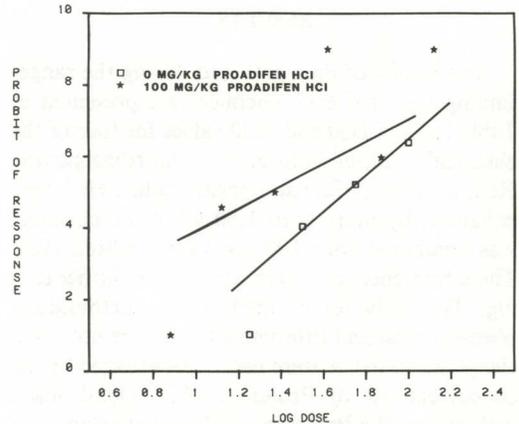


Fig. 2. Probit regression lines for the LD50 response to phencyclidine HCl.

intervals for RP contained the value 1.00 (no synergistic effect). The LD50 of chlordiazepoxide HCl was not synergized at the maximum dose tested, 1000 mg/kg.

Proadifen HCl also affected the TI50 responses of the four chemicals. When 100 mg/kg Proadifen HCl was administered with *alpha*-chloralose, the quantity of *alpha*-chloralose needed for a given level of response was reduced by approximately one-half (Table 4) and probit regression lines were parallel (Fig. 4). For the three levels of Proadifen HCl administered with nicotine sulfate, the regression lines were also parallel (Fig. 5) and significant levels of synergism were achieved. They ranged from a calculated RP of 0.612 at 10.0 mg/kg to 0.335 at 100 mg/kg (Table 4). As was the case with the LD50 for nicotine sulfate, results indicated that approximately 50.0 mg/kg of Proadifen HCl was

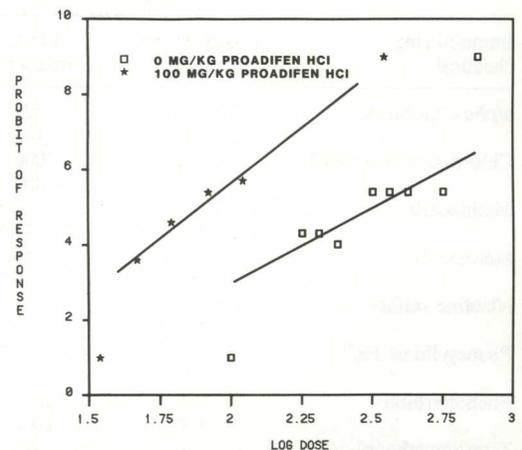


Fig. 3. Probit regression lines for the LD50 response to nicotine sulfate.

Table 3. Relative potency (RP) of the LD50 of chemicals that displayed synergistic responses with Proadifen HCl (n = 12)

Immobilizing chemical	Proadifen HCl (mg/kg)	RP ^a	95% Confidence interval
<i>alpha</i> -Chloralose	100	0.639	0.531-0.765
Nicotine sulfate	100	0.248	0.199-0.312
	31.6	0.313	0.248-0.402
	10.0	0.598	0.486-0.735
Phencyclidine HCl	100	0.424	0.284-0.619
Chlordiazipoxide HCl	100	^b	^b

$$^a \text{Relative Potency} = \frac{\log \text{LD50 (chemical + Proadifen HCl)}}{\log \text{LD50 (chemical)}}$$

^bCould not be calculated because only minimal lethal effects occurred at the maximum dose level (1000 mg/kg).

Table 4. Relative potency (RP) of the TI50 of chemicals that displayed synergistic responses with Proadifen HCl (n = 12)

Immobilizing chemical	Proadifen HCl (mg/kg)	RP ^a	95% Confidence interval
<i>alpha</i> -Chloralose	100	0.519	0.412-0.654
Nicotine sulfate	100	0.335	0.243-0.484
	31.6	0.381	0.272-0.558
	10.0	0.612	0.448-0.835
Chlordiazipoxide HCl	100	^b	^b
Phencyclidine HCl	100	^b	^b

$$^a \text{Relative Potency} = \frac{\log \text{LD50 (chemical + Proadifen HCl)}}{\log \text{LD50 (chemical)}}$$

^bRP could not be calculated because of nonparallelism of probit regression lines.

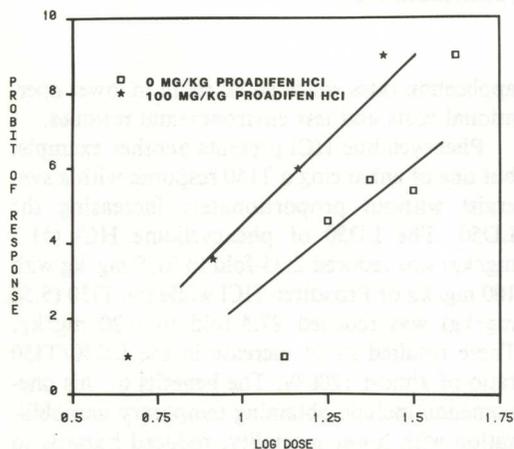


Fig. 4. Probit regression lines for the TI50 response to *alpha*-chloralose.

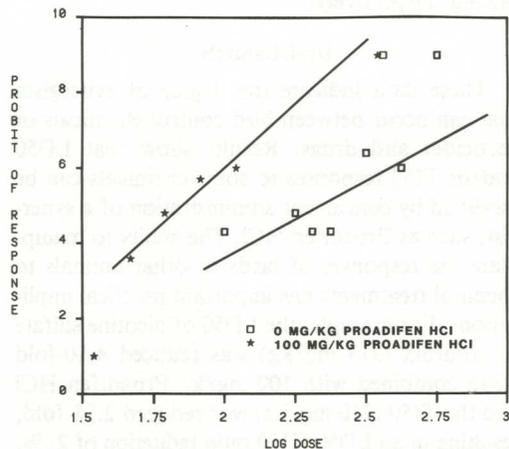


Fig. 5. Probit regression lines for the TI50 response to nicotine sulfate.

an optimum synergistic level with respect to the temporary immobilization response. Overlapping confidence intervals indicated that a negligible increase in synergism was achieved when Proadifen HCl doses were increased from 31.6 to 100 mg/kg.

RP values for chlordiazepoxide HCl and phenacyclidine HCl could not be estimated due to the rejection of the parallelism hypothesis. Figure 6 illustrates that administration of 100 mg/kg of Proadifen HCl did not influence the TI50 response with chlordiazepoxide HCl at low dose levels, but as the level of response increased so did the synergistic effect. For example, the TI20 values of chlordiazepoxide HCl with and without Proadifen HCl were 11.1 and 10.5 mg/kg, respectively; while the values for the TI50 were 16.2 and 40.0 mg/kg; and at the TI80 the synergistic response continued to increase, with values of 23.7 and 152 mg/kg. Because the TI50 level is the lowest level at which confidence intervals for the two estimates do not overlap, any comments concerning the synergistic effect of Proadifen HCl on chlordiazepoxide HCl must be conditioned to the level of response. The same kind of conditional statements are necessary for phenacyclidine HCl; however, unlike the results for chlordiazepoxide HCl that indicated no synergism at low levels of response, extreme differences in TI50 values between phenacyclidine HCl with and without Proadifen HCl are indicated at all levels of response (Fig. 7). Consider that the calculated TI10 and TI90 values for phenacyclidine HCl with 100 mg/kg of Proadifen HCl are 0.17 and 0.24 mg/kg respectively, while the same values for phenacyclidine HCl alone are 28.4 and 92.5 mg/kg, respectively.

DISCUSSION

These data indicate the degree of synergism that can occur between bird control chemicals or pesticides and drugs. Results show that LD50 and/or TI50 responses to some chemicals can be modified by concurrent administration of a synergist, such as Proadifen HCl. The ability to manipulate the responses of birds or other animals to chemical treatments has important practical implications. For example, the LD50 of nicotine sulfate to coturnix (313 mg/kg) was reduced 4.10-fold when combined with 100 mg/kg Proadifen HCl and the TI50 (210 mg/kg) was reduced 2.98-fold, resulting in an LD50/TI50 ratio reduction of 27%. Reducing both the LD50 and TI50 may be of value in achieving efficacy with lower chemical

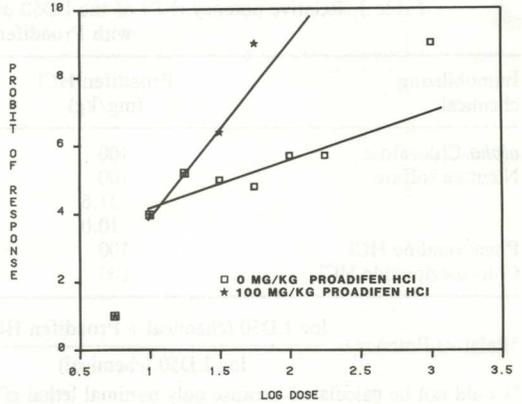


Fig. 6. Probit regression lines for the TI50 response to chlordiazepoxide HCl.

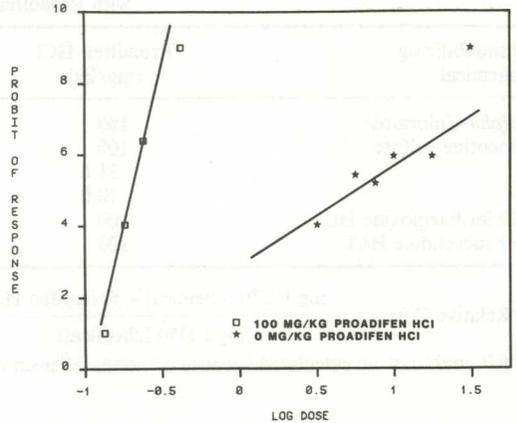


Fig. 7. Probit regression lines for the TI50 response to phenacyclidine HCl.

application rates which could result in lower operational costs and less environmental residues.

Phenacyclidine HCl presents another example, but one of enhancing a TI50 response with a synergist without proportionately increasing the LD50. The LD50 of phenacyclidine HCl (51.3 mg/kg) was reduced 2.35-fold to 21.8 mg/kg with 100 mg/kg of Proadifen HCl while the TI50 (5.50 mg/kg) was reduced 27.5-fold to 0.20 mg/kg. These resulted in an increase in the LD50/TI50 ratio of almost 1200%. The benefits of this phenomenon include obtaining temporary immobilization with lower mortality, reduced hazards to nontarget species, and a more favorable cost ratio. It implies that other chemicals may have many

desirable characteristics but were formerly considered marginal or were eliminated from further development because of low levels of effectiveness, intolerable safety or cost problems. The results of these experiments also indicate that it is prudent for wildlife managers to consider synergistic effects of chemicals before using them as management tools.

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