1092

Imported Fire Ant Toxic Bait Studies: Evaluation of Toxicants[1]

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ABSTRACT

Test methods were developed by which it is possible to evaluate quickly large numbers of chemicals for their potential as delayed-action ant bait toxicants. From these tests it has been determined that an effective toxicant must exhibit delayed action over a wide dosage range (preferably 100-fold or greater, be readily transferred from one ant to another and not be repellent to the ants when incorporated in a bait. Laboratory and field test data with 4 toxicants are presented. Mirex was the only toxicant which fulfilled these requirements. Kepone® (decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalen-2-one and HRS-1243 (1,1a,3,3a,4,5,5,5a,5b,6-decachloro-2-(2,3-dehydroxypropoxy) octahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*] pentalen-2-ol) were the next most effective toxicants.

While toxic baits have been employed for many years for the control of ants, relatively little effort has been spent on developing new toxicants. The standard toxicants employed have been either thallium sulfate or sodium arsenate. Recently, Hays and Arant (1960) reported on tests with 29 toxicants which were conducted in the field with individual colonies of the imported fire ant, *Solenopsis saevissima richteri* Forel. The toxicants were offered to the ants in tankage-cooking oil bait. Observations were made on the acceptability of the toxic baits over a 12-hr period and on their toxicity as evidenced by the presence of ants in the mounds after 2 weeks. Kepone® was the most effective material tested. Bartlett and Lofgren (1961) in concurrent field studies also found Kepone to be a good imported fire ant bait toxicant.

The need of a laboratory method for rapidly screening a large number of bait toxicants against imported fire ants became very evident in our early work with baits. Many test methods for evaluating bait toxicants on other insects have been reported; however, these methods are primarily designed to select insecticides which give a quick kill. The objective of a test for selecting toxicants for use in baits against a colonial insect, such as the imported fire ant, must be to detect insecticides which are characterized by a delayed killing action. The reason is that the foraging ants must not become alarmed by the presence of the toxicant or be killed before the bait is brought back to the colony. Observations in the field have shown that once the ants become aware that they have taken a poisoned bait, they will usually cease feeding on it and move the colony.

The purpose of this paper is to describe a series of laboratory and field test procedures for evaluating bait toxicants and to illustrate their use by results of tests with the following 4 compounds:

Bayer 30911	*O*-2,4-dichlorophenyl *O*-methyl methylphosphonothioate
HRS-1243	1,1a,3,3a,4,5,5,5a,5b,6-decachloro-2-(2,3-dehydroxypropoxy) octahydro-1,3,4-metheno-2*H*-cyclobuta[cd]pentalen-2-ol
Kepone	decachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[cd]pentalen-2-one
Mirex	

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Laboratory Tests.—*Procedures*.—The following general procedures are employed in the laboratory studies. The test chambers are small plastic flower pots 42 mm in diameter at the base and 63 mm at the top with an upright rim 16 mm high. The rim forms a narrow ridge at its base inside the pot. There are 3 small holes in the bottom. A layer about ¼-in. thick of plaster of paris mixed with cement (9:1 ratio) is poured over the bottom of the pot and allowed to harden. When the pots are placed on wet peat moss, the plaster of paris acts as a wick to draw up water and maintain a high humidity, which is essential for survival of the ants. The cement is added to make a harder mixture which the ants cannot tunnel through for escape. The ants are confined in the containers by small plate-glass discs with a diameter slightly less than that of the rim, so that they rest on the ridge between the rim and the tapered portion of the chamber. The insides of the pots are dusted lightly with talc. The talc must be used sparingly because ants are easily killed by it, especially in the absence of moisture or if they are in a weakened condition.

In preparing the toxic bait, the candidate insecticides are dissolved directly in the food material; i.e., either peanut oil or 10% sucrose solution, depending on the solubility of the chemical. The bait is offered to the ants on cotton plugs in small vial lids. The ants used in the tests are collected in the field with a portion of their mound and placed in large galvanized tubs. They are maintained in the laboratory 3–7 days without food prior to the test. Worker ants are collected with the aid of CO_2 and an aspirator and placed in the test containers the afternoon of the day preceding the test. This time interval permits the ants to recover from the effects of the CO_2 and to orientate themselves to the container before the start of the test. Ants that die during this 24-hr period are replaced with others similarly conditioned.

As mentioned previously, the objective of the tests is to determine if a toxicant will give delayed toxicity. In these tests, delayed toxicity is defined as less than 15% mortality after 24 hr exposure and more than 89% mortality at the end of the test period.

During these studies, 3 different test methods have been used. They are the:

(1) Continuous feeding test.—In this procedure ad libitum feeding by the ants is permitted throughout the test period. Two replications with 20 worker ants each are made at each dosage level. Observations for dead ants are usually made over a 14-day period. It is recognized that this test does not differentiate between delayed toxicity, temporary repellency, fumi-

gant action, or accumulative toxicity during the prolonged feeding period.

(2) Limited feeding test.—In this test the previously described procedure was modified so that the ants are allowed access to the bait for the initial 24 hr of the test period. Then the toxic bait is removed and the ants are starved for 24 hr. The ants are then provided with uncontaminated peanut oil as food for the remainder of the test period. The limited exposure to the toxicant insures that any toxicity exhibited is due to delayed action rather than accumulative action.

(3) Bait transfer test.—Imported fire ants are characterized by a communal stomach and readily pass food from one individual to another. A food transfer test was designed to determine how effictively toxic baits are passed between worker ants. Worker ants are separated into 2 groups according to size. Major or minor worker ants are fed bait for 24 hr and then transferred to a clean test chamber containing unfed ants of the opposite size. This sizing procedure permits a determination of which ants have been prefed. The ants can either be left together throughout the test period or the prefed ants can be removed after a predetermined period.

Two sets of experiments were conducted. In the first experiment the delayed toxicity range for the 4 toxicants was determined by the continuous and limited feeding tests as well as the effective toxicity range in the bait transfer test. Two tests with 2 replications each were conducted. Twenty worker ants were used in each replication in the continuous and limited feeding tests. In the bait transfer test, 10 major workers transferred bait to 10 minor workers in each replication. The major workers were kept with the minor workers throughout the test but mortality counts were made only on the minor workers. Observations for mortality were made over a 20-day period. The concentrations of each toxicant in the peanut oil are recorded in Table 1.

The second experiment was conducted with the bait transfer test to (1) compare the toxicity of the 4 toxicants when 20 ants were prefed and allowed to feed 10 unfed ants and (2) determine the difference

Table 1.—Toxicity range of 4 bait toxicants in peanut oil to imported fire ant workers as determined by 3 different test methods.[a]

		Lethal time (days) required to kill 15% and 90% of ants with each of the following test methods:					
		Continuous feeding		Limited feeding		Transfer feeding	
Toxicant	Conc. (%)	LT_{15}	LT_{90}	LT_{15}	LT_{90}	LT_{15}	LT_{90}
Mirex	0.001	15	>20	11	>20		
	.0025	9	17	9	20		
	.005	6	13	7	15		
	.01	6	11	5	11	6	>20
	.025	4	7	3	9	3	>20
	.05	3	7	3	6	6	>20
	.1	3	4	3	6	5	15
	.25	2	4	2	3	4	9
	.5	2	3	2	3	3	11
	1.0	2	3	2	2	4	6
HRS-1243	0.005	9	>20	13	>20		
	.01	11	20	11	20		
	.025	5	10	5	9		
	.05	8	7	3	7	6	>20
	.1	3	6	3	7	5	>20
	.25	2	6	2	4	4	>20
	.5	2	4	1	4	4	>20
	1.0	1	4	1	4	3	>20
Kepone	0.0025	13	>20	14	>20		
	.005	9	20	21	>20		
	.01	10	15	10	>20		
	.025	5	8	7	10		
	.05	5	8	5	10		
	.1	2	8	2	7	11	>20
	.25	2	4	2	8	4	>20
	.5	1	6	1	5	11	>20
	1.0	1	5	1	4	5	>20
Bayer 30911	0.0025	6	>20	>20	>20		
	.005	5	10	9	>20	13	>20
	.01	3	6	3	11	7	>20
	.025	1	4	2	3	4	>20
	.05	1	2	1	2	3	>20
	.1	1	2	1		3	11
Check	—	17	>20	>20	>20	15	>20

[a] The delayed toxicity range of each toxicant in the continuous and limited-feeding tests is indicated by a vertical line adjacent to the LT_{90} values. Effective delayed toxicity is defined as less than 15% mortality after 24 hours but more than 89% after 20 days. Results adjusted for check mortality by Abbot's formula.

in their toxicity to major workers and minor workers. The toxicant concentrations tested were the same as in the previous test. The prefed ants were separated from the unfed ants after 3 days. Observations for mortality were made over a 31-day period.

Results.—The data for the first experiment (Table 1) show that mirex gave delayed toxicity in the continuous feeding and limited feeding tests over a 400-fold dosage range (1.0% to 0.0025%); in the bait transfer test it gave kill over a 10-fold range (1.0% to 0.1%). Kepone and HRS-1243 were about equal in effectiveness and exhibited delayed action in the continuous and limited feeding tests over a 10- to 20-fold dosage range. Neither compound gave more than 90% kill in the bait transfer test. Bayer 30911 gave delayed action over a 1- to 2-fold range in the continuous and limited feeding tests and produced more than 90% kill at the 0.1% level in the transfer test.

The results of the second experiment are presented in Table 2. Data is presented only for sufficient concentrations to show where satisfactory transfer of bait occurred as indicated by the LT_{90}. In this test the observation period was 31 days compared with 20 days in the previous test; however, the results of the 2 tests can be compared if only the LT_{90} values of less than 21 days are considered. On this basis all the toxicants with the exception of Bayer 30911 gave kill of the minor workers over a greater range of dosages when the ratio of prefed major workers to unfed minor workers was 2 to 1 than when it was 1 to 1. In fact, the increased toxicity was greater than would be theoretically expected. Presumably, twice as much bait and toxicant was available to the minor workers and, therefore, the lowest dosage causing mortality might drop by one-half. Actually, it dropped by a factor of 4 to 10. Since field colonies were used in these tests, the increased toxicity must be due to differences such as the degree of hunger of the ants, body weight, or quantity of bait actually transferred.

In the test where minor workers transferred bait to major workers, only mirex was toxic enough to give kill over a range of dosages (0.1–1.0%).

Table 2.—Imported fire ant bait transfer tests with various toxicants in peanut oil.[a]

Toxicant	Conc. (%)	10 minor workers fed by 20 major workers. Days required to obtain following kill		10 major workers fed by 20 minor workers. Days required to obtain following kill	
		15%	90%	15%	90%
Mirex	0.005	26	>31	31	>31
	.01	3	21	>31	>31
	.025	9	17	>31	>31
	.05	6	11	10	31
	.1	6	11	9	28
	.25	4	10	7	28
	.5	4	6	4	14
	1.0	2	4	3	13
HRS-1243	0.01	11	>31	>31	>31
	.025	10	17	>31	>31
	.05	8	19	>31	>31
	.1	24	31	>31	>31
	.25	4	11	10	31
	.5	3	7	>31	>31
	1.0	2	7	>31	>31
Kepone	0.025	21	31	5	>31
	.05	10	31	19	>31
	.1	10	31	26	>31
	.25	3	12	6	>31
	.5	2	8	>31	>31
	1.0	2	6	>31	>31
Bayer 30911	0.005	17	>31	>31	>31
	.01	17	31	>31	>31
	.025	12	>31	>31	>31
	.05	19	31	11	>31
	.1	3	28	>31	>31
Check	—	19	31	17	31

[a] Results adjusted for check mortality by Abbott's formula.

REPELLENCY TESTS.—*Procedures.*—In early field studies it was found that most toxicants exhibited some degree of repellency when combined with a bait. As a result, all promising toxicants are evaluated for their repellency before field control tests are conducted. The procedure employed is essentially the same as that described by Lofgren et al. (1962) for evaluating food materials. The toxicants are dissolved in the oil in the bait at concentration levels which bracket the upper levels of the delayed toxicity range. A bait, soybean oil (40%)-white flour (60%), is used for the tests because the oil is readily available to the ants, making rapid distribution throughout the colony possible. One hundred grams of bait are placed around each mound. Worker ants are collected after 24-hr exposure to the bait and examined for the presence of dye. Repellency is determined by comparison with feeding activity on bait containing no toxicant and with a standard toxicant, usually mirex. Some of the apparent repellency exhibited by highly toxic compounds or at the higher dosage levels is undoubtedly a result of death of ants which ate the bait. However, general observations of colonies used in this study have shown that this is not the case with the toxicants reported in this investigation.

Results.—All the toxicants imparted some degree of repellency to the imported fire ant at the highest concentrations tested. Mirex, however, was the least repellent with 64% of the ants taking the bait at 3 concentrations (0.05, 0.1, and 0.25%). The data are presented graphically in Fig. 1.

CONTROL TESTS.—*Procedures.*—Two sets of control experiments were conducted in the field against natural infestations of imported fire ant to evaluate the 4 toxicants. The plot design and the formulation and application procedures were the same as described by Lofgren et al. (1963).

The soybean oil-flour baits were applied at the rate of 6 lb/acre with a specially designed applicator which pumped the bait out each end of a 10-ft boom and dropped it directly to the ground in strips 10 ft apart. (C. E. Stringer, C. S. Lofgren, and F. J. Bartlett, unpublished data). The granular baits were applied at the rate of 10 lb/acre with a jeep-mounted Buffalo turbine blower at a swath spacing of 30 ft.

In Experiment I, the toxicants were evaluated in 2 tests in a bait consisting of soybean oil (47%), monoglycerides (3%), and white flour (50%). The monoglycerides were included in the formulation as a stabilizer. The concentrations of each insecticide evaluated are recorded in Table 3. In all formulations the concentration of toxicant was based on total

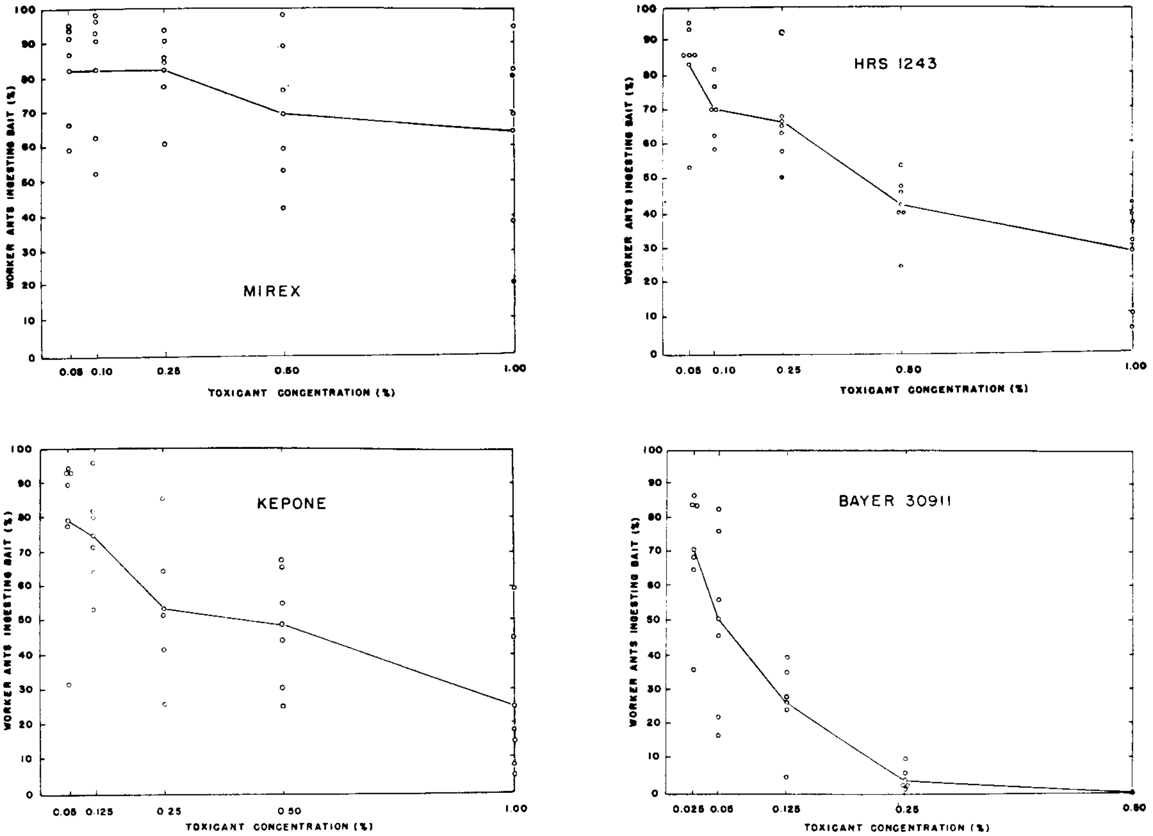

FIG. 1.—The acceptability of flour-soybean oil baits containing different concentrations of 4 toxicants.

formulation weight. Kepone was included in both tests as the standard for comparison.

Subsequent to the previous experiment, a bait superior to the soybean oil-white flour bait was developed (Lofgren et al. 1963). It consisted of soybean oil impregnated on corncob grits. This bait was readily adaptable to the various formulation and application procedure used in the Imported Fire Ant Eradication Program with granular insecticides. Experiment II was conducted to re-evaluate each of the toxicants with the soybean oil-corncob grits bait. Lofgren et al. (1963) indicated that it was necessary to employ the highest possible concentration of the toxicant for effectiveness with the corncob grits bait. This was due to the lesser amount of oil available to the ants. As a result, the concentration of the toxicants tested was increased 2- to 10-fold over that in the previous test. A series of 3 tests was conducted in this experiment. The concentrations of each toxicant tested are presented in Table 4.

Results.—The data for Experiment I are recorded in Table 3. They show that the most effective toxicant was mirex. It gave 100% kill of the ants at both dosages tested (0.05% and 0.1%). HRS-1243 gave complete control at the highest concentration (0.25%) and 99% at the lower level (0.1%). Bayer 30911 killed all the ants at a dosage of 0.05% but produced a reduction of only 89% at the 0.02% concentration. The decline in control after 16 weeks in Test 1 and after 8 weeks in Test 2 was from reinfestation from mating flights either just prior to or just after the bait was applied. This count was made in September, when new colonies begin to build mounds with the onset of cooler weather.

The results of Experiment II are given in Table 4. In Test 1, mirex was the only toxicant which gave any appreciable amount of control (96% after 16 weeks). HRS-1243 gave a maximum control of 60% after 16 weeks. The test was initiated in August of 1961. The treatments were interrupted several times by light showers. Since this bait will lose some of the oil and toxicant when it comes in contact with water, the rain may have accounted for the poor control obtained with all of the toxicants except mirex. The reason for control with mirex can be attributed to its greater toxicity as demonstrated in the laboratory tests.

In Test 2, it will be noted that the plots started to become reinfested after 16 weeks and in Test 3 after 8 weeks. Both these counts were made in September, at the time when incipient imported fire ant colonies are most easily found. In addition, there was some migration in Test 3 of mature colonies across the 50-ft buffer zone onto the count areas. For this reason, the best evaluation of the results can be made by comparing the 2- to 8-week results in Test 2 with the 2- to 4-week results in Test 3. On this basis, Kepone and mirex at a concentration of 0.075% both gave complete control. At the 0.15% level, however, mirex gave 100% control in Test 2 and a high of 99% in Test 3 compared to 95% and 99% respectively for Kepone. The lesser control by Kepone at the higher

Table 3.—The effectiveness of 4 toxicants in flour-soybean oil bait for controlling natural infestations of imported fire ants.[a]

Toxicant	Conc. (%)	Avg pretreatment count of active ant mounds	Percent reduction in active mounds after following weeks: 2	4	8	16
		Test 1				
Mirex	0.05	31	93	100	100	99
	.1	15	100	100	100	96
HRS-1243	.1	16	87	94	99	0
	.25	14	97	100	100	0
Kepone	.25	11	96	100	98	8
Check	—	14	13	5	5	0
		Test 2				
Bayer 30911	0.02	18	62	89	55	—
	.05	15	98	100	85	—
Kepone	.25	14	83	100	100	—
Check	—	11	40	27	8	—

[a] Actual bait composition was white flour 56 or 50%, soybean oil 43 or 47%, and monoglycerides 1 or 3%. Average results from three 1-acre areas within 6-acre plot. Application rate 6 lb/acre.

Table 4.—The effectiveness of 4 toxicants in soybean oil-corncob grits bait for controlling natural infestations of imported fire ants.[a]

Toxicant	Conc. (%)	Pretreatment count in active ant mounds	Percent reduction in active mounds after following weeks: 2	4	8	16
		Test 1				
Bayer 30911	0.015	40	1	0	—	—
	.03	21	30	14	—	—
HRS-1243	.075	41	20	0	0	—
	.15	39	33	49	44	60
Mirex	.075	39	57	96	95	96
Check	—	19	3	0	—	—
		Test 2				
Kepone	0.075	51	74	100	100	99
	.15	44	52	95	96	85
HRS-1243	.15	37	54	82	95	94
	.3	35	87	96	97	84
Mirex	.075	20	98	100	100	100
	.15	36	100	100	100	100
Check	—	22	0	11	31	42
		Test 3				
Kepone	0.075	31	71	100	93	93
	.15	22	99	91	96	48[b]
HRS-1243	.3	26	90	99	90	73[b]
Mirex	.075	25	99	100	95	95
	.15	31	99	98	96	93[b]
Check	—	15	0	24	0	0

[a] Average results from 3 count areas in a 6-acre plot; application rate 10 lb/acre.
[b] These figures include mounds introduced into the area from mating flights or migration subsequent to application of the toxic bait.

dosage level is presumed to be due to its greater repellency at this concentration (1% in the oil). HRS-1243 gave 95% and 97% control, respectively, at concentrations of 0.075% and 0.15% in Test 2 and 99% control at the 0.15% level in Test 3.

DISCUSSION AND CONCLUSIONS.—With the preceding tests it is possible to evaluate large numbers of insecticides for their potential as delayed action ant bait toxicants. An analysis of the results illustrates that an effective toxicant must, (1) exhibit delayed action over at least a 10- to 100-fold dosage range and preferably greater, (2) be readily transferred between ants and kill the recipient and, (3) not be repellent to ants when combined with a bait. Of the toxicants tested, mirex most satisfactorily meets these requirements. Kepone and HRS-1243 are the next most effective compounds. Both give delayed kill over a 10- to 20-fold dosage range but both are repellent at high concentration levels. Numerous compounds have been found which give delayed kill over a 2- to 10-fold dosage range. Bayer 30911 is characteristic of this group. In general, the control in field tests with the latter compounds has been erratic. The reason appears to be associated with the high concentrations of toxicant needed to insure that ants consuming a small quantity of bait receive sufficient toxicant to be killed. These compounds become increasingly repellent at the higher concentrations and may also kill too fast. Field observations have shown that ants vary greatly in their degree of hunger. Therefore, very hungry ants may feed on a bait which will be repellent to those less hungry. All these factors contribute to the erratic performance of marginal toxicants. It has been found also that these less effective compounds will perform much better in a slurry or mash type oil bait or a granular bait which contains a high percentage of oil. This fact is probably due to the ease with which the ants can extract the oil from these baits.

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