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Development of Chemicals to Control Forest Wildlife Damage

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THERE ARE two basic approaches in screening chemicals for biological activity. One is the empirical procedure of evaluating compounds against a standard. This system is generally associated with major programs which involve vast numbers of chemicals and a wide array of organisms. The second approach is more sophisticated and involves correlating biological activity with chemical structure. This method is generally practical in programs with limited application.

Seldom is either the empirical or the correlation method of screening employed in its pure form; most programs incorporate varying degrees of both. The chemical screening and development project at the Denver Wildlife Research Center is of this nature. Compounds identified by code designation are currently accepted for evaluation, but the supplier makes selections through correlation activity and screening empirically. In effect, the Denver project receives "prescreened" compounds known to be biologically active. The objective of this program is to define more accurately the activity of these materials as it relates to forest wildlife problems.

Frequently, the question is asked, why is a government agency involved in chemical development? For members of the chemical industry to undertake this research would require a market capable of defraying the cost. Since this cannot presently be assured, the only solution is the active participation of land management and conservation groups that likewise stand to benefit by improved chemical "tools." As a conservation agency, the U. S. Bureau of Sport Fisheries and Wildlife has a responsibility in developing materials and methods that can be used effectively and safely in alleviating wildlife damage problems.

Chemical evaluation within the Bureau had its genesis within the Bu- War II. Though some chemical developmental research was done earlier, the work was greatly accelerated during the war years when the foreign supply of common rodenticides was curtailed. Under funds provided by the Office of Scientific Research and Development and the Office of the Quartermaster General, the Bureau laboratories at Patuxent, Maryland, and Denver, Colorado, cooperatively evaluated several thousand chemicals (*4*). Early investigations by the Forest Wildlife Damage Project were conducted on compounds individually solicited. Later, in 1960, a chemical screening program was established that provided a continual flow of compounds from participating chemical companies (*6*). This program received approximately 800 compounds annually and operated for a period of 2 years. Based on this experience, the current program was established in 1963. Since that time there have been numerous refinements and more will be forthcoming. The goal is for greater simplification and increased sensitivity. The number of steps in the evaluation program and the complexities of each preclude more than just the highlighting of the procedures.

Program

In general, two types of compounds are sought, viz., effective lethal agents that are also specific and broad spectrum repellents. With rodenticides, selectivity is desired but this need not apply among species of rodents but rather among major groups such as birds and rodents. With repellents the opposite effect is desired—one compound repellent to rodents, birds, and larger herbivores. Another important parameter is safety; repellency is too often considered synonymous with the avoidance associated with sublethal toxicity.

A screening test is primarily an effort toward efficiency. The objective is the rejection with a minimum of testing of most unpromising chemicals without loss of active compounds. The procedure involves subjecting compounds to a standard series of tests, each designed to disclose more specific information about the material. Some of the tests are critical in that the compounds are rejected if they do not meet predetermined requirements.

There are three levels of investigation in the chemical evaluation programs: (1) the laboratory phase which identifies the potential candidates, (2) the enclosure studies, designed to evaluate repellent compounds on problem species under semi-field conditions, and (3) field evaluation which in effect is the test under actual use conditions. Three years are required for a compound to progress through the program. Additional samples of compounds surviving the laboratory studies are requested annually in time to be scheduled in the seasonal pen studies. Those compounds proving better than the standards in the pen studies are likewise scheduled for the following season's field studies.

Laboratory Evaluations

Initial bioassay—The first test determines if the candidate chemical possesses sufficient biological activity to warrant further testing. It is a simple test of offering five individually

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caged animals *(Peromyscus maniculatus)* 25 kernels of wheat each day for 3 consecutive days (sustaining ration of pelleted food and water available). The chemical is applied to sized, white wheat at an arbitrary level of 2 percent by weight. The animals are kept under observation 4 additional days to observe chronic effects. If a compound produces 60 percent mortality of the test animals or a 40 percent reduction in food intake, it meets minimum requirements acceptable for further testing.

Approximate lethal dose (ALD) determination.—If a high degree of mortality occurs on the initial bioassay, toxicity is obvious; if the seed is avoided, however, the property of the compound is not apparent. In order more clearly to define this activity an ALD is determined. The procedure is a slight modification of the method described by Deichman and LeBlanc (*3*). This method determines the order of toxicity of a compound within 30 percent of the LD_{50} with only 6 animals. The chemical is administered by gavage at standard dosages, each 50 percent higher than the preceding one. The ALD is the lowest concentration producing death.

Toxicity and repellency are vague, qualitative terms. Rather than attempt to define them and classify all active chemicals accordingly, a rating system has been developed that takes into consideration three important functions: (1) the toxicity of the compound (ALD), (2) acceptance in relation to toxicity, and (3) mortality of the test species. Each of these qualities can be measured and assigned a value, thus permitting comparisons of compounds.

The ALD is the basis of the rating system. Compound consumption calculated on first day's acceptance (initial bioassay) is expressed in amounts consumed based on the ALD. To be considered a promising repellent, compounds must have a low order of toxicity, be poorly accepted, and possess an adequate margin of safety. A compound with a rating of 1000-0.05-0 would be relatively nontoxic (1000 mg/kg), poorly accepted (0.05 of a lethal dose), and have no apparent adverse effect on the test animals (0 percent mortality). A rating of 5-10-100 would indicate a highly toxic compound (5 mg/kg) that is well accepted (10 times a lethal dose) and effective as a lethal agent (100 percent mortality).

Seed phytotoxicity.—The third step in the evaluation series is to determine if the candidate chemical affects seed viability. A portion of the seed treated for bioassay is reserved for germination tests.

Douglas-fir bioassay.—Theoretically, conifer seed can be protected from rodents through treatment with either repellents or lethal agents. Consequently, both types of candidate compounds are tested by following the same procedure outlined for the initial bioassay. The only difference is that Douglas-fir *(Pseudotsuga menziesii* [Mirb.] Franco) seeds treated at a one percent concentration are offered daily. Subsequent evaluation procedures are specific for the lethal agents and repellents.

Concentration-effect bioassay (lethal agents).—Because of differences in order of toxicity of the compounds, comparisons of rating indices based on initial bioassay results can be misleading. For example, treating wheat at the 2 percent level with a chemical having an ALD of 1 mg/kg renders each kernel a carrier of approximately 50 times a lethal dose for a 20-gram mouse compared to each kernel carrying slightly more than one lethal dose when treating with chemicals having an ALD of 42 mg/kg. Therefore, a concentration-effect bioassay (following the initial procedure) is conducted by treating the wheat to produce a lethal formulation on five seeds. This establishes a common denominator for comparing degrees of acceptance and permits rejection based upon standards.

Quail ALD (lethal agents).—The next test is an ALD determination on Coturnix quail *(Coturnix coturnix japonica)*. This animal is used to indicate specificity between birds and rodents. It is not necessarily a test for rejection of compounds but does have a bearing on the nature of additonal studies. Advanced evaluations of lethal agents are primarily governed by the properties of the compound and the conditions under which they will be employed.

Foliar phytotoxicity (repellents).—To have utility as a contact foliar repellent, the chemical must be tolerated by the plant at effective repellent levels. Laboratory-greenhouse phytotoxicity tests are conducted on two species of potted plants; one-year-old (1-0) Douglas-fir seedlings[1] with newly produced foliage and one-week-old bean seedlings are used. Six and one percent suspensions of the chemicals are sprayed on two containers of each species (pots contain a minimum of four Douglas-fir or four bean seedlings) until the leaves are wetted to capacity. Following application, the seedlings are held for observation in the greenhouse for 2 weeks although phytotoxicity, when occurring, is usually apparent within one week.

Rabbit ALD (repellents). — Animal species' differences regarding toxicity of candidate repellent chemicals have been responsible for biasing enclosure studies. To guard against the placement of toxic compounds in the repellent studies, an ALD test using domestic rabbits (Californian) is conducted on all compounds scheduled for enclosure evaluation. The cost and availability of snowshoe hare and deer, which are the actual target animals, preclude their use in these toxicity determinations. Those compounds having an ALD of 210 mg/kg or less on rabbits are rejected. This level was selected as the cut-off point for two reasons: (1) the order of toxicity is approaching a potential hazard for use as a repellent because the margin of safety is minimal; and (2) TMTD (Tetramethylthiuram disulfide), which is the current standard for repellent studies, has an LD_{50} of 210 mg/kg for domestic rabbits (*1*).

Translocation studies.—A systemic repellent would have a decided advantage over contact repellents since it would protect new foliage. To measure this potential, a rapid procedure for translocation evaluation is being developed. The compounds to be evaluated will be only those proven to be effective repellents in the enclosure studies. These chemicals will be applied to the soil of potted plants in the greenhouse. Foliage from the seedlings will be processed for "partial clean-up" following a flow chart which has been developed by processing a series of standards. The analysis will be done by thin-layer and gas chromatography.

Enclosure Studies

The enclosure studies are conducted at the Center's field station in Olympia, Washington. The physical facilities and the testing methods are designed to permit rapid evaluation of numerous chemicals under semi-field conditions. Eight treatments can be evaluated at one time and the procedure is the same for both black-tailed deer and snowshoe hares except for the size of the units. The hare enclosure is 1 acre and deer enclosure is 2½ acres.

Two-year-old (2-0) Douglas-fir seedlings are the standard carriers for the candidate repellents; all tests are conducted during the fall and winter months. To insure uniform coverage, the seedlings are treated by immersion into a mixture containing 6 percent candidate chemicals and 10 percent Rhoplex AC-33[2] adhesive. After

[1]Douglas-fir seedlings supplied by the L. T. Webster Nursery, Washington State Department of Natural Resources.

[2]Trade names referred to in this publication do not imply Government endorsement of commercial products.

drying, the seedlings are transplanted into the enclosures and exposed to the animals until 60 to 80 percent of the untreated control seedlings are clipped or browsed which indicates sufficient feeding pressure. The experimental treatments are compared to a 6 percent TMTD treatment, the chemical standard, since it is currently employed operationally as a contact repellent (2) (5). To overcome individual behaviorism, a minimum of 10 animals is employed in each test.

The test design within each enclosure consists of ten blocks, each providing 80 tree locations spaced at 3-foot intervals. Therefore, each treatment is represented by 100 seedlings per test. Two permanent block patterns are employed alternately, each having the chemical-tree locations assigned at random. Frequency of cutting or browsing is recorded and these data are transformed to arcsine and the null hypothesis is tested by analysis of variance. If the analysis of variance indicates differences among treatments, Scheffe's S-method is employed for mean separations.

During the treating process, an additional 25 seedlings are included for each chemical. These seedlings are planted in a field nursery plot and examined periodically for evidence of long term phytotoxicity. The tests are not terminated until midway through the following growing season.

Field Studies

In an attempt to simplify and standardize field testing procedures in the Pacific Northwest, a cooperative study was conducted to evaluate a series of plot designs (unpublished report, N. W. Pest Action Council, Wildlife Problems Committee). Based on results of this study, the current design was established. The basic unit is a plot containing ten rows of 25 seedlings each with rows and seedlings placed on 8-foot intervals. Treatments are assigned at random to rows within each plot. Five replications are required to give sufficient strength for statistical analysis. To assure achieving a critical test several 5-plot series are installed for each species of animal evaluated. Incidence of damage is recorded at the end of the growing season and again several weeks after the buds have burst.

Discussion and Results

The number of compounds received annually for screening is declining but the quality is progressively improving. Of 891 compounds received during the first 2 years of the program, 502 or 56 percent were sufficiently active to warrant conducting ALD tests. During the first 7 months of 1965, the third year of the program, 293 compounds were received of which 188, or 82 percent, were considered active. If compounds are received at the present rate for the remaining 5 months of this year, the 1965 total will be 394 compounds compared to 469 in 1963 and 421 in 1964. The fate of the 891 compounds received during 1963 and 1964 is summarized in Figure 1.

Of the 502 compounds considered active after the initial bioassay, 254, or 51 percent, were accepted for further study. Forty-nine percent of the compounds were rejected, primarily because of their acceptance rating. The ALD line represents chemicals accepted in amounts equal to an approximate lethal dose (Fig. 2). The

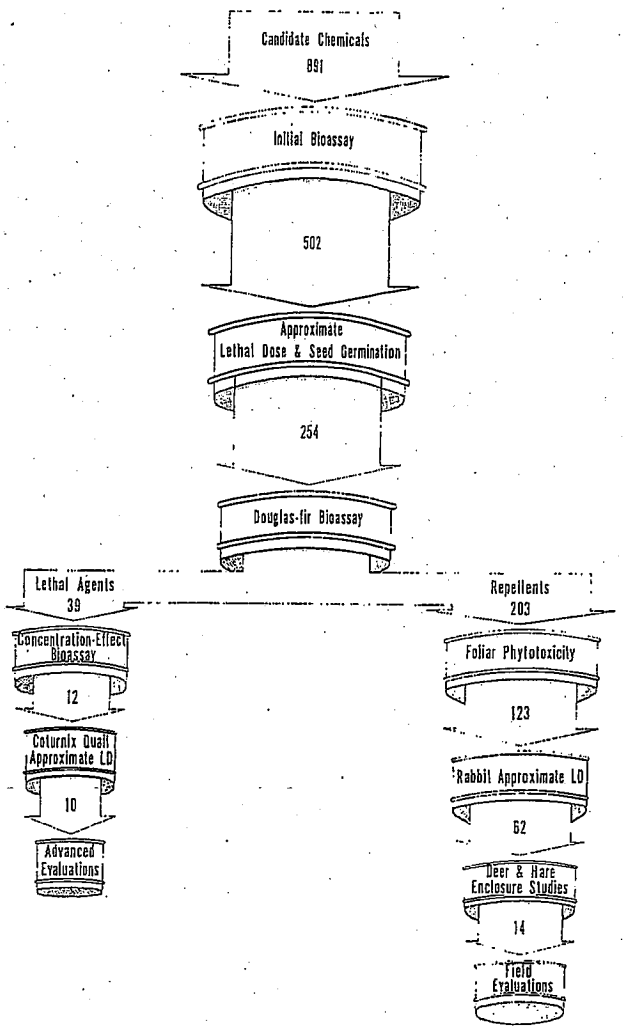

Fig. 1 (left).—Fate of compounds as lethal agents and repellents. The numbers are compounds entering each test of the screening procedure after surviving the previous test.

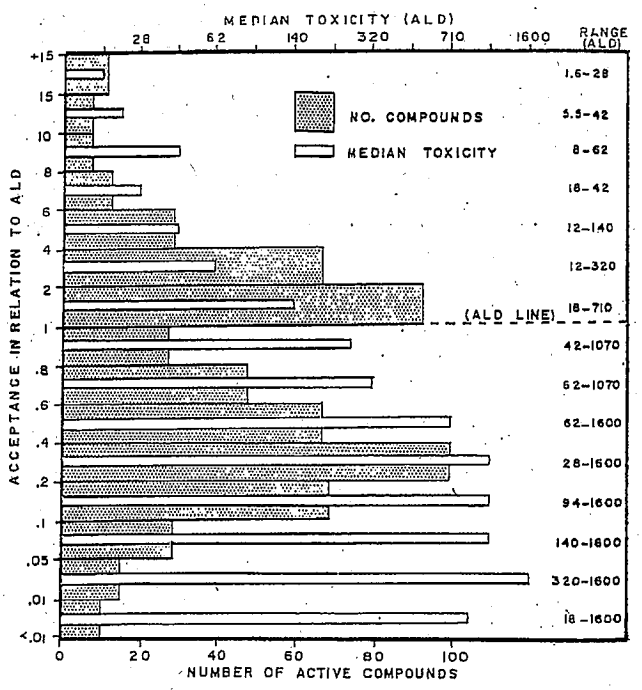

Fig. 2 (above).—Number of active compounds as they relate to acceptance and median toxicity.

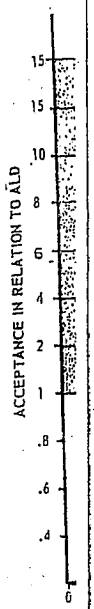

Fig. 3 in the

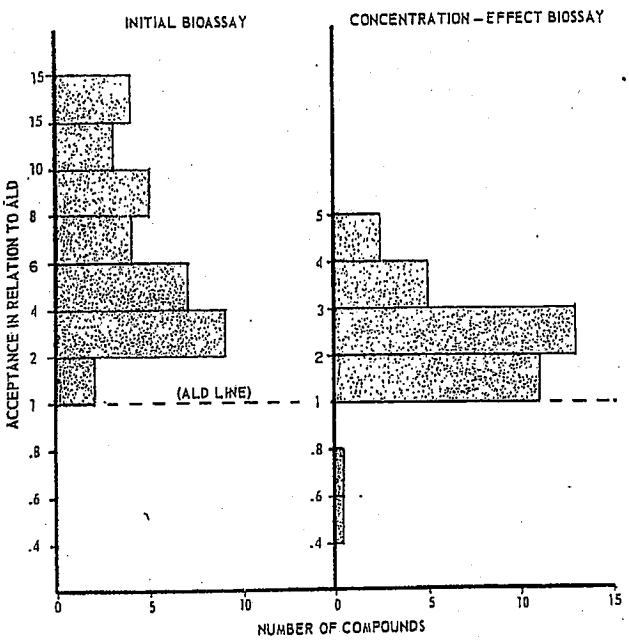

Fig. 3 —Relationship of initial and concentration-effect bioassays in the evaluation of candidate lethal agents.

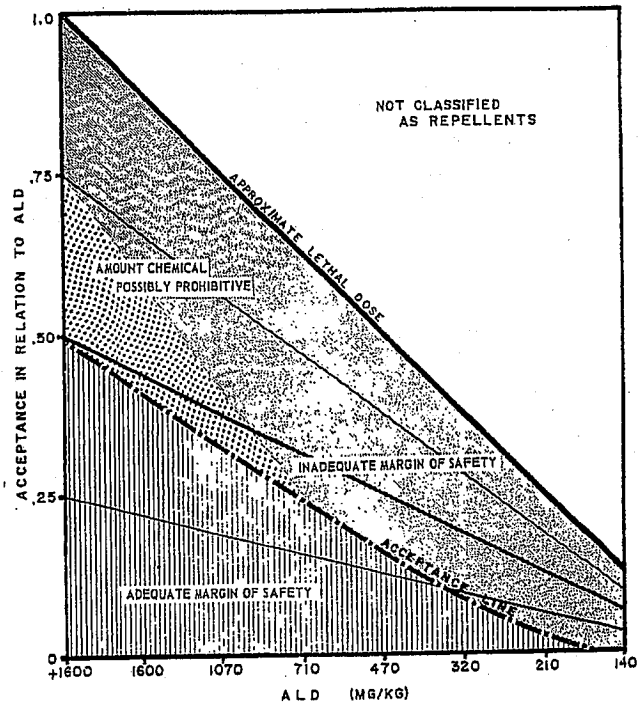

Fig. 4 —Schedule of acceptance of candidate repellent compounds. The converging lines indicate relative levels of acceptance.

compounds falling just above the line would be considered lethal agents poorly accepted by the mice, whereas those just below would be compounds with inadequate margin of safety precluding their value as repellents. The further a compound lies in either direction from the line, the greater its potential. For lethal agents to remain active, acceptance must exceed two times a lethal dose and a high rate of mortality must occur. For repellents, the point of rejection varies with differences in order of toxicity. In general, the more toxic a compound the greater its acceptance rating and as the median toxicity decreases the acceptance rating decreases.

The profile of compounds in Fig. 2 is as expected for those above the ALD line. Most of these chemicals are poorly accepted as shown by their location just above the line. It was anticipated that this same frequency relationship would apply below the line resulting in a "normal curve" with the ALD line transecting at the apex. This did not occur, however, for the majority of the chemicals below the line fall in acceptance between 0.1 and 0.6 of an ALD. This frequency pattern is a manifestation of the rules of rejection. Compounds producing 60 percent mortality of test animals in the initial bioassay are accepted for an ALD, whereas, compounds producing less mortality would be rejected. This accounts for the smaller number of compounds with acceptance indices between 0.6 and 1.0.

Following the ALD evaluation and subsequent computation of a rating index, many of the compounds falling above the line are rejected. Additional rejections occur as a result of the concentration-effect bioassay. This test, an important link in the evaluation chain, provides for a more accurate comparison of oral acceptance by reducing all chemicals to an equal level based on toxicity. In Figure 3, the relationship between the initial and concentration-effect bioassays is graphically portrayed.

The effect of the concentration-effect bioassay is to reduce the oral acceptance rating of the candidate lethal agents. Also, it should be noted that the acceptance rating cannot exceed five times an ALD. This is the upper limit because the test offers only 25 seeds which have been formulated to produce a five-seed lethal treatment. Acceptance of only two compounds was reduced to where they fall below the ALD line. The majority of the compounds ranged between one and three times an ALD at the lower concentration.

The severity of this test becomes apparent since Compound 1080, a highly effective rodenticide, rated an acceptance of only 0.8 of an ALD at the five-seed lethal concentration. However, even at this low level of consumption, all of the test animals died. The effect, of course, weighs heavily in the final decision of rejection. In some instances, when the animal is capable of rapidly metabolizing the compound, consumption exceeds an ALD several fold with little effect on the test animals. In the bioassay studies the animals have nearly a full day to consume the chemical compared to an immediate receipt of the compound when it is administered by stomach intubation in the ALD studies.

The advanced evaluations of the lethal agents depend largely upon the properties of the compounds and circumstances under which they will be employed. For instance, a highly toxic rodenticide that is equally toxic to birds would have utility only in situations where its use would not present a hazard to birds. At present, only one candidate lethal agent is being field evaluated but additional developmental work is scheduled for others.

About 40 percent of the candidate repellents were rejected due to foliar phytotoxicity. The rabbit ALD test has only been in effect for one year. It appears that this test will not appreciably reduce the number of compounds otherwise scheduled for enclosure evaluations. A greater loss of chemicals occurs from problems associated with supply. Out of the 891 compounds received and tested, only 62 (7 percent) survived the laboratory evaluations.

The safest repellents are compounds with a low order of toxicity and a large differential between the amount of chemicals required to produce the repellent effect and the lethal level. In Figure 4, this would be those

compounds plotted in the lower left hand corner. Compounds accepted in amounts greater than an ALD cannot be considered repellents even if they are relatively nontoxic. Similarly, compounds that are highly toxic cannot be considered repellents even when acceptance is poor. In general, the higher the toxicity of a compound the greater must be the avoidance. For example, a compound with an ALD of 1600 mg/kg that is consumed at the 0.5 level would have an 800 mg/kg safety margin. In contrast, a compound with an ALD of 10 mg/kg would have a safety margin of only 5 mg/kg. When the margin of safety in amounts of chemicals is reduced, the chance of hazard is increased. For these reasons, the point at which compounds are acceptable as candidate repellents is dependent upon acceptance in relation to ALD and the order of toxicity. A sliding scale, as shown in Figure 4, is employed in the decisions.

Based only on margin of safety, it is likely that compounds having an ALD greater than 1600 mg/kg could be employed at a higher than 0.5 acceptance level. However, there is a point where the volume of chemical applied becomes a limiting factor. This limitation could be excessive cost of the product, formulating difficulties or interference with the physiology of the seedling.

During the winter of 1962-63, the procedures for the enclosure evaluation were developed and tested in preparation for the candidate materials expected the following year. During the 1963-64 season 47 chemicals (some from sources other than this screening program) were evaluated as repellents on both black-tailed deer and snowshoe hare (Table 1). Unfortunately, the pen design was not adequate for statistical separation of treatments. Therefore, based primarily on rank rather than significant differences, nine compounds were selected for field evaluation on deer and five of them were also selected for snowshoe hare. These compounds were field evaluated during the 1964-65 season with the result that all proved as good as the TMTD standard repellent, but none was considered better even at the 0.25 level of significance.

During the 1964-65 season, 35 compounds were evaluated as repellents in the enclosures using an improved test design. Six of those compounds proved better than TMTD on deer at the 0.05 level of significance. Furthermore, five of these six compounds, though not significantly better, ranked higher than TMTD on snowshoe hare. These chemicals are scheduled for field evaluation this coming season on deer, hare, and elk. Of further interest, is the fact that 21 of the 35 compounds tested on deer were as good as the standard and 17 of the 35 were as good as the standard on hare.

Table 1.—Summary of Advanced Studies of Candidate Repellents

	Pen studies		Field studies	
	Better than	Good as	Better than	Good as
Season and animal	TMTD	TMTD	TMTD	TMTD
Pen 1963-64, Field 1964-65				
Deer	9/47[1]	—	0/7	7/7
Hare	5/47	—	0/6	6/6
Pen 1964-65, Field 1965-66				
Deer	6/35	21/35	–/6	–/6
Hare	0/35	17/35	0	–/6

[1]Number compounds better than TMTD / number compounds tested.

All chemicals scheduled for enclosure studies or field evaluation require formulating to insure weatherability over the exposure period. Many of the compounds are compatible with Rhoplex AC-33 and are therefore formulated in an identical manner as the TMTD standard. Others, however, because of incompatability with Rhoplex AC-33 require special formulation.

Studies are now being conducted to provide more and better adhesives for use in formulating candidate chemicals. The materials studied represent eight recognized adhesive groups. They are being subjected to a series of tests to screen out the least effective. The tests include laboratory and greenhouse studies to evaluate: (1) phytotoxicity, (2) film flexibility, (3) film coverage, and (4) weatherability using a commercial weatherometer. The advanced studies of the promising materials include field weatherability and laboratory controlled environment studies on film setting and retention under varying temperatures and relative humidity.

Summary and Conclusions

Recent changes in the chemical screening and development program at the Denver Wildlife Research Center have improved its efficiency, effectiveness and reliability, and further improvements are contemplated. Compounds submitted by participating chemical companies are evaluated in three steps: (1) the laboratory to define the activity, (2) enclosure studies to determine the effect on target species, and (3) cooperative field evaluation which is the ultimate test of worth.

Although the quantity of compounds received has diminished, the quality is improving. The outlook for an improved contact repellent that is effective on both deer and hare is favorable. Six compounds have proven better than the standard at the 0.05 level of significance on deer in the enclosure studies, and five of these ranked better than the standard on snowshoe hare. Several promising candidate lethal agents have also emerged from the program. Advanced evaluation will be "customized" for each compound depending upon its specific properties.

It is our goal to help assure that the renewable resources of timber and wildlife are managed to produce optimum yields now and in the future.

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