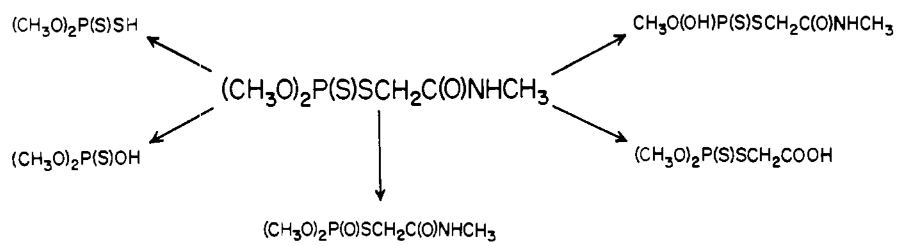

Figure 7. Proposed initial sites of metabolic attack of the Dimethoate molecule

degradation was more extensive with Dimethoate than with the Dimethoate–P=O (with male rats, 81% of the administered dose was excreted in the first 24 hours following Dimethoate treatment compared with 19% for the Dimethoate-P=O treatment). Therefore Dimethoate is more readily attacked at the amide C—N bond than is the Dimethoate-P=O, and this must be the principal route of degradation of Dimethoate.

The selective toxicity of Dimethoate may be dependent on the ability of the mammal to attack the C—N bond more vigorously than can the insect.

As the des-methyl derivative was excreted intact after administration to rats and initially excreted in the urine of rats and cows in small amounts, the hydrolytic attack at the alkoxy group must be a minor pathway in the detoxification of Dimethoate in mammals.

Of the identified metabolites, the two hitherto undescribed are the carboxy and des-methyl derivatives. Hydrolytic attack at the alkoxy group has been reported for several compounds (*7, 11*). However, this is the first example of the hydrolysis of a carbamoyl phosphate to yield an identified carboxy phosphate metabolite.

Dimethoate is of short persistence in cows after oral administration. It appears to partition readily from the blood into and out of tissues and is rapidly metabolized and excreted.

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LABORATORY TESTING OF RAT REPELLENTS

Quantitative Evaluation of Repellency of Chemical Coatings on Paperboard

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THE SEARCH FOR EFFECTIVE REPELLENTS for rodent-proofing corrugated paperboard boxes and other containers has been handicapped by lack of a simple, quantitative laboratory test for repellency. Existing methods of testing rodent repellents, both in the laboratory and in the field, have been reviewed by Welch (*12*). Laboratory testing has been done by food acceptance tests (*1*) and barrier tests (*2*). In food acceptance tests, a candidate repellent was mixed with food, and its effectiveness was measured by a comparison of the amount of treated and untreated food eaten by a rat within a given period of time. In barrier tests, hungry rats were trained to gnaw through laminated paper barriers coated with repellent to obtain food, and the effectiveness was determined by a comparison of the time required for rats to penetrate coated and plain barriers. Unfortunately, some chemicals, although highly effective in making food unacceptable, were not repellent to rats gnawing on treated barriers. Therefore, food acceptance tests were useful only as preliminaries (*13*). A possible explanation was, that rats, when gnawing paper, did not eat or chew it, but tore and shredded it with their incisors (*13*).

To express repellency of a chemical quantitatively, any test used becomes a biological assay, and must meet certain requirements (*4*). Especially germane to this problem is that the results obtained in the test parallel field effectiveness when the repellent is applied as a coating to a paperboard box, and express activity in terms of an effective repellent as a reference standard. The values obtained must be reproducible on repeat tests. Lastly, the precision of results must be analyzed by suitable statistical methods. Other desirable features would be minimal training of experimental animals and a simple method of applying coatings using small amounts of chemicals.

For these studies, the highly repellent antibiotic substance cycloheximide Actidione, Upjohn, [3-2-(3,5-dimethyl-2-oxo-cyclohexyl)-2-hydroxyethyl] glu-

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A new method was developed to express quantitatively the repellency of chemicals to rats when applied as a coating to corrugated paperboard. A row of peanuts was covered by a 2-inch wide piece of corrugated paperboard, untreated at one end and then coated with 3/4-inch wide strips of repellent chemical, the concentration increasing logarithmically from one strip to the next. Rats gnawed along the paperboard until the concentration of the repellent coating was so great that they ignored the remaining peanuts. The average maximum concentration gnawed through was calculated and the repellent activity was expressed as a percentage of the activity of cycloheximide (Actidione), an extremely potent repellent.

tarimide, purity 85 to 100%, was used as the reference standard (*10*).

Barrier Tests

Kaseoru (*8*) carried out a series of studies aimed at improving and refining the barrier test method of Bellack and DeWitt (*2*) using corrugated paperboard instead of laminated paper barriers. In one study, she compared the performance of trained rats gnawing plain barriers to barriers coated with cycloheximide in concentrations of 0.01, 0.032, and 0.10 mg. per sq. cm. The percentage of rats which penetrated the barrier within 60 minutes (after which there was seldom any further gnawing) decreased with increasing concentration of repellent. (Plain barriers were penetrated in about 15 minutes.) In freshly trained rats, limiting consideration only to rats penetrating barriers within 60 minutes, penetration times increased with increasing concentrations of cycloheximide. Four months later, when the same group of rats was tested in the same manner using the highest and lowest concentrations of cycloheximide, about the same percentages of rats failed to penetrate as before. However, this time there was no significant difference between penetration times for coated and plain barriers. Apparently, with very experienced rats, the decision became only one of whether to gnaw the barrier.

In the method of Bellack and DeWitt, if a barrier were not penetrated at the end of 60 minutes, the rat was replaced with another, and this process repeated until the barrier was penetrated. Because rats vary considerably in their reaction to repellent-treated barriers, some of them at times gnawing hardly at all and others gnawing readily, penetration times in replicate experiments might vary greatly, depending upon the gnawing activity of the first rats selected. Such variations would reduce the accuracy of the measurement of repellency.

Traub *et al.* (*10*) utilized a barrier with two adjacent coatings; the rat presumably would gnaw the less repellent. Kaseoru investigated this method by using two separate barriers placed side by side. She also presumed that the rat would gnaw the less repellent. Then, by using one cycloheximide coating as a reference, an unknown repellent coating could be rated as stronger or weaker. While training rats to gnaw barriers, she noted that any individual rat consistently gnawed either the left or the right barrier, the majority of rats the left. Such position "preferences" have been reported in random choice experiments (*9*). Accordingly, the rats were trained using one paperboard and one metal barrier, their positions were reversed in every test. Nevertheless, when confronted by two barriers coated with different concentrations of cycloheximide, an individual rat's choice, if he gnawed at all, was little influenced by the coatings, but he selected instead predominantly the barrier on one side. As before, rats selected more left barriers than right. A similar phenomenon was reported in which rats were to select between drinking fountains containing water or 15% ethyl alcohol (*7*).

The present investigation was undertaken, because of the conclusive demonstration of the limitations of barrier test methods.

Graded Strip Test

Essentially, this test method involved clamping a 2-inch wide piece of corrugated paperboard over a row of Spanish peanuts (salted and roasted). The paperboard was left untreated at one end, and then coated with 3/4-inch wide strips of the chemical to be used, the concentration increasing logarithmically from one strip to the next. One peanut was placed under each strip. Rats, after a starvation period, gnawed along the paperboard until the concentration of repellent coating was so great that they ignored the remaining peanuts. The average of the highest concentrations gnawed (peanut obtained) was calculated, and the repellent activity was stated in terms of the activity of cycloheximide.

Each piece of paperboard had 11 strips. The first three were left uncoated, the second three coated only with the adhesive used for applying chemicals, and the last five coated in sequence at concentrations of 0.32, 1.0, 3.2, 10, and 18 mg. per sq. cm. The highest concentration was not in the same logarithmic increment, because greater concentrations were so thick that they would chip and flake. Leaving the first six strips without repellent not only maintained the state of training of the rats, but also indicated whether some other factor such as a minor illness, might have impaired gnawing ability.

Six rats were used for the initial screening tests, while for more accurate evaluation of active compounds 18 rats were used.

Carrying Frame. The frame was made of tempered hardboard with a narrow metal strip across one end to block access to the peanut at the end of the strip coated with the highest concentration. To hold the peanuts in place, a row of conical depressions was drilled along the midline of the frame. Strips of test coatings were 3/4 inch wide with a 1/32-inch space between each pair, so that the coatings would not run into each other during application. Each peanut was 1/8 inch from the border of a coated strip, which required that, to obtain the peanut, the entire 3/4-inch width of coated strip had to be gnawed.

The paperboard, usually as a single piece, was held in place by two rows of flat-jawed paper clips (Everhandy clip No. 1, Hunt Pen Co., Camden, N. J.). When in place, 1 1/4 inches of paperboard were exposed to the rat, leaving margins of 3/8 inch covered by the clips.

Conduct of Test. After having been starved for 24 hours, the rats were put into small individual cages with one test board each in the late afternoon and allowed to work overnight. Rats started gnawing immediately, and cursory observations indicated that there was little if any further gnawing after the first 2 or 3 hours. Water was always available. Rats were offered test boards no oftener than once every third day. On such a program they gained weight and remained healthy.

Coating of Paperboard. The paperboard must be coated on both sides with chemical to be effective. When only the upper surface was coated, there was little contact between the coating and the rat's tongue; when only the lower surface was coated, rats gnawed away the top facing and fluting and obtained the peanut by making a small hole directly over it, avoiding the coating.

Two general methods of applying coatings have been used: suspension in an adhesive starch dispersion, and impregnation of filter paper using a solution of the chemical in a volatile solvent. Starch dispersion was used for solids, while liquids and resinous solids or semisolids were applied by filter paper impregnation. Of various methods of laboratory application of coatings, starch was reported to give the greatest activity to repellents (*12*).

The starch used was Superfilm No. 25 (Stein, Hall and Co., New York 17, N. Y.), prepared by mixing 20 grams of finely powdered material in 80 ml. of water at 74° C., and maintaining the mixture at that temperature for 20 minutes. The filter paper used was Whatman 3 MM, cut to exactly 3/4-inch width.

To minimize loss of chemical and for ease of handling, solids were prepared first by making a heavy initial suspension in a syringe barrel and then, after inserting the plunger, transferring this heavy suspension to other syringes for application directly or for diluting with additional starch dispersion. All dilutions were made by first drawing the requisite amount of starch dispersion into a syringe, and then transferring the suspension into it through a short nipple of rubber tubing. Coatings were spread evenly using a small camel's hair brush, followed by air drying.

Training. In contrast to barrier tests, only a minimal period of training was necessary, because the peanuts served as a strong lure. For this study, male albino rats, of Wistar strain origin, were used. An initial weight of 150 grams was most satisfactory. During training, each rat was offered two identical frames covered by paperboard (a total of 22 peanuts), the last five strips of which were coated with starch adhesive. Training was considered complete after a rat successfully obtained all 22 peanuts in three successive trials. Few rats were rejected, and most rats over 150 grams obtained all peanuts in the first test. No definite retraining schedule was followed, but it seemed that a rat should be retested with two training frames once every 4 to 6 weeks.

After rats had been used regularly for 6 months, some were discarded, because they ceased to gnaw vigorously.

Quantitative Expression of Repellency

The repellency of a chemical was expressed as per cent of the activity of cycloheximide, as calculated from the ratio of the average highest concentration of cycloheximide gnawed to that of the test chemical. For a concentration to be considered gnawed, the entire peanut had to be removed. All calculations were carried out using concentrations as log milligram per square centimeter. Statistical evaluation was by the usual methods of biological assay for graded responses (*5*).

Table I. Repellency of Cycloheximide, Aniline–1,3,5-Trinitrobenzene Complex and Zinc Dimethyldithiocarbamate–Cyclohexylamine Complex[a]

Cycloheximide		*Aniline-1,3,5-Trinitrobenzene Complex*		*Zinc Dimethyldithiocarbamate–Cyclohexylamine Complex*	
Strip coating, log mg./sq. cm.	*Highest concns. gnawed*	*Strip coating, log mg./sq. cm.*	*Highest concns. gnawed*	*Strip coating, log mg./sq. cm.*	*Highest concns. gnawed*
0.50	1	1.25	5	1.25	9
0.00	2	1.00	7	1.00	7
$\bar{1}.50$	5	0.50	5	0.50	2
$\bar{1}.00$	5	0.00	1	0.00	0
$\bar{2}.50$	5	$\bar{1}.50$	0	$\bar{1}.50$	0
$\bar{2}.00$	0				
$\bar{3}.50$	0				
$\bar{3}.00$	0				
Average Highest Concentration Gnawed					
$\bar{1}.20 \pm 0.14^b$ log mg./sq. cm. (0.16 mg./sq. cm.)		0.88 ± 0.11^b log mg./sq. cm. (7.6 mg./sq. cm.)		1.07 ± 0.08^b log mg./sq. cm. (11.8 mg./sq. cm.)	
Cycloheximide Activity, %					
100		2.1		1.4	

[a] All chemicals applied in starch adhesive. 24-hour starvation before tests. 18 test boards used for each compound.

[b] Standard error, $\sqrt{\frac{S(y-\bar{y})^2}{n(n-1)}}$, where y = individual highest concn. gnawed, $\bar{y}$ = average highest concn. gnawed, and n = number of boards tested (*5*).

Because rats showed considerable variation in the highest concentration they would gnaw, concentrations of cycloheximide ranging from 0.001 to 3.2 mg. per sq. cm. in increments of log 0.5, a total of eight strips, were used for standardizing rats. Two strips were coated only with adhesive and the first strip was left uncoated. The value obtained was remarkably constant, there being no significant difference between average values obtained using young, freshly trained rats and old rats which had been working for 5 months. Neither was there any significant difference whether the cycloheximide was applied in starch adhesive or dissolved in ethyl alcohol and applied to filter paper strips, which were then pasted on paperboard. The average highest concentration of cycloheximide gnawed, based upon a total of 49 tests using 31 rats, was $\bar{1}.24 \pm 0.083$ log mg. per sq. cm. [standard error (*5*)].

Results of three typical tests, one a cycloheximide standardization and two using compounds proved to be effective rodent repellents in field tests (*12*, *14*) are given in Table I. [The 1,3,5-trinitrobenzeneaniline complex was prepared as directed by DeWitt *et al.* (*6*). The zinc dimethyldithiocarbamate–cyclohexylamine complex, was supplied as B. F. Goodrich and Co. Z.A.C. in water suspension. It was dried and applied as described for the starch adhesive.]

Several of the rats gnawed the highest concentrations coated. For the purpose of calculations, it was assumed, although it was unlikely to be true, that this was the highest concentration that would have been gnawed. Therefore, it is probable that the average highest concentrations gnawed as calculated were actually too low, and consequently the cycloheximide percentages were too high.

When the graded strip was first devised (*11*), food was withheld from rats only 8 instead of 24 hours prior to use. Further experience showed that, after working for several weeks, some rats were not sufficiently motivated to gnaw for all the peanuts using uncoated paperboard during the test period. The shorter starvation period, and hence the diminished hunger drive, reduced the average highest concentration of cycloheximide gnawed from 0.17 to 0.035 mg. per sq. cm. As might have been expected, activities of compounds expressed as cycloheximide percentages by the two methods did not differ significantly, because the rats gnawed correspondingly lesser concentrations of test compounds (see examples in Table II).

Comparison with Other Test Methods. It is difficult to make critical comparisons of the graded strip with other test methods, because other methods are qualitative estimates of repellency, or, as with food acceptance tests, may not reflect activity as a coated barrier. There are given in Table II several compounds which have been tested by other methods. About the best that can be said is that, considering compounds already tested by other methods, those which have had proved activity in the field have been active in the graded strip test. The test can detect only compounds with strong repellent activity; weak compounds would be recorded as inactive. However, it would seem that only compounds with activities on the order of at least 20% that of cycloheximide would be suitable as practical repellents.

The average highest concentration gnawed in the graded strip test was of the same order as the effective coating concentration in field tests. Thus, for the compounds given in Table I (7.6 and 12 mg. per sq. cm., respectively), in field tests on coated boxes concentrations of 7.7 mg. per sq. cm. were effective repellents. Cycloheximide itself (0.16 mg. per sq. cm.) showed activity at 0.38 mg. per sq. cm., but was even more effective at higher concentrations (*12*). Although individual rats gnawed concentrations much higher than the averages, in the experimental test, the rat was a highly trained, strongly activated (hungry) animal with a strong lure (peanut) always less than an inch from his nose. On the other hand, the rat under field conditions had the alternative of looking for food elsewhere.

Although it has not been possible to carry out field tests using compounds whose activity was first revealed by this assay method, the graded strip offers a simple, reliable method not only for detecting rat repellent activity as coatings on paperboard, but also for a quantitative comparison with known effective repellents.

Table II. Comparison of Four Repellency Test Methods

Chemical	*Graded Strip Cycloheximide Activity, %*	*Food Repellency Index (2% in Diet)*	*Barrier Tests, Penetration Time Treated/Control (50 Mg./Sq. In.)*	*Field Tests, Penetration Time Treated/Control (50 Mg./Sq. In.)*
Cycloheximide	100	100 (*3*)		>31 at 30 and 10, 24 at 5, 5.6 at 2.5 (*12*)[a]
Aniline–1,3,5-trinitrobenzene complex	3.0[b] 2.1[c]	91.5 (*3*) 100 (*6*)	1.5 (*6*)	10.4 (*6*)
o-Anisidine–1,3,5-trinitrobenzene complex	0.6[d]	98.6 (*3*) 99.3 (*6*)	2.6 (*6*)	9.5 (*6*)
o-Toluidine–1,3,5-trinitrobenzene complex	1.5[d]	100 (*3, 6*)	7.3 (*6*)	2.0 (*6*)
1-Naphthylamine–1,3,5-trinitrobenzene complex	0.4[d]	100 (*3, 6*)	3.5 (*6*)	2.6 (*6*)
Zinc dimethyldithiocarbamate–cyclohexylamine complex (Z.A.C.)	1.2[e] 1.4[c]			9.5 3.0 at 20 (*14*)[a]
Bis (dimethylthiocarbamyl) disulfide (tetramethylthiuramdisulfide)	1.3[b]	73, 90, 96 (*3*)	12 (*2*)	3 to 4 at 20 (*14*)[a]
Bis (diethylthiocarbamyl) disulfide (tetraethylthiuramdisulfide)	2.0[b]	69 (*3*)[f]		
Pentabromophenol	0.3[d]	49 (*3*)		
2-Phenylindole	0.3[d]	85, 100 (*3*)		

[a] Recalculated from days until treated and control boxes were penetrated. Undamaged boxes calculated as if penetrated on last day of test. [b] Determined on 18 rats, 8-hour starvation. [c] Determined on 18 rats, 24-hour starvation. [d] Determined on 6 rats, 8-hour starvation. [e] Determined on 12 rats, 8-hour starvation. [f] Value of 91 given by J. B. DeWitt on rodent repellents. Other data agreed with (*3*).

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FUMIGANT REACTIONS WITH FOODS

Hydroxyethyl Derivatives in Prunes Fumigated with C^{14}-Ethylene Oxide

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Fumigation with volatile alkylating agents such as methyl bromide or ethylene oxide is an effective way to protect stored food products from insect and fungus damage. Such chemical treatment usually causes some chemical modification of the food product. Winteringham (*18*) has shown that wheat fumigated with methyl bromide reacts chemically with the fumigant with the formation of water-soluble bromide and methylated products.

Dried fruits (prunes, raisins, apricots, etc.) may require fumigation in some instances, but little is known about the reactive constituents in fruit that are likely to be modified by fumigants. This investigation was designed to explore the effect of fumigating dried prunes with ethylene oxide under controlled conditions in order to provide data on the quantity and identity of these relatively unknown food constituents. The fruit selected for this investigation was the dried plum of the French variety grown in the Santa Clara Valley in California. The major constituents