and calculated (spiked) organic chloride concentrations in Table IV demonstrated the validity of the method.

This neutron activation procedure offers several distinct advantages for the determination of total organic chloride concentration in milk and its derivatives. The chloride sensitivity of this neutron activation method is about 10 p.p.b. of total organic chloride. With higher neutron fluxes, the sensitivity is proportionately increased. Thus, the lower limit of detection for total organic chloride is inversely proportional to the neutron flux. The procedure described is also applicable for the determination of the total organic bromide or iodide concentration (and in turn the calculated concentrations of brominated or iodinated organic molecules) in butterfat. A similar method for the determination of organic Br in orange peel and orange juice has been employed by Castro and Schmitt (*1*). The method of neutron activation is also applicable in the analysis of chlorinated or brominated pesticides in foods and fodder, viz., leafy vegetables and alfalfa. In brief, the procedure consists of extracting organic chlorinated (or brominated, etc.) pesticides from the food or fodder matrix by a pure solvent such as mixed hexane, volatilizing most of the solvent, and neutron activating the resultant reduced solvent volume (*2*). The time required for such an analysis is less than 1 hour for a single sample but can be reduced considerably if many specimens are processed simultaneously.

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RODENT REPELLENCY

A Quantitative Method for Evaluating Chemicals as Rodent Repellents on Packaging Materials

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A new method, a concentration-repellent effect determination, was devised to express quantitatively rodent repellency of chemicals on packaging materials. The technique uses time, chemicals, and test animals efficiently, and yields an expression of effectiveness in terms of the concentration required to repel 50% of test rodents, an R_{50}. The method also yields the confidence limits (95%) of the R_{50} and a regression line that allows the estimation of the concentration that repels any given percentage of test animals. β-Nitrostyrene, tributyltin chloride, cycloheximide, and α-cyano-β-phenylacrylonitrile were the most effective repellents for commensal rodents among those given early trials. Although effectiveness is the first and most important consideration in the development of a successful rodent repellent for packaging use, stability, use hazards, and cost must also be favorable.

EVALUATION of chemicals as repellents for protecting paper, plastic, and textile packaging materials from rodent damage has been in progress for more than a decade. Techniques for appraising repellents for packaging have been reported by Bellack, DeWitt, and Triechler (*1*), Weeks (*6*), and Welch (*7*). A review of previous work in this field clearly shows the need for a quantitative and statistically sound expression of the repellent activity of chemicals that can be better projected to field conditions, and can be expressed in readily comparable terms, such as those employed in rating the toxicity of chemicals. This article presents such an expression, the testing technique employed in attaining it, and some of the initial results obtained.

Expression of Rodent Repellent Activity

A widely used value for rating toxicities is the LD_{50}, that is, the amount of chemical that is lethal to 50% of the test animals when administered in a single dose. This value is expressed in milligrams of chemical per kilogram of body weight of the test animal. In this article, the rodent repellency of chemicals is expressed in an analogous term, an R_{50}. The R_{50} is the amount of a chemical applied to a given packaging material (mg. per sq. inch) that repels 50% of the test animals during a stated period of time under given conditions.

Experimental Procedure

Training of Test Animals. When the repellent activity of large numbers of chemicals is to be determined and related, a single kind of test animal, type of packaging material, and testing period must be selected for an initial evaluation. In addition, acquisition and training of test animals, application of chemicals, and exposures of treated materials must be kept relatively simple. The authors found house mice (*Mus musculus*) and burlap bags to be the most practical combination of test animal and test material. Penetration of untreated burlap bags containing food was regularly accomplished by individually caged house mice within several hours after exposure. An overnight exposure period of 16 to 18 hours for treated materials was found to be most satisfactory.

To ensure that all mice used in tests possessed the ability to penetrate untreated

Table I. Concentration-Repellent Effect Determinations for Chemicals Applied to 10-Ounce Burlap and Tested with Rodents

Repellents Tested	R_{50}	Confidence Limits, 95%, Mg./Sq. Inch	Slope Function	R_{80}, Mg./Sq. Inch	R_{99}, Mg./Sq. Inch
		House Mice[a]			
β-Nitrostyrene	2.9	(2.4–3.5)	1.47	4.1	7.3
Tributyltin chloride	3.6	(2.5–5.2)	2.06	6.7	20.0
α-Cyano-β-phenylacrylonitrile	6.2	(4.4–8.7)	1.71	10	22
Cycloheximide	8.0	(5.2–12.3)	2.76	19	86
Trinitrobenzene-aniline	13.1	(10.9–15.7)	1.37	18.1	27.5
Compound SW-1	13.8	(8.9–21.5)	5.90	54	144
α-Trithiobis (*N*-diethylmethylthioformamide)	16.0	(8.9–27.2)	3.79	50	125
Tetrakis(laurylammino)boronium chloride	23.8	(17.4–32.6)	1.60	35.5	73
Trinitrotoluene	36.0	(30.5–42.5)	1.39	47	78
Triphenyltin chloride	44.0	(28.4–68.1)	2.35	96	129
Tetramethylthiuram disulfide	180.0	(101–320)	3.00	455	720
		Norway Rats[b]			
β-Nitrostyrene	1.25	(0.56–2.8)	3.76	3.9	12
Cycloheximide	1.45	(0.8–2.6)	3.14	3.7	15+
Tributyltin chloride	7.2	(4.5–11.5)	1.91	10.9	48
Trinitrobenzene-aniline	30.2	(25.3–36.5)	1.20	39.5	62.5
Tetrakis(laurylammino)boronium chloride	43.5	(36.9–51.4)	1.31	54	80
		White Mice Males			
β-Nitrostyrene	1.3	(0.9–1.9)	1.96	2.3	6.2
Tributyltin chloride	1.4	(0.8–2.3)	2.22	2.7	8.8
Trinitrobenzene-aniline	6.5	(4.7–9.0)	1.90	11.5	31.2
		Females			
Tributyltin chloride	1.4	(1.0–1.9)	1.78	2.2	5.2
β-Nitrostyrene	2.0	(1.5–2.9)	1.75	3.3	7.5
Trinitrobenzene-aniline	3.5	(1.8–6.9)	2.96	8.7	44.0
		Mixed Sex[c]			
Tributyltin chloride	2.3	(1.7–3.1)	1.35	3.4	7.0
β-Nitrostyrene	2.5	(1.5–4.3)	2.23	4.8	10.0
Tetrakis(laurylammino)boronium chloride	3.7	(2.0–6.6)	2.00	6.5	11.6

[a] Weight range = 15.5 to 28 gms. Mean weight = 21 gms.
[b] Weight range = 151 to 392 gms. Mean weight = 272 gms.
[c] Weight range = 25 to 41 gms. Mean weight = 29 gms.

Table II. Concentration-Repellent Effect Determinations for Tetrakis(laurylammino)boronium Chloride Applied to Burlap and Cotton and Tested with House Mice

Material	R_{50}	Confidence Limits, 95% Mg./Sq. Inch	R_{80}, Mg./Sq. Inch	R_{99}, Mg./Sq. Inch
10-Ounce burlap	23.8	(17.4–32.6)	35.5	73
4.25-Ounce/yard cotton	4.0	(2.9–5.4)	8.8	27

bags in an overnight exposure, the test animal must first qualify by penetrating untreated bags for two consecutive nights. Mice were held individually in 60-cage bioassay racks (LC-75, George O. Wahman, Baltimore, Md.). Bags were of 10-ounce burlap, 4 × 4 inches in size, and each contained one tablespoonful of a mixture of rolled oats and fox chow meal (Ralston Purina Co., St. Louis, Mo.), 1:1 by volume. The bag was deemed penetrated when the hole was sufficiently large for the test animal to obtain food.

Selection of Repellent Chemical Compounds. Several of the most active rodent repellents reported in the literature were among those selected for initial evaluation (*2, 6, 7*). Others tested were solicited from or selected by cooperating chemical companies on the basis of their containing known or likely repellent elements, radicals, or structural configurations (*7*).

Application of Chemical to Burlap Bags. Most candidate repellents were applied to burlap in organic or aqueous solvents. Application was accomplished by determining the amount of solvent that a given weight of burlap would absorb and then dissolving the desired amount of the candidate compound in a slight excess of this quantity of solvent to ensure saturation of the bag. Insoluble chemicals were applied in aqueous suspensions, passing the treated burlap through wringer rolls at a constant pressure to achieve desired concentrations of the candidate on the fabric.

Concentration-Repellent Effect Test. To determine the concentration of a chemical necessary to repel 50% of the test animals, an initial concentration was selected and applied to 10 bags. Ten milligrams per square inch was the concentration most frequently selected. Each of 10 house mice was then offered a single treated bag overnight. No other food was present during the test. Depending upon the results obtained, the concentration of the candidate was increased or decreased in a logarithmic sequence in subsequent trials with additional groups of 10 animals.

By utilizing an adaptation of the Litchfield and Wilcoxon (*3*) method for determining dose-effect measurements, three to seven concentrations were usually necessary to statistically determine the R_{50} and its confidence limits (95%). When concentration and repellent effects for several concentrations were plotted on two- or three-cycle, logarithm-probability paper, a regression line was formed, from which concentrations that would repel any given percentage of animals (R_1 to R_{99}) could be estimated (*5*).

In further trials, concentration-repellent effects determinations proved adaptable to other species of rodents and other packaging materials.

Results and Discussion

Data on the comparative effectiveness of several repellent chemicals when applied to packaging materials and tested against house mice, Norway rats (*Rattus norvegicus*), or white mice are presented in Table I.

In the early trials, β-nitrostyrene, tributyltin chloride, cycloheximide, and α-cyano-β-phenylacrylonitrile were the most effective repellents for commensal mice and rats. Other compounds were less effective, but several may possess developmental potential as packaging repellents because of favorable cost or low mammalian toxicity. Tetramethylthiuram disulfide, a broad-spectrum repellent for numerous species of mammals and birds and considered by many persons to have utility as a package protectant, was found to have only $^1/_{50}$th of the repellent activity of the better house mouse repellents at the R_{50} concentration.

The R_{50} of the cycloheximide on burlap was found to be 1.45 mg. per sq. inch for Norway rats and 8.0 for house mice. This correlates with the extreme variation in toxicity of this compound to these two species. Oral LD_{50} of cycloheximide for white rats is 1 mg. per kg. and 135 to 150 for white mice (*4*).

Three R_{50}'s were obtained for trinitrobenzene-aniline on three groups of house mice trapped in the wild at three locations within a 70-mile radius of Denver, Colo. The R_{50}'s for the three groups of mice were 12.7, 13.8, and 15.9 mg. per sq. inch, all falling within the allowable confidence limits. This greatly increases confidence in the method for predicting results with w ld rodent populations.

The results of tests with tetrakis(laurylammino)boronium chloride show that much less chemical was required to protect cotton than burlap (Table II). The R_{50} of tetrakis(laurylammino)boronium chloride for house mice was 23.8 mg. per sq. inch for 10-ounce burlap and only 4.0 mg. per sq. inch for $4^1/_4$ ounces per yard cotton. In other tests with house mice not herein reported, paper (multiwall bags) required less repellent per square inch than cotton, and polyethylene (1.5 to 6 mil) required more than burlap. This difference is attributed to the natural resistance of these materials.

There did not appear to be a great difference between reactions of male and female mice to repellents. Differences in ages of male, female, and mixed sexes may have accounted for the slight, but not significantly different, results obtained, in tests with white mice. It was hoped that white mice, which are easier to obtain and handle, could be used interchangeably with house mice, but white mice obtained from two different sources reacted differently. The limits of the R_{50} for tetrakis(laurylammino)boronium chloride varied from 2 to 6 mg. per sq. inch for mice from one source and 9 to 15 mg. per sq. inch for mice from another source.

Relationships of Effectiveness to Other Factors. Effectiveness is the first and most important consideration in the development of a chemical as a successful rodent repellent treatment for packaging, but other properties such as stability, use hazards, and cost must also be favorable. Thus, a compound that possesses only one-fifth or one-tenth the activity of the most effective rodent repellent may prove to be a more useful packaging protectant.

Results obtained show that the concentration-repellent effect technique can be used to measure reliably the comparative effectiveness of various repellents for protecting packaging materials against rodent damage.

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INSECTICIDE RESIDUES

Estimation of Insecticide Residues in Foods through Parallel Screening Methods

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A parallel screening system has been developed which provides a basis for detection, characterization, and estimation of most of the insecticides that inhibit cholinesterase or contain chlorine. The system consists of bioassay, organic chlorine, and acetylcholinesterase inhibition analyses of the same extract. Further information is obtained by mathematical treatment of the data which provides a high degree of selectivity. Data are presented for toxicants that are representative of the two major classes of insecticides.

THE DETERMINATION of insecticide residues in food products presents a complex challenge because it is necessary to show that no tolerance is exceeded. The problem is greatly magnified when complete spray history information is unavailable, which often is the situation confronting the food processor. The processor is also faced with the further complication that the analytical work on many products must be completed within a short time so that quality and factory operation are unaffected. To expect specific methods to be applicable on a routine basis is impractical mainly because of the large number of compounds and variety of materials to be analyzed.

The analytical system described herein provides a basis for detection, characterization, and estimation of most of the insecticides that inhibit cholinesterase or contain chlorine. The system consists of bioassy, organic chlorine, and acetylcholinesterase inhibition tests on each extract; hence the term parallel screening. In addition to the data from each determination, two additional information factors are obtained that aid the analyst in estimating the significance and identity of the residue present: the product of the LD_{50} value and parts per million organic chlorine, and the ratio of LD_{50} to $AChEI_{50}$. These two factors relate the in vivo system with the chemical determinations. The LD_{50} is the dose required to kill 50% of the fly population, and the $AChEI_{50}$ is the amount of toxicant required to decrease acetylcholinesterase enzyme activity 50%, hereafter designated as I_{50}.

Procedures

Sample Preparation. The stripping procedure varies with the particular crop and its mechanical-physical properties. Maceration with 2 ml. of benzene per gram of sample followed by either filtration or centrifugation is preferred when a suitable volume of extract is recovered. In the event of poor extract recovery, benzene–2-propanol (2:1) is used at the rate of 3 ml. per gram of sample. The 2-propanol is removed by water-washing