

**Table I. Recovery of Thiodan Added to Various Crops**

Sample	Extraction	Cleanup	Sample Size	µg./Sample		% Recovery
				Added	Found	
Sugar beet leaves	Total	None	179 <sup>a</sup>	5	4.7	94.0
			179	10	11.7	117.0
			716	10	9.6	96.0
Alfalfa	Total	Ether solution decolorized with Nuchar	100 <sup>b</sup>	200	180.0	90.6
			100	500	508.0	101.6
			200		458.5	91.7
			200		478.5	95.7
Strawberry	Surface	None	100 <sup>b</sup>	75	82.0	109.3
			100		70.5	94.0

<sup>a</sup> Square centimeters of leaf surface.

<sup>b</sup> Grams of sample.

50° C. with a gentle stream of air. For ether extracts, the volume was reduced to about 5 ml. with the aid of a three-ball Snyder column, transferred to a test tube, and carefully evaporated to dryness. Five milliliters of the pyridine solution and 2 ml. of 5*N* sodium hydroxide were added, the tube was stoppered, and the mixture heated in a 100° C. oil bath for 3 minutes with occasional swirling, cooled for 1 minute in an ice-water bath, filtered through a loosely packed cotton plug, and the absorbance determined with a spectrophotometer at 525 mµ. Samples of the crop being analyzed that had not been treated with Thiodan were also analyzed as a check or control.

**Recovery of Thiodan from Extracts**

Known amounts of purified Thiodan were added to check or control samples prior to extraction with *n*-hexane and the percentage recovery of the added Thiodan was determined. The results are presented in Table I. In an effort to approximate the hexane extracts of air, two samples (500 ml. of *n*-hexane) were fortified with 5 and 25 µg. of Thiodan,

respectively. These samples were concentrated, with the aid of a three-ball Snyder column, on a steam bath, transferred to test tubes, and analyzed in the manner described. Recoveries were 5.4 µg. (108%) and 24.8 µg. (99.2%), respectively.

**Interferences**

Other compounds may possibly interfere with this analytical method (1). One hundred micrograms of a number of insecticides were analyzed by this method to check for possible interferences. No interference was found for ovex, DDT, aldrin, Tedion (2,4,4',5-tetrachlorodiphenyl sulfone), dieldrin, endrin, methoxychlor, and Kepone [decachlorooctahydro - 1,3,4 - metheno-2*H* - cyclobuta(cd)pentalen - 2 - one]. Chlorobenzilate (ethyl 4,4' - dichlorobenzilate), heptachlor epoxide, and toxaphene gave interferences equivalent to 0.4, 0.7, and 4.0 µg. of Thiodan. Chlordane and heptachlor gave interferences equivalent to 11.0 and 25.0 µg. of Thiodan, and Kelthane [4,4' - dichloro - *alpha* - (trichloromethyl)-benzhydrol] gave an interference equivalent to 73.5 µg.

**PESTICIDE RESIDUES**

**Total Organic Chloride Content in Butterfat by a Rapid Method of Neutron Activation Analysis**

CHLORINATED insecticides are chiefly aryl organic molecules that have attached chlorine atoms. For example, the insecticide DDT is 50% chlorine by weight and only about 18% chlorine in atom per cent; the insecticide toxaphene is 68% chlorine by weight and about 30% chlorine in atom per cent. If these chlorinated organic compounds are present, even at low concentrations, in fodder and are ingested by dairy animals, some

of the chlorinated organics may be released in milk by these animals (7). Therefore, determinations of total organic chloride in butterfat yield the total chlorinated insecticide content of milk. Neutron activation analysis would be suitable for a quick screening test for possible insecticides in milk and dairy products such as cheese and ice cream.

At present, analytical procedures such as chromatographic (4) and colorimetric

**Discussion**

Ammonium hydroxide, aluminum hydroxide, and barium hydroxide, when substituted for the sodium hydroxide, gave no color even when the heating time was increased. Sodium hydroxide and potassium hydroxide from 0.5 to 3.0*N* produced the desired color, but a precipitate formed very quickly and this interfered with the reading of the absorbance. Sodium hydroxide gave a higher absorbance than potassium hydroxide. Sodium hydroxide above 5*N* gave colors in the orange range and a lower absorbance. Three minutes of heating at 100° C. with 5*N* sodium hydroxide gave the optimum absorbance.

The method requires no special apparatus or reagents, is rapid, and is adaptable to routine microdeterminations of Thiodan residues in or on food and forage crops.

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Received for review October 12, 1961. Accepted January 25, 1962. Division of Agricultural and Food Chemistry, 140th Meeting, ACS, Chicago, September 1961.

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(5, 6) techniques are used to ascertain the insecticide content of milk and dairy products. The information derived from these products is specific for quite a number of insecticides (3). Specific sensitivities, as low as 0.03 p.p.m. of DDT, are claimed for chromatographic techniques and about 1 p.p.m. for the total chlorine coulometric technique.

Neutron activation analysis of chlorine induces radioactivity in chlorine nuclei.

A rapid neutron activation method has been developed and applied successfully for the determination of the total organic chloride content in milk products. This neutron activation procedure consists of irradiating butterfat and a chlorine reference standard in a nuclear reactor for a short time and subsequently measuring and comparing the induced  $\text{Cl}^{38}$  radioactivity. From the total organic chloride content in milk products, an upper limit for the content of chlorinated pesticides in milk products may be estimated.

Both isotopes of chlorine,  $\text{Cl}^{35}$  and  $\text{Cl}^{37}$ , absorb thermal neutrons and become  $\text{Cl}^{36}$  and  $\text{Cl}^{38}$ , respectively. The former,  $\text{Cl}^{36}$ , has a half-life of  $3 \times 10^5$  years while  $\text{Cl}^{38}$  has a half-life of 37.3 minutes. Since the number of radioactive disintegrations in a chlorinated specimen is inversely proportional to the half-life of the species, the radioisotope  $\text{Cl}^{38}$  is pertinent for neutron activation analysis of chlorine. The long-lived radioisotope  $\text{Cl}^{36}$  is useful for tracer studies where the involved times are over many hours or days. Decay of 37.3-minute  $\text{Cl}^{38}$  is via emission of energetic beta particles and accompanying gamma rays of 2.16- and 1.61-m.e.v. energies. The resultant nucleus or atom from decay of  $\text{Cl}^{38}$  is  $\text{Ar}^{38}$ , a stable atom.

Radioactivity of  $\text{Cl}^{38}$  is usually recorded by a standard NaI (TI) scintillation spectrometer, which detects the highly energetic 2.16-m.e.v. and 1.61-m.e.v. gamma rays. Other radioactivities are easily discriminated against with a scintillation spectrometer equipped with a differential discriminator.

The flux in a TRIGA reactor operating at 250 kw. of power, at which these experiments were carried out, is  $1.8 \times 10^{12}$  neutrons per  $\text{cm}^2$  per second. When exposed to this flux for 15 minutes, the induced radioactivity of  $\text{Cl}^{38}$  is 0.03 microcurie per microgram of natural chlorine—i.e., about  $6 \times 10^4$  disintegrations of  $\text{Cl}^{38}$  per minute at the end of neutron irradiation. Since 500 disintegrations per minute may be easily detected by scintillation spectrometric techniques, sensitivities of 10 p.p.b. (parts per billion) of natural chlorine by weight may be achieved.

In addition to the induced radioactivity in chlorine atoms, neutron activation of butterfat also induces radioactivities in other elements that are present in fat. Radioactivities induced by neutron absorption in the main constituents, hydrogen, oxygen, and carbon are completely negligible. The other elements present in milk and butterfat, Na, K, Ca, Mg, Fe, S, and P, absorb thermal neutrons and become radioactive. However, their resultant radioactivities are immensely reduced by separation of the organic and inorganic constituents prior to neutron irradiation and also reduced sharply by discriminately detecting the prominent  $\text{Cl}^{38}$  gamma rays.

The total organic chloride content in butterfat is determined by comparing directly the induced  $\text{Cl}^{38}$  radioactivity in a butterfat specimen (inorganic chloride removed) and in a chlorine standard ( $\text{NH}_4\text{Cl}$  solution) that are exposed to the same thermal-neutron bath or flux. The neutron spectrum must be identical in the two samples; therefore, the hydrogen densities in the samples should be nearly identical to avoid unnecessary calibration of chlorine neutron captures as a function of hydrogen densities. The developed procedure, reported herein, has obviated such calibrations.

#### Experimental Procedure

Place about 5 to 10 ml. of butter in a conical centrifuge tube and add about 10 ml. of demineralized water. Place in a water container on a hot plate to melt the butterfat. Stir for about 20 seconds to cause precipitation and settling of the protein. Centrifuge.

Pipet 3.0 ml. of clear butterfat (the pipet should be placed near side of test tube and above protein phase) into 7 ml. of pure mixed hexanes (having less than 0.5  $\mu\text{g}$ . of Cl per 7 ml.) in a 60-ml., cylindrical separatory funnel (Ultramax-Teflon stopcock with solid penny-head stoppers). The stopcock should be tight to prevent solution loss on shaking.

Add about 10 ml. of demineralized water, and place a piece of masking tape over the stopper to hold it in place while being shaken. Place the funnel on the shaker and shake for 2 minutes (Burrell-Wrist-Action-Shaker Model BB). Carefully open the stopcock, and then drain the water. When the butterfat phase just touches the Teflon, close the stopcock and add a few milliliters of water. Again let out the water until the butterfat just touches the Teflon.

Add about 10 ml. of water and replace stopper and tape. Shake again for 2 minutes. Again remove the stopper and drain water until the butterfat just touches the Teflon.

Drain mixed hexane-butterfat into a 4-dram polyethylene vial. Heat-seal the vial. Prepare a chlorine standard (about 30  $\mu\text{g}$ . of Cl) in a 4-dram polyvial and bring up to volume of butterfat-hexane polyvial. Also pipet 7 ml. of the mixed hexane in a 4-dram polyvial for determination of the organic chloride background. To prevent organic chlo-

**Table I. Total Organic Chloride Content in Commercial Butter by Neutron Activation Analysis<sup>a</sup>**

Grams of Butter	No. of 10-ml. Water Washes	Total Organic Chloride, P.P.M.
2.23	4	1.0
2.33	4	1.1
2.45	6	0.9
2.30	6	1.1
2.27	6	0.8
2.16	6	0.9
4.2 (1.60) <sup>b</sup>	4	1.1
4.2 (1.94) <sup>b</sup>	4	1.6 <sup>c</sup>
4.2 (1.87) <sup>b</sup>	4	0.9
4.2 (1.82) <sup>b</sup>	4	1.1

Av.  $1.0 \pm 0.1$   
p.p.m.

<sup>a</sup> Butter samples were water-washed in an ultrasonic bath before irradiations.

<sup>b</sup> Water washes were done on pads of butter, about 4.2 grams each. Weights in parentheses are exact amounts of butterfat that were irradiated.

<sup>c</sup> This value was not included in the average because of possible chloride impurity in the polyvial.

ride uptake in the polyvial, transfer the mixed hexane-butterfat to the vial in less than 1 hour before irradiation. Irradiate for 15 minutes in a neutron flux of about  $10^{12}$  neutrons per  $\text{cm}^2$  per second.

After irradiation, transfer all samples to unirradiated polyvials. Heat-seal and obtain a gamma-ray spectrum with a multichannel gamma-ray spectrometer [3 × 3 inch NaI (TI) solid crystal]. Calculate the total chloride in the butterfat by comparing the 1.61- or the 2.16-m.e.v. peak heights. Three milliliters of butterfat weigh 2.46 grams.

#### Discussion and Results

One of the most difficult problems in procedural development involved the removal of all inorganic chlorine from the fat phase. Commercial butter contains about 13,000  $\mu\text{g}$ . of inorganic chlorine per gram of butter. The first attempt was directed toward removal of the inorganic chlorine by washing melted butter with water. Two water washings with hand stirring of the water and the melted butter reduced the chlorine content to about 8 p.p.m. Two additional 10-ml. water washes of a few grams of melted butter reduced the total chloride content to about 2 p.p.m. A

**Table II. Total Organic Chloride Content in DDT and Toxaphene (TPH) Spiked Commercial Butter by Neutron Activation Analysis<sup>a</sup>**

Grams of Butter	Added, P.P.M.	Calc. Total Organic Cl, $\mu\text{g.}$	Obs. Total Cl, $\mu\text{g.}$	Ratio of Obs. Cl/Calc. Cl
DDT				
4.59	0.78	6.4	6.3	0.9
4.13	0.86	5.9	6.1	1.03
4.23	0.99	6.3	9.7	1.54 <sup>b</sup>
Av. 1.01 $\pm$ 0.02				
4.15	37.6	82.1	76.5	0.93
4.65	30.4	75.4	79.1	1.05
4.56	31.3	76.2	75.1	0.99
Av. 0.99 $\pm$ 0.05				
TPH				
5.08	1.25	9.3	9.0	0.97
4.42	1.84	9.8	9.1	0.93
4.78	1.54	9.6	8.6	0.90
Av. 0.93 $\pm$ 0.03				
4.71	14.7	50.4	46.3	0.92
4.71	14.7	50.4	44.6	0.89
Av. 0.97 $\pm$ 0.03				
4.83	14.8	51.9	50.1	0.97

<sup>a</sup> Butter samples were washed four times with 10 ml. of water in ultrasonic bath before neutron irradiation.

<sup>b</sup> Because of chloride contamination, this value was not included in average.

**Table III. Total Chloride Content in Butter by Neutron Activation Analysis**

Butter A				Butter B			
Procedure I Hand Stirring of Melted Butter plus 10 ml. of Water		Procedure II Procedure I and Ultrasonic Energy		Procedure II Procedure I and Ultrasonic Energy		Procedure III Butterfat-heptane plus 10 ml. of Water Autoshaking	
Washes	Cl, p.p.m.	Washes	Cl, p.p.m.	Washes	Cl, p.p.m.	Washes	Cl, p.p.m.
0	13,000	0	13,000	...	...	2 1-min.	1.3 $\pm$ 0.1
2	8	2	8	...	...	2 3-min.	1.4 $\pm$ 0.1
4	2	4	1.0 $\pm$ 0.1	4	1.3 $\pm$ 0.1	2 5-min.	1.3 $\pm$ 0.1
6	1.5	5	1.0 $\pm$ 0.1	...	...	4 5-min.	1.3 $\pm$ 0.1
						6 5-min.	1.2 $\pm$ 0.1

few more water washings reduced the chloride content to about 1.5 p.p.m. Such a slight reduction in chloride content from 8 to 2 to 1.5 p.p.m. in the final washes was attributed to incomplete mixing of the fat and water phases. To effect better mixing and complete removal of inorganic chloride in a few water washes, ultrasonic agitation was used in conjunction with hand stirring. With ultrasonic and hand agitation, the total chloride in salted butter was reduced from an initial value of about 13,000 p.p.m. to 1.0 p.p.m. in four to six washings.

The total organic chloride content (shown in Table I) was determined in butter as a function of butter mass and number of water washes with an ultrasonic device. The data clearly indicated that four water washes were sufficient for total inorganic chloride removal and that at least 4 grams of butterfat were easily processed by this procedure. Also, the reproducibility in the total organic chloride of 1.0  $\pm$  0.1 p.p.m. in this particular bath of butter was quite consistent. For this butter, the total organic chloride content calculated as DDT was 2.0 p.p.m. (51% Cl).

Since all organic compounds have a finite (though small) solubility in water,

it was imperative to determine under the present experimental conditions the partition coefficient of two representative insecticides, DDT and toxaphene (TPH), in water and the fat phase. Experimental conditions and results are listed in Table II. The quantities of added DDT varied from 0.8 to 38 p.p.m. (i.e., 0.4 to 19 p.p.m. of added organic chloride), while concentrations of added toxaphene varied between 1.3 and 15 p.p.m. (0.9 and 10 p.p.m. of organic chloride, respectively). The partition coefficients in fat to water phases calculated from the data listed in the last column are found to be greater than 20 to 1 and greater than 13 to 1, respectively. On the average, less than 1% DDT and about 2% TPH are lost in every water wash.

An alternative and simpler method was developed for the extraction of all inorganic chloride from butterfat. After the protein was removed from the butter by washing it once with water, butterfat (3.0 ml.) was dissolved in about 7 ml. of pure heptane (or pure mixed hexane). The background organic chloride content in pure heptane or pure mixed hexane was determined by neutron activation analysis and found to be about 50 to 80 p.p.b. All inorganic

**Table IV. Total Organic Chloride Content in Organic Chloride Spiked Butterfat by Neutron Activation Analysis<sup>a</sup>**

Total Organic Chloride Added ( $\mu\text{g. Cl/Ml.}$ )	Total Organic Chloride Found ( $\mu\text{g. Cl/Ml.}$ )
1.5 as Toxaphene	1.8 $\pm$ 0.2 <sup>b</sup>
3.0 as Toxaphene	3.3 $\pm$ 0.4 <sup>b</sup>
6.1 as Toxaphene	5.2 $\pm$ 0.9 <sup>b</sup>
6.7 as Toxaphene	7.1 $\pm$ 1.2 <sup>c</sup>
4.6 as DDT	5.1 $\pm$ 0.6 <sup>c</sup>

<sup>a</sup> Via two 2-minute cycles of autoshaking of 2.46 grams of butterfat, 7 ml. of mixed hexanes, and water phases. Duplicate analysis of each sample.

<sup>b</sup> Total organic chloride background in the unsalted butter of 1.4  $\pm$  0.1  $\mu\text{g. Cl}$  per ml. and of 0.6  $\mu\text{g. Cl}$  per ml. of mixed hexanes was subtracted to obtain the net organic chloride values.

<sup>c</sup> Total organic chloride background in the butterfat of 1.0  $\pm$  0.1  $\mu\text{g. Cl}$  per ml. and of 0.5  $\mu\text{g. Cl}$  per ml. mixed hexanes was subtracted to obtain net organic chloride values.

chloride was extracted into 10 ml. of demineralized water by vigorously shaking the butterfat-heptane-water mixture with an automatic shaker at about 300 shakes per minute. Two 2-minute shaking periods were required for total removal of all inorganic chloride (Table III). The total organic chloride determined by neutron activation of the butterfat-heptane-water autoshake procedure was identical to that determined by the butterfat-ultrasonic-water procedure on the same butterfat. Six 5-minute autoshaking periods removed the same quantity of inorganic chloride as two 1-minute autoshaking periods. It was concluded, therefore, that all the inorganic chloride was removed from the butterfat-heptane phase by two 2-minute autoshaking periods. Because of the simplicity of the butterfat-heptane (or hexane)-water autoshake procedure, the authors have adopted that procedure for routine inorganic chloride removal.

Additional experiments clearly demonstrated that the total organic chloride may be determined using either heptane or mixed hexanes as the organic phase in the butterfat-autoshaking method. A butter sample was dissolved in hexane and washed with water by autoshaking. The amount of organic chloride found was 0.81  $\pm$  0.06  $\mu\text{g.}$  per ml. of butterfat. When heptane was used as solvent, the result was 0.83  $\pm$  0.03  $\mu\text{g.}$  per ml. of butterfat.

To demonstrate that this simple and rapid procedure of butterfat-mixed hexane-water autoshake followed by neutron activation analysis determined the total organic chloride present in the butterfat, known amounts of butterfat were spiked with definite quantities of DDT and TPH. The agreement within experimental error between the observed

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.

#### Acknowledgment

The authors acknowledge the assistance of J. D. Buchanan, J. C. Migliore, J. E. Lasch, and D. A. Olehy in analysis of the butter and butterfat samples and E. L. Pye of the University of California

at Davis for spiking the unsalted butter samples.

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Received for review November 24, 1961. Accepted February 21, 1962. Division of Agricultural and Food Chemistry, 140th Meeting, ACS, Chicago, September 1961.

## 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.  $\beta$ -Nitrostyrene, tributyltin chloride, cycloheximide, and  $\alpha$ -cyano- $\beta$ -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 Triehler (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