the weights of the cats used for the assay should be as nearly as possible the same for both the preparation being assayed and the reference standard.

6. None of the methods provided evidence of loss of potency in the tinctures during a period of approximately a year.

7. The U. S. P. X process of extraction of the drug fails to produce tinctures meeting the official potency requirements. To achieve the required potency, a quantity of drug in excess of that specified by the U. S. P. X must be used.

8. It has not been possible to obtain specimens of strophanthus which would meet U. S. P. X potency requirements no matter how completely the drug was extracted. The fault therefore lies, not in the specified procedure of extraction, but in the fact that the required amount of potency is not contained in available lots of the drug. It is concluded, therefore, that the official potency requirements for the drug and the tincture should be reduced to 50 or 60 per cent of U. S. P. X requirements.

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RED SQUILL IV. BIOASSAY METHODS.*[1]

BY JAMES C. MUNCH, JUSTUS C. WARD, ERNEST M. MILLS, ROBERT E. BUCK AND FRANK N. JARVIS.

In connection with our studies on red squill powders and preparations great variations in potency have been observed. The methods of chemical assay have not furnished adequate indications of physiological potency, so bioassays are required. After studying the pharmacodynamic action on various animals, a standard technique was developed using the white rat (1, 2, 3). In this series of toxicity determinations every effort was made to conform rigidly to the following specifications: Normal male rats, not previously used for any other test and weighing between 100 and 200 Gm., were starved for eighteen hours, after having been fed on a stock diet for at least one week. The squill powder tested was weighed and mixed with the stock diet, then the quantity for each rat was weighed and placed in an individual dish, which was offered to the animal. The bait was consumed within fifteen minutes; squill symptoms developed before death, which occurred within five days, approximately.

The detailed results with five lots of commercial red squill powder furnished by John L. Hopkins, International Sales Corporation, S. B. Penick and K-R-O Company are recorded in Table I. Equal weights of each of these five powders were thoroughly mixed, in the preparation of "BSS-1." The results obtained with the five squill powders represent assays made in October and November 1935; the tests on BSS-1 were made in March and April 1936.

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[1] Control Methods Research Laboratory, Bureau of Biological Survey, Denver, Colorado.

TABLE I.—TOXICITY OF RED SQUILL POWDERS TO MALE RATS.

Product.	Dose, Mg./Kg.	Lab. A.	Lab. G.	Lab. D.
Sq. 1	250	11/12		
	400	8/8	1/5	9/10
	500		3/10	
	600		8/10	
Sq. 2	250	10/12		
	400	8/8	1/5	6/9
	500		0/10	
	600		6/10	
Sq. 3	250	8/12		
	400	8/8		9/10
	500		1/10	
	600		6/10	
Sq. 4	250	7/11		
	400	6/8	0/5	6/9
	500		0/10	
	600		7/13	
Sq. 5	250	1/7		
	400	6/8	0/5	3/9
	500		1/10	
	600		2/13	
	700		7/10	
BSS-1	100	0/8	0/9	0/10
	200	2/10	4/10	0/10
	300	5/10	5/10	2/10
	400	7/10	5/10	5/10
	500	8/8	7/10	5/10
	600		7/10	8/10
	750		7/7	10/10
Est. $LD_{50\%}$:mg./Kg.		300	350	450

Statistical studies of these results indicate that the susceptibility of these rats to these red squill powders follows the "standard curve" (4). While such a relationship was anticipated, no previous studies had determined the degree of variability in assays conducted in the same laboratory, or in different laboratories studying the same samples. It is believed that the differences in potency between the estimated value from mixing the five squill powders and the values observed in feeding such a mixture are due to this animal variability.

In practical rodent control it is desired to kill 100 per cent of the animals in an infested area. For statistical reasons, however, the $LD_{50\%}$ has been calculated to permit more precise evaluation of results in the three laboratories listed. The estimated $LD_{50\%}$ for laboratory A is 300 mg./Kg.; for laboratory G, 350 mg./Kg. and for laboratory D, 450 mg./Kg., when BSS-1 is considered. To prevent improper interpretations of experimental findings in feeding tests upon rats it appears necessary to adopt a specific red squill preparation as a reference standard for toxicity determinations, to determine the "characteristic curve" for each laboratory, and to feed the standard red squill at the same time that any unknown squill powder, preparation or extract is bioassayed. By this method dependable results may be obtained. This embodies a change in the psychology of these bioassays: It is necessary to consider a specific red squill *product* and not the *white rat* as the nonvariant factor.

CONCLUSION.

1. The susceptibility of white rats to red squill powder follows the "standard curve."

2. By using a reference standard red squill powder and evaluating the potency of red squill preparations in terms of such a standard more precise results will be obtained than if attempts are made to determine potency in terms of dosage of body weight of white rats.

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STRYCHNINE VII. THE TOXICITY OF NUX VOMICA PREPARATIONS.*

JAMES C. MUNCH,[1] JUSTUS C. WARD[2] AND F. E. GARLOUGH.[3]

In other papers of this series the variation in physiological activity of strychnine alkaloids obtained from the same or different manufacturers during the last four years has been reported (1, 2). Simultaneous determinations of total alkaloidal content of the tincture, fluidextract and powdered extracts of nux vomica by the U.S.P.X method and the toxicity of these samples in form of tinctures were undertaken. In order to determine whether the same variations might be found in these galenicals as in the strychnine alkaloids studied, the same methods of bioassay were used.

The detailed results obtained in tests on nine tinctures and three fluidextracts of nux vomica commercially manufactured between 1929 and 1935 are given in Table I. For the taste tests the method previously used (3, 4, 5) was employed. In the determinations of toxicity, the $LD_{100\%}$ was determined on at least five, and in many instances up to twenty, mice, guinea pigs or rabbits. Since the susceptibility of these animals followed the "standard curve," the final conclusions are based on at least ten animals at the killing dose. The tinctures and fluidextracts were diluted with water to reduce the alcohol content below 5%: In some tests, a portion of the alcohol was evaporated off on a water-bath, but this did not affect the toxicity. Injections were made subcutaneously into mice and guinea pigs, and intravenously to rabbits. The mice weighed approximately 20 Gm., the guinea pigs 250 Gm. and the rabbits 2 Kg.

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* Scientific Section, A. PH. A., Dallas meeting, 1936.

[1] Senior Pharmacologist, Control Methods Research Laboratory.

[2] Associate Pharmacologist, Control Methods Research Laboratory.

[3] Biologist, Control Methods Research Laboratory.

U. S. Biological Survey, Division of Game Management.