

AN ASSAY OF SEVIN* FORMULATIONS USING MICRO STEAM DISTILLATION AND ITS APPLICATION TO STUDY OF INSECTICIDE DISTRIBUTION IN GRANULES

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In a sample of granules, the content of Sevin (1-naphthyl-*N*-methyl carbamate) as shown by analysis was less than stated and the granules were of noticeably different sizes. It was suspected not that the toxicant content was low but that Sevin was distributed unevenly between the several sizes of granules, rendering it difficult to obtain a representative sample. To check this supposition more analyses were needed than could reasonably be made by the standard methods of Sevin analysis which are time consuming. Assay of Sevin depends upon the hydrolysis of the insecticide followed by the estimation of one or other of the two main hydrolysis products, i.e. 1-naphthol and methylamine. Assay by estimation of 1-naphthol by the colorimetric method of Miskus *et al.*¹ depends upon the formation of a diazonium dye-stuff. It is not only time consuming but has the added disadvantage that the diazonium colour reagent is unstable and is not readily available. The colour absorbance has to be read at a definite time after its formation, thereby adding complications to its use. Assay of Sevin by alkaline hydrolysis followed by distillation and titration of methylamine as described by Whitehurst and Johnson² is only suitable for 50% formulations, and it is also necessary to extract and concentrate the toxicant using Soxhlet extractors for lesser concentrations, thus adding to the time and equipment required for each assay. In this method the apparatus for distillation of methylamine requires large amounts of material. It is slow and it seemed likely, that a small scale distillation apparatus such as used for distillation of ammonia in a micro-Kjeldahl method, might both speed the method and make it applicable to smaller quantities. One form of microdistillation was available and its use for the assay of Sevin was investigated and then applied to the examination of the distribution of the insecticide in different sizes of granules in a granular formulation. An attempt was also made to reach a reasonable estimate of the mean Sevin content of a large bulk of granules.

*Carbaryl (1-naphthyl-*N*-methyl carbamate)

†From Rothamstead Experimental Station, Harpenden, U.K. on secondment to the Department of Plant Protection, Karachi under U.K. Colombo Plan arrangements from September 1967-68.

Experimental

Assay of Sevin

The method is based on the steam distillation of the methylamine formed by the alkaline hydrolysis of Sevin. The methylamine is absorbed in boric acid and titrated with 0.01N hydrochloric acid using a mixed indicator (methyl red-bromocresol green).

Reagent

Boric Acid-Mixed Indicator Solution.—For absorption of methylamine, 20 ml 0.1% ethanolic bromocresol green was mixed with 4 ml methyl red and added to 500 ml 2% boric acid solution in water.

Apparatus

Microdistillation assembly—Kjeldahl type—used in these tests was similar to the Quickfit-Quartz Assembly Cat. No. 21/100 MC consisting essentially of a 100-ml kjeldahl flask, a distillation head with steam inlet and a Leibig condenser (Fig. 1). Details are given in the diagram, although similar equipment of a different design could be used if tests were made to ensure complete distillation of the amine and that no alkali is splashed over.

Procedure

Approximately 4 mg Sevin was introduced into the distillation apparatus. Two methods were used depending upon the physical state of the sample. Solid samples were weighed directly into the distillation kjeldahl flask which was then fixed to the head of the apparatus. Liquid samples were pipetted into the flask through the ground joint in the head, used later in the procedure to introduce steam. It was then rinsed with about 1 ml water. Subsequent operations were the same whichever the form of Sevin sample, and were as follows. Sodium hydroxide 40% (2.5 ml) was added through the joint in the head (as for liquid samples) followed by a rinse with about 1 ml water. Steam was then passed through

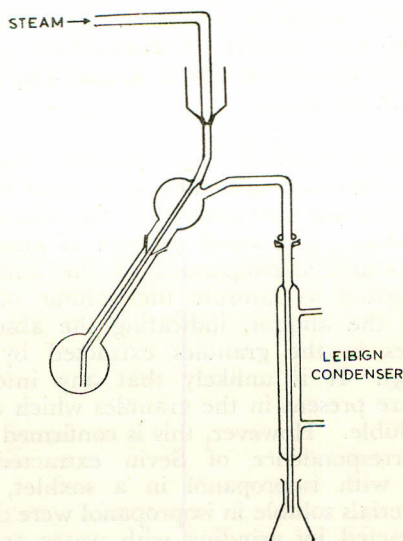


Fig. 1.—Diagram of distillation apparatus. (Not to scale).

the apparatus and 10 ml of the distillate collected in a 25-ml conical flask containing 2 ml boric acid indicator mixture. The contents of the flasks were titrated using 0.01N hydrochloric acid from a 5-ml burette. No more amine was found when a second 10 ml distillate was collected, indicating complete recovery.

Blank determinations were made by using the same procedure but no Sevin was added to the apparatus.

Calculation of Results

On hydrolysis of sevin one molecule of methylamine is liberated from each molecule of Sevin thus the acid used for titration is equivalent to the Sevin hydrolysed. Hence if x ml of 0.01N HCl are used for the titration then this is equivalent to $\frac{x}{1000} \times \frac{1}{100}$ moles of sevin. or $201 \times \frac{x}{1000} \times \frac{1}{100}$ g of Sevin (mol wt 201) = $2.01 \times \text{mg Sevin}$.

Standardization of Method with Pure Sevin

A sample of analytical grade Sevin* (99.9% purity) was used to standardize the method by preparing solutions containing about 0.2 g Sevin, accurately weighed, in 100 ml isopropanol 2 ml portions of the solution were assayed by alkaline hydrolysis and titration of the methylamine separated by steam distillation, using the standard

*Supplied by Messrs Union Carbide of U.S.A.

TABLE I.—CHECK ON ASSAY USING PURE SEVIN SOLUTION IN ISOPROPANOL

Sevin test solution conc mg/ml	Vol. test solution for assay/ml	Titre* 0.01208N Hcl	Sevin found as % used
2.33	2.00	1.93	100.50
2.33	2.00	1.93	100.50
2.33	2.00	1.93	100.50
2.13	2.00	1.75	100.00
2.13	2.00	1.78	100.05
2.13	2.00	1.75	100.00
2.00	2.00	1.63	99.80
2.00	2.00	1.63	99.80
2.00	2.00	1.63	99.80

*Titre corrected for distillation blank 0.07 ml.

method for solutions. The results (Table I) show a reproducible and almost complete hydrolysis of Sevin and distillation of hydroxylamine, indicating that accurate measurement of Sevin is possible.

Preparation of Granules for Test

Small samples of granules containing about 4 mg Sevin were weighed directly into the distillation apparatus.

Large samples of granules (approximately 1.0 g) were ground in a macerator (MSE top drive) with a mixture of 1 ml water and 9 ml isopropanol for about 6 min. The mixture was transferred to a test tube and the homogenizer washed out with 10 ml isopropanol. The mixture was shaken and allowed to settle. An aliquot (2 ml) containing 4 mg Sevin was used for assay.

Larger samples (10 g) of granules were exhaustively extracted with isopropanol as solvent (as in the standard assay procedure of Whitehurst and Johnson²) using a soxhlet extractor. The extracts were made up to volume with solvent and 2 ml aliquot containing about 4 mg Sevin were measured into the distillation apparatus.

The results of assay of Sevin contents (see Table 2) on the several sizes of samples, indicate that the maceration with propanol-water extracts Sevin from granules rapidly and completely. The greater variation between results of assay using the smaller samples indicate that Sevin is not uniformly distributed in the granules.

Check on Possibility of Materials Present in Filler Interfering with Assay Procedure

Because the assay is based on acid titration of steam volatile amines, ammonium salts present

TABLE 2.—SEVIN ASSAYED IN A SERIES OF SAMPLES OF DIFFERING WEIGHTS TAKEN FROM A BATCH OF GRANULES OF UNIFORM SIZE (500–1000 MICRONS).

Sample wt g	Titre ml 0.01208N HCl	% Sevin	Mean
(A) 10-g sample extracted in a soxhlet with isopropanol and volume made up to 250 ml.			
10.114	1.80	3.90	3.57 ± .28
10.314	1.60	3.30	
10.066	1.57	3.20	
10.405	1.85	3.94	
10.043	1.78	3.86	
10.001	1.79	3.90	
10.120	1.60	3.30	
10.112	1.88	3.45	
10.000	1.65	3.50	
10.004	1.61	3.35	

*Not corrected for distillation blank 0.05 ml 0.01208N HCl.

(B) 1-g sample ground with 20 ml propanol water (18:2)†			
1.165	2.025	3.14	3.57 ± .28
1.141	2.07	3.34	
1.049	2.225	3.99	
1.253	2.34	3.57	
0.998	1.82	3.20	
1.060	2.01	3.46	
1.033	2.13	3.84	
1.018	2.03	3.66	
1.212	2.27	3.55	
1.114	2.30	3.92	

†Not corrected for distillation blank 0.50 ml 0.01208N HCl.

(C) 0.1-g samples placed directly in the distillation apparatus.‡			
0.062	1.75	4.90	3.78 ± 1.94
0.105	2.25	4.05	
0.118	2.60	4.32	
0.101	1.70	2.89	
0.103	1.66	2.73	
0.109	1.85	3.01	
0.110	1.97	3.25	
0.068	1.80	4.65	
0.106	1.81	3.05	
0.059	1.70	4.94	

‡Not corrected for distillation blank 0.50 ml. 0.01208N HCl.

in the filler would be assayed as Sevin and give spuriously high results. Unfortunately no filler for the granules was available so that only indirect tests could be made.

To check whether the granules contained soluble ammonium salts they were extracted with water which was then assayed by the microdistillation method. The small amount of amine was distilled which corresponded to the amount of Sevin needed to saturate the volume of water taken in the aliquot, indicating the absence of substances in the granules extracted by water (Table 3). It is unlikely that any interfering amines are present in the granules which are not water soluble. However, this is confirmed by the close correspondence of Sevin extracted from granules with isopropanol in a soxhlet, where only materials soluble in isopropanol were distilled and extracted by grinding with water and propanol and the amount of Sevin found in entire granules (Table 2 a and c).

Sevin Contents of Different Sizes of Granules

When the rapid microdistillation method for Sevin assay had been shown to be satisfactory it was used to investigate Sevin content of granules in a 56-lb bag of material in which the sizes of granules was obviously variable.

No facilities were available for either taking a representative sample from the sack or for sieving all of the contents and it was necessary to devise a method for taking samples, with facilities and materials available. A series of small shutes were made which successfully divided material into two parts until 32 fractions were obtained. The device is represented diagrammatically in Fig. 2. In this way the bag of sevin granules was divided into 32

TABLE 3.—CHECK ON ABSENCE OF INTERFERING SUBSTANCES FROM GRANULES FILLERS BY COMPARISON OF SOLUBILITY OF SEVIN IN WATER.

Standard solutions of Sevin in water were prepared from pure sevin and Sevin granules.

Vol test soln	Titre* ml 0.0100N HCl	
	Sevin granules	Pure Sevin
2.00	00.32	00.33
2.00	00.32	00.32
2.00	00.30	00.31
2.00	00.41	00.30
2.00	00.32	00.30

*Titre not corrected for blank 0.15 ml.

batches. To test the uniformity of the 32 batches five were selected at random and sieved to give four different size groups, viz. particles larger than 1000 microns, between 1000 and 500 or 500 and 295 microns and less than 295 microns. The proportions of granules larger than 1000 and from 500 to 1000 microns in the five batches differed, indicating that the batches were not truly representative of whole bag. However, the proportions of granule greater than 500 microns were similar in all the five batches and accounted for over 95% of the sample. The test was therefore continued to examine Sevin content of granules of different sizes, and preliminary results showed that Sevin content of the larger sizes did not differ greatly although the particles less than 295 microns were much richer in Sevin. The test was therefore, continued and samples from each of the four fractions from the five sieved samples were assayed by grinding 1-g portions with water and propanol, (total volume 20 ml) and steam distilling 2 ml aliquots with sodium hydroxide (see methods). Three 1-g portions were assayed for each fraction. There was little variation between replicate assays, but considerable variations between the fractions of different sizes in each sample. The differences were constant from sample to sample thus the smallest particles (less than 295 microns) all contain a concentration (13-15%) of Sevin two to three times greater than the larger particles (4-7% Sevin) of the other sizes of particles, those between 500 and 1000 microns consistently contained less Sevin (4-5%) than the larger (greater than 1000 microns) and smaller (between 295 and 500 microns) particles which contained about equal amounts (5-6%) of Sevin.

Discussion

Substitution of a microdistillation assembly and a titration technique used for ammonia in microkjeldahl methods for nitrogen assay for macrodistillation and titration as used in the standard Sevin assay described by Whitehurst and Johnson² greatly shortens the time needed for the assay and the distillation can be completed in 6 instead of 90 min. Test with analytical grade of samples of Sevin showed that the method is accurate and reproducible (Table 1). The method has the advantage that it requires as little as 4 mg Sevin for distillation and titration. There do not appear to be any substances in the granular formulation which interferes with the method so that it may be applied to small groups of granules directly, with the 5% formulation tested 80 mg granules, about ten individual granules, sufficed.

The sieving tests on batches taken from a 56-lb sack showed the difficulty of obtaining truly re-

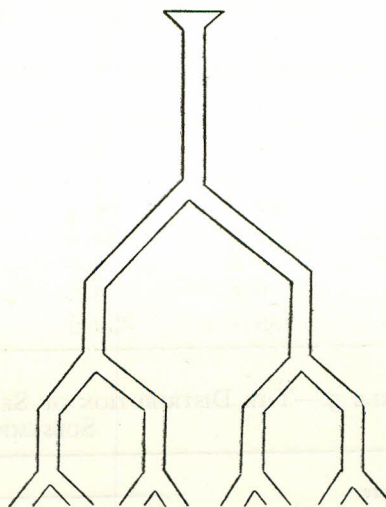


Fig. 2.—Diagram of the shutes used in subsampling.

presentative samples from such a bulk of material (Table 4). Analysis of the fractions obtained further emphasized the difficulty because although replicate determinations on fractions from individual batches are closely similar the results vary from batch to batch (Table 5). Also the results indicate a systematic variation in Sevin contents of the four different particle size fractions which is larger than experimental variations. In all the five batches tested the 500-100 micron fraction contained the least and the smallest particle size (less than 295 micron) fraction contained the highest concentration of Sevin.

In all the five batches tested the two largest fraction (over 1000 microns and 1000 to 500 microns) made up over 95% of the bulk of the material, and the concentration of Sevin in the fraction of larger particles was consistently 25 to 30% greater than in the fractions of smaller particles. This difference in Sevin content of the particle sizes which make up the greater part of the formulation is unlikely to have any serious effect on the use of the material, but it does pose a sampling problem since even in the tests on the 5 batches examined the proportion of the two sizes range from 2:2 to 1:2 (Table 4).

The fine particles (less than 295 microns) obviously pose sampling and analytical problems since they contain up to 15% insecticide, three times the stated concentration so that a sample containing too large a proportion of these would give spuriously high values for insecticide content. In addition these fine particles may result in practical difficulties, especially with aerial application methods, despite their forming only a small proportion 1-2% of the bulk of the formulation.

TABLE 4.—THE RELATIVE AMOUNTS OF PARTICLES OF DIFFERENT SIZES IN FIVE SUBSAMPLES OF SEVIN GRANULES.

Sieve size	Subsamples					Mean
	S ₁	S ₂	S ₃	S ₄	S ₅	
1000	65.0	66.4	36.8	34.1	33.0	47.1 ± 15.3
500-1000	31.8	30.8	61.5	63.4	65.7	50.6 ± 15.9
295-500	0.8	0.8	1.1	0.6	0.4	0.74 ± 0.2
295	0.8	0.8	1.5	1.6	0.8	1.1 ± 0.4
Total g	239.0	239.9	767.4	1036.3	1732.0	

TABLE 5.—THE DISTRIBUTION OF SEVIN BETWEEN PARTICLES OF DIFFERENT SIZES IN FIVE SUBSAMPLES OF SEVIN GRANULES.

Sieve size	Subsamples					Mean
	S ₁	S ₂	S ₃	S ₄	S ₅	
1000	5.70	5.75	6.86	6.69	6.49	6.06 ± 0.43
	5.60	5.65	6.63	5.96	6.17	
	5.40	5.50	6.70	5.67	6.18	
	Mean	5.56 ± 0.12	5.63 ± 0.10	6.73 ± 0.09	6.11 ± 0.43	
500-1000	4.30	4.25	4.85	5.70	5.23	4.74 ± 0.36
	4.30	4.00	5.16	4.38	4.88	
	4.40	4.60	4.86	5.29	4.86	
	Mean	4.33 ± 0.05	4.28 ± 0.25	4.96 ± 0.14	5.12 ± 0.55	
295-500	6.00	6.05	6.19	6.25	5.81	5.94 ± 0.42
	5.80	6.00	6.87	5.38	5.30	
	6.20	5.90	6.84	5.33	5.11	
	Mean	6.00 ± 0.16	5.98* ± 0.06	6.64 ± 0.31	5.62 ± 0.4	
295	13.00	12.90	14.65	15.80	15.62	14.17 ± 1.13
	13.12	12.50	15.00	14.40	15.18	
	13.50	12.00	14.85	14.88	15.30	
	Mean	13.21 ± 0.21	12.47 ± 0.37	14.83 ± 0.14	15.03 ± 0.58	

Having a relatively small particle size this fraction will be liable to drift and the high Sevin content (about 15%) may cause damage to sensitive plants.

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