Table I. Recovery of 0.1 P.P.M. EPTC from Potatoes

Code	Check Value, γ	Recovery, % (Corrected for Check)
A	0.3	100.0
B	0.7	96.0
C	0.8	93.5
D	0.3	94.0
E	0.2	99.0
F	0.7	99.0

Extraction Procedure

Thoroughly macerate about a dozen potatoes in their own liquid in a 1-gallon blender. Weigh a 200-gram portion of the blended tissue, transfer to a 1-quart blender, and blend with 200 ml. of isopropyl alcohol (reagent grade) for 30 seconds. Add 300 ml. of Skellysolve B (technical) to the mixture and blend for 2 minutes. Pour the contents into a large, coarse porosity, sintered-glass funnel, and filter by suction. Use 25 ml. of hexane (used hereafter to designate technical Skellysolve B) to rinse the blender and funnel. Then transfer the contents of the suction flask to a 500-ml. separatory funnel and allow the layers to separate. Drain the aqueous isopropyl alcohol (lower) layer into a beaker and the upper hexane layer into a 500-ml. Erlenmeyer flask with a 24/40 standard-taper joint. Re-extract the isopropyl alcohol–water mixture twice with 50-ml. portions of hexane, using the latter to rinse the suction flask and beaker each time. Combine the hexane extracts, and distill off the solvent as in step 2 of the hexane extraction method (*1*). Then use the subsequent steps of the method (*1*) for cleanup and determination of the herbicide.

Results and Discussion

Recovery studies with potatoes were made using the isopropyl alcohol–hexane extraction procedure. Twenty micrograms (0.1 p.p.m.) of EPTC in hexane was added to each of six 200-gram portions of blended potato tissue. The herbicide was then blended in, 200 ml. of isopropyl alcohol was added, and the procedure was completed as described. Check samples were also carried through the procedure. Table I shows the recoveries and check values obtained.

The average of the six recoveries was 96.9%. The average of the six check values was 0.5 γ of EPTC.

Potato plants, 8 to 10 inches tall, were treated with EPTC in 1958 for weed control. Using a randomized block design, a comparison was made between two granular formulations, clay and vermiculite, and an emulsifiable concentrate. One application of each formulation was made on July 23 using a hand sprayer. The granular clay formulation was applied at a rate of 3 pounds per acre. The granular vermiculite and emulsion formulations were applied at both 3 and 4.5 pounds per acre. The potato plants were also treated with DDT, parathion, Maneb, and sodium arsenite. The tubers were harvested on October 11. Analyses of the tubers for EPTC from each of the treatments using the method described showed no residue.

Acknowledgment

This experiment was done in cooperation with R. D. Sweet, Vegetable Crops Department, New York State College of Agriculture, Ithaca, N. Y.

Literature Cited

(1) Batchelder, G. H., Patchett, G. G., J. Agr. Food Chem. **8**, 214 (1960).

Received for review June 25, 1959. Accepted January 8, 1960.

SEED DISINFECTION

Fungicide and Dye Distribution in Liquid Seed Treatment

OLLE LINDSTRÖM

AB Casco, Stockholm 11, Sweden, and Research Department, Panogen Co., Woodstock, Ill.

The mercurial distribution produced in liquid seed treatment (Panogen process) was determined by a new chemical method which permits mercury assay of individual kernels of seed. Distribution statistics based on whole kernel assay differs but slightly from the statistics with the radioactive method, where only part of the kernel surface was examined. Because of vapor action, here illustrated by simultaneous treatment with nonvolatile tracer and labeled mercurial, a reasonably uniform mercurial distribution is obtained in laboratory and commercial treatment, irrespective of treatment conditions and liquid dosage. More or less uniformly colored treated seed is of no value in judging the mercurial distribution.

The mechanism of liquid seed treatment (Panogen process) was recently studied by radioactive tracer methods with particular attention to distribution (*3*). This method accounts only for the amount of fungicide present on that part of the kernel facing the window of the Geiger tube, roughly one sixth of the total kernel surface. Since then methods have been developed for the assay of the total mercury content of single kernels of treated seed. Gamma counting is performed at near 4 π geometry by scintillation techniques (*2*). Irradiation of treated seed in a nuclear reactor produces by neutron activation the radioactive isotope mercury-197, which is determined by radioactive techniques (*5*). Finally, a new chemical method with spectrophotometric mercury determination has been developed for the mercury assay of single kernels of treated seed (*2*). This method, called flame analysis, is used in the present investigation.

The effects of important variables in seed treatment were studied, particularly the liquid dosage and treatment conditions. Commercially treated seeds were included. The relationships between distribution statistics based on beta counting and whole kernel assay are illuminated. Regression analysis is used to get the relation between fungicide uptake and kernel size. The importance of vapor action is demonstrated in an experiment involving simultaneous treatment with a nonvolatile tracer and the labeled mercurial fungi-

cide. Estimation of the dye distribution on the treated seed is frequently (and erroneously) used in the field to judge the effectiveness of the seed treatment procedure. This was illustrated by means of an objective method for evaluation of the distribution of the dye.

Materials and Methods

Seed Treatment Materials. Radioactive distribution studies were performed with radioactive Swedish Panogen 4925, containing 0.8% weight by volume of mercury as cyano(methylmercuri)guanidine labeled with mercury-203 (*3*). In a demonstration of vapor action 1.5% thallium nitrate tagged with thallium-204 was added to this Panogen formulation then containing two radioisotopes. Experimental formulations with 0.8, 1.5, 3.0, and 4.5% weight by volume of mercury as methylmercurihydroxide and 1.6 and 4.7% as cyano(methylmercuri)guanidine were used in studies of the influence of the liquid dosage on fungicide and dye distribution. Dye concentrations and solvents were varied in these experimental formulations. The following solvent-water ratios were used: methanol 1 to 4 (T_1); ethylene glycol 1 to 4 (T_2), 1 to 2 (T_3), and 2 to 1 (T_4). Distribution measurements were also performed on seeds commercially treated with Swedish Panogen 4925 containing 0.8% weight by volume of mercury as cyano(methylmercuri)guanidine.

Seed Treatment Procedures. Laboratory treatments were performed with 100-gram batches of seed in a rotating bottle (*3*). Two techniques were used for the addition of the disinfectant: liquid poured directly on seeds (poor initial distribution) and liquid poured on walls of the bottle.

The reproducibility of the mechanical mixing process of the laboratory procedures is poor because of the minute amount of liquid added to the small batch of seed. Larger batches are therefore required for tests in which small differences in dye distribution are to be detected. Tests were performed with 20-kg. batches in a drum (diameter 23 inches and length 20 inches), revolving at 42 r.p.m. (This batch size was considered sufficient for reproducibility but is less than the optimum batch to get a good initial coverage in this drum.) The required amount of the disinfectant was added to the seed in the revolving drum in 1 minute. The drum was revolved 10 minutes, after which samples were taken. This mixing time was considered necessary to get representative samples.

Commercially treated seed samples were also examined. The Panogen seed treaters used in these tests were: Models J and JS (capacity 3 tons of small grain per hour); Model K (capacity 10 tons of small grain per hour); and Model A (capacity 4.5 tons of small grain per hour). [Conversion factors: 1 ton (metric) = 1000 kg. = 2205 pounds ~ 36.4 U.S. bushels of wheat; 1 fluid ounce per bushel (wheat) ~ 108 ml. per kg.]

A. Discharge of treated seed
B. Mixing drum
C. Distributing fingers
D. Dipper
E. Constant-level reservoir
F. Inlet spout
G. Scale hopper
H. Feed pump
I. Exhaust fan
J. Electric motor
K. Pipe for exhaust gas

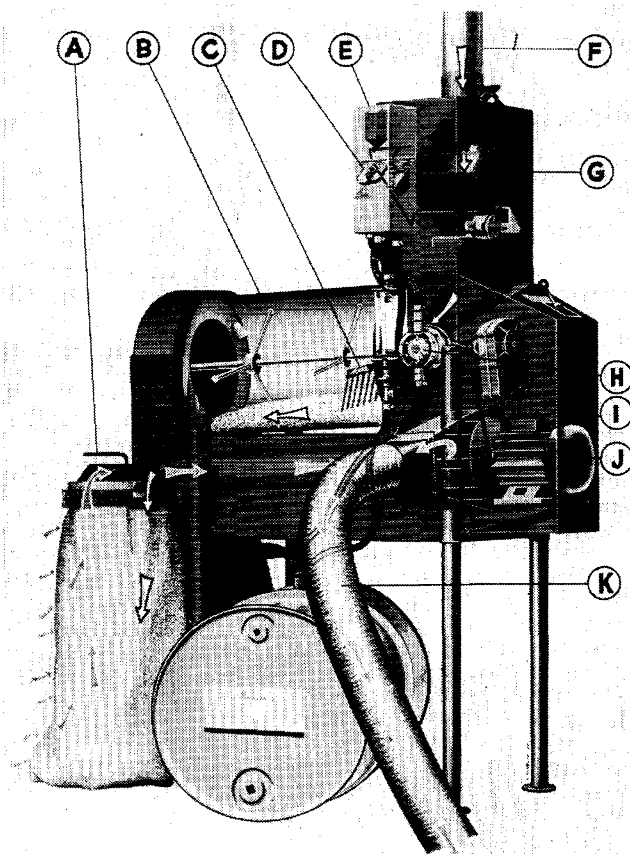

Figure 1. Section through Panogen seed treater, Model A illustrating principle of operation

Figure 1 shows the design and function of the Panogen seed treater, Model A.

Nucleonic Techniques. Beta counting was performed as described (*3*). In case of seeds treated with doubly labeled Panogen containing mercury-203 and thallium-204 the two beta activities were separated by means of a 30 mg. per sq. cm. absorber. Figure 2 shows transmission curves for seed samples treated with mercury-203 or thallium-204, and a mixed sample with 43% mercury-203 and 57% thallium-204 activity. The absorber reduces the mercury-203 activity by 50% and the thallium-204 activity by 10%. If C_{no} denotes the activity observed with no absorber and C_{ab} the activity observed with the 30 mg. per sq. cm. absorber, the appropriate activities, C, are

$$C_{Hg203} = \{C_{no} - 2.0 \times C_{ab}\}/0.8 \quad (1)$$

$$C_{Tl204} = C_{no} - \{C_{no} - 2.0 \times C_{ab}\}/0.8 \quad (2)$$

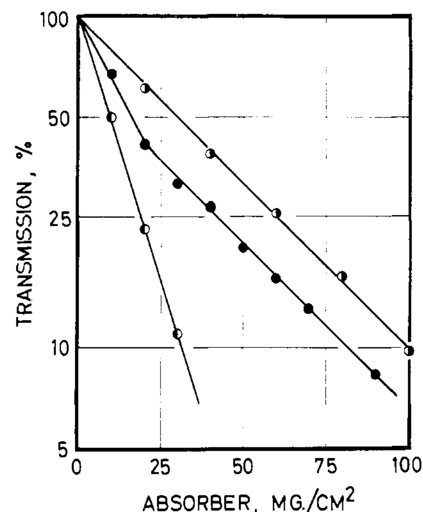

Figure 2. Transmission curves for beta activity of seeds treated with mercury-203 and thallium-204

◐ Only Hg^{203}
◑ Only Tl^{204}
● Mixture of Hg^{203} (43%) and Tl^{204} (57%)

Chemical Analytical Methods. Mercury in single kernels of treated wheat was determined by flame analysis (*2*). The mercurial is located in the surface layer of the seed and is easily brought into solution by digestion of the surface layer with a drop of nitric acid. The technique previously described in detail for analysis of wheat may be used also with oats, rye, barley, and cotton.

Table I. Evaluation of Dye Distribution on Treated Wheat Seed by Pairwise Comparison

(10 experienced and 10 inexperienced examiners)

	Treatment Conditions				Score (Total Number of Preferred Choices)	
Code[a]	Dosage, ml./100 g.	Solvent	Dye concn., % w./v.	Dye concn. on seed	Experienced	Inexperienced
$F_1T_1H_1$[b]	0.2	T_1	0.35	7	65	65
$F_1T_1H_2$[b]	0.2	T_1	0.40	8	52	51
$F_1T_1H_1$[c]	0.2	T_1	0.35	7	51	50
$F_1T_2H_1$	0.2	T_2	0.35	7	41	43
$F_1T_1H_2$[c]	0.2	T_1	0.40	8	41	41
$F_2T_1H_2$	0.04	T_1	2.00	8	16	17
$F_2T_2H_1$	0.04	T_2	1.75	7	12	12
$F_2T_1H_1$	0.04	T_1	1.75	7	2	1

[a] Dosage: F_1 0.2 ml./100 grams; F_2 0.04 ml./100 grams. Solvent: T_1 methanol-water 1:4; T_2 glycol-water 1:4. Dye applied to seed: H_1 7 p.p.m.; H_2 8 p.p.m.
[b,c] Duplicates of indicated factor combination.

Table II. Seed Treatment with Radioactive Panogen, Active Mercurial Labeled with Mercury-203, and Thallium-204 Nitrate Used as Nonvolatile Tracer

	Observed Activity, C.P.M.		Calculated Tracer Activities			
			Hg^{203}		Tl^{204}	
Kernel No.	C_{no} (no absorber)	C_{ab} (30 mg./sq. cm. absorber)	C.p.m.	Rel. activity	C.p.m.	Rel. activity
		Seeds Counted 2 to 5 Hours after Treatment				
1	206	66	92	1.0	114	0.8
2	234	76	103	1.1	131	0.9
3	265	101	79	0.8	186	1.3
4	215	69	96	1.0	119	0.9
5	210	66	98	1.0	112	0.8
6	232	87	73	0.8	159	1.1
7	310	108	118	1.2	192	1.4
8	207	64	99	1.0	108	0.8
9	242	82	98	1.0	144	1.0
10	1206	475	320	3.4	900	6.4
		Seeds Counted 4 Days after Treatment				
11	161	55	64	1.0	97	0.6
12	292	118	70	1.1	222	1.4
13	287	112	79	1.2	208	1.3
14	202	83	45	0.7	157	1.0
15	189	70	61	0.9	128	0.8
16	181	72	46	0.7	135	0.9
17	184	65	68	1.0	116	0.7
18	1194	497	125	1.9	1069	6.9
19	306	117	90	1.4	216	1.4
20	195	69	71	1.1	124	0.8

(Cottonseeds are cut into smaller pieces prior to nitric acid extraction.) After rinsing with a small portion of distilled water, the aqueous solution is atomized and burned in an oxyhydrogen flame. The combustion gases are purified and assayed for mercury by means of a mercury vapor spectrophotometer. The coefficient of variation for the total analysis is about 7%. This variation is connected with the sample preparation, as only 1% variation is associated with the assay of the mercury content of the aqueous solution (*2*). The detection limit is about 0.02 γ of mercury in this particular application.

Control analyses of larger seed samples were performed by conventional wet digestion and colorimetric dithizone assay (*3*).

Distribution Parameters. The mercurial distribution is characterized by the same kind of distribution parameters that were introduced for the beta counting populations (*2*, *4*). The main group of normal kernels, N-kernels, is obtained by exclusion of the few rich kernels, R-kernels, carrying more than twice the average N-kernel mercurial content. The group of N-kernels is treated as a normal distribution and is characterized by its coefficient of variation, also called the spreading error. The R-kernel population is described by three parameters: (1) R = number of R-kernels divided by total number of kernels; (2) S = total quantity of fungicide carried in excess by the R-kernels present, above the N-kernel average, divided by the amount of fungicide added in the process; (3) $T = S/R + 1$, which is the relative dose for the R-kernels referred to the N-kernel average.

Evaluation of Dye Distribution. Commercial seed dressings contain dye for coloring the treated seed to distinguish it from nontreated seed. Frequently the outcome of the seed treatment procedure is considered equivalent to the more or less uniform dye distribution achieved in the mixing process. Some experiments were therefore made to compare dye distribution with fungicide distribution.

Dye distribution should be evaluated as it is in the field—by visual examination. To get reliable and objective results, a statistical procedure is required with a sufficient number of independent examiners and a randomized testing sequence. No distribution parameters are obtained as for the analytical methods, but a ranking list is used.

Samples are compared by twos (all possible different combinations) and the examiner has to decide which is most uniformly colored—a decision has to be made even if the two samples are actually identical. If there are n samples $\left\{n^2 - \sum_{1}^{n} v\right\}$ different comparisons have to be made. The tester has to examine the pairs in a randomized order without knowing the identity of the samples. The test is conducted by an interviewer, who records the results. Scores are obtained by summing the total number of comparisons in favor of the actual sample.

Table I reports the result of a test involving two groups of test persons, one group with a long experience in this particular field and a second group with none. Although detectable differences between the seed samples were slight, scores reported by the two groups of examiners were essentially the same. This indicates that the method should give fairly objective results if a sufficiently large number of examiners are consulted. [A closer inspection of the data in Table I demonstrates that the variation between the replicates (laboratory-treated seeds) apparently is larger than the differences to be expected between the two levels of dye concentration in the disinfectant, 0.40 and 0.35%. A poorer dye distribution is obtained for the low volume dosages.]

Experimental Results and Discussion

Demonstration of Vapor Action. The role played by vapor action (*3*) in the Panogen process was demonstrated in a straightforward way by means of a formulation containing nonvolatile thallium nitrate tracer and radioactive mercurial. Table II contains data for wheat seeds treated with this formulation; 3200 counts were taken for each reading, the background being 25 c.p.m. Mercury-203 and thallium-204 activities were calculated by Equations 1 and 2.

The mercurial distribution is more uniform than the distribution of the thallium tracer. The relative activity figures—actual activity divided by the

Table III. Distribution Study Involving Beta Counting and Whole Kernel Assay of Wheat Treated with Radioactive Panogen

Kernel No.	Kernel Weight, W, Mg.	Beta Activity B, C.P.M.	Kernel Assay A, γ Hg	B/W, C.P.M./Mg.	$B/W^{2/3}$, C.P.M./$(Mg.)^{2/3}$	$B/W^{1/3}$, C.P.M./$(Mg.)^{1/3}$	$A/W \times 10^3$, P.P.M.	$A/W^{2/3}$ γ Hg/$(Mg.)^{2/3}$	$A/W^{1/3}$ γ Hg/$(Mg.)^{1/3}$
1	33.5	117	0.51	3.5	11	36	15	0.049	0.16
2	45.8	202	0.94	4.4	16	57	21	0.073	0.26
3	35.2	150	0.65	4.3	14	46	18	0.061	0.20
4	26.0	126	0.64	4.8	15	43	26	0.074	0.22
5	38.2	109	0.59	2.9	10	32	15	0.053	0.18
6	48.2	155	0.73	3.2	12	43	15	0.055	0.20
7	41.8	95	0.78	2.3	8	27	19	0.065	0.23
8	41.7	129	0.66	3.1	11	37	16	0.055	0.19
9	40.1	121	0.63	3.0	10	35	16	0.054	0.18
10	56.8	123	0.80	2.2	8	32	14	0.054	0.21
11	49.9	121	0.85	2.4	9	33	17	0.063	0.23
12	44.9	100	0.53	2.2	8	28	12	0.042	0.15
13	50.8	127	0.67	2.5	9	34	13	0.049	0.18
14	40.2	124	0.63	3.1	11	36	16	0.054	0.18
15	46.5	93	0.66	2.0	7	26	14	0.051	0.18
16	43.4	98	0.56	2.3	8	28	13	0.045	0.16
17	37.7	92	0.38	2.4	8	27	10	0.034	0.11
18	48.5	131	0.81	2.7	10	36	17	0.061	0.22
19	51.7	108	0.47	2.1	8	29	9	0.034	0.13
20	36.0	100	0.49	2.8	9	30	14	0.045	0.15
21	37.0	80	0.46	2.2	7	24	12	0.041	0.14
22	47.1	87	0.38	1.8	7	24	8	0.029	0.11
23	36.0	92	0.38	2.6	8	28	11	0.035	0.12
24	43.1	89	0.40	2.1	7	25	9	0.032	0.11
25	51.5	110	0.53	2.1	8	30	10	0.038	0.14
26	46.2	77	0.42	1.7	6	21	9	0.033	0.12
27	44.0	119	0.71	2.7	10	34	16	0.057	0.20
28	36.9	116	0.64	3.1	10	35	18	0.057	0.19
29	41.8	93	0.58	2.2	8	27	14	0.048	0.18
30	55.3	95	0.24	1.7	7	25	4	0.017	0.06
Av.	43.2	113	0.59	2.7	9.3	32	14	0.049	0.17
Coeff. of variation	0.16	0.23	0.27	0.29	0.26	0.24	0.31	0.27	0.26

average for the N-kernels—furnish striking evidence. Kernels 10 and 18 have very high relative thallium-204 activities of the order of 6 to 7, indicating large initial doses of disinfectant. Because of vapor action, the relative mercury-203 activities are lower, approaching unity with increasing storage time—the relative mercury-203 activity for No. 18 is 1.9 compared to 6.9 for thallium-204, indicating that in this particular case more than 70% of the initial mercury-203 loading has volatilized.

Whole Kernel Assay *vs.* Beta Counting. Different distribution parameters are to be expected for statistics based on whole kernel assay compared to beta counting, which accounts only for the mercurial present on parts of the kernel facing the window of the Geiger tube. Table III reports analytical data for a sample of wheat seed treated with radioactive Panogen. These seeds were first subjected to beta counting and then analyzed by the flame method (2560 counts were taken with a counting error of 2 to 3%). Variation coefficients are reported in the table for primary analytical data and for primary data divided by the kernel weight, W, and by $W^{2/3}$ and $W^{1/3}$. These statistics are discussed below. Whole kernel assay gives larger coefficients of variation than beta counting in this particular example.

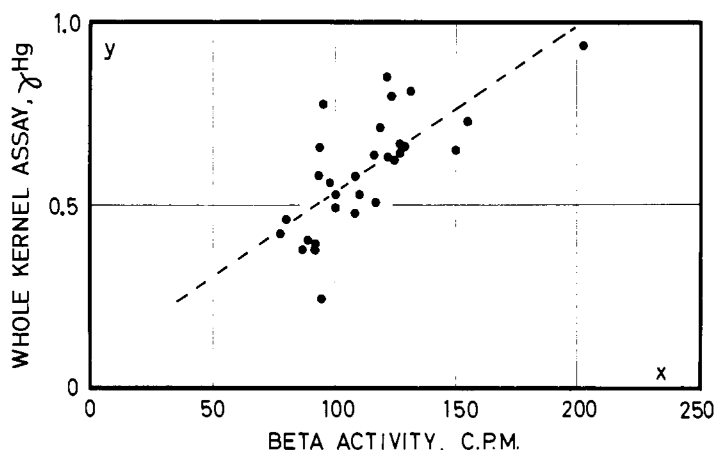

Figure 3. Whole kernel assay vs. beta counting

In Figure 3 total mercury contents are plotted against the observed beta activity. The equation of the regression line is $y = (0.0046 \pm 0.0017)\ x + 0.078$ (95% confidence limits), which indicates a significant though weak correlation between these two analytical quantities. The correlation coefficient is $r = 0.72$ with the 95% confidence limits 0.49 to 0.86.

It is assumed that no R-kernels are present, that all kernels are the same size, and that beta counting accounts for the mercury content of $1/f$ of the total kernel surface, f being an integer. Two distribution models are used.

In one model the f surface fractions of a kernel are assumed to be independent and randomized with respect to mercury content. Each kernel is the sum of f surface fractions picked up at random from a large universe. The coefficient of variation based on whole kernel assay is then $\sqrt{1/f}$ of the corresponding statistics based on beta counting (normal distribution). As f is estimated to be about $f = 6$, the ratio between the corresponding coefficients of variation would be ~ 0.4.

The other extreme is that the f sur-

face fractions of a particular kernel are not independent but are carrying equal amounts of mercury; variations may, however, occur between different kernels. This gives identical statistics for the two populations. A perfect correlation between beta counting and whole kernel assay is then to be expected, which is not the case as shown in Figure 3.

There is, however, always a small proportion of R-kernels found in the beta counting population. This R-kernel population has a major influence on the relations between the two populations studied.

If surface fractions carrying R-kernel activities ("R-surface fractions") are randomly distributed, there is but a small chance that a kernel will carry more than one R-surface fraction. The R-kernel population obtained in the beta counting will then be almost completely eliminated in the statistics based on whole kernel assay of the same sample of seed. The spreading error based on whole kernel assay then becomes smaller or larger than the corresponding beta counting statistics, depending on the properties of the beta R-kernel population.

In practice certain kernels are carrying more than the statistical share of R-surface fractions because of mechanical damage and/or improper mixing. This bias again would tend to give similar R-kernel statistics in the two populations but with the R-kernel dosage (defined by the T factor) smaller for whole kernel assay than for beta counting.

No exact relationship can be derived between the statistics based on whole kernel assay and on beta counting. The distribution studies performed, however, indicate somewhat larger spreading errors, similar R- factors, and smaller S- and T- factors for statistics based on whole kernel assay. This is probably due to a partial transformation of the beta R-kernel population into the N-kernel population observed in the whole kernel assay as discussed above.

Distribution statistics may serve as a guide for evaluating the effectiveness of the seed treatment. Beta counting would be preferred for this purpose because distribution parameters should be evaluated by means of the smallest possible samples of the object. For comparison between different treatments, whole kernel assay is also more convenient.

Comparison between Dye and Fungicide Distribution. The dye distribution obtained in laboratory seed treatment is much influenced by how the few drops of disinfectant are added to the seed batch. This variability, apparent in Table I, may also have an influence on tests, where small differences in fungicide distribution are to be detected. Drum treatment with 20-kg. batches was therefore used in the following experiments, designed to establish: factors influencing the dye distribution; the relationship between dye distribution and mercurial distribution, if any; and the effect of volume dosage on the mercurial distribution. (This treatment did not give a good initial distribution because of the small charge of seed compared to the capacity of the drum and the mode of addition of the disinfectant. It was, however, desired to enlarge any differences between the formulations studied.)

Experimental formulations containing various solvents and varying amounts of dye and fungicide were used as reported in Table IV. The largest and the smallest rate of application differed by a fac-

Table IV. Dye Distribution on Wheat for Various Experimental Formulations

Formulation	Solvent	Hg Concn., % W./V.	Dye Concn., % W./V.	Viscosity (20° C.), Cp.	Surface Tension, (20° C.), Dyn./Cm.	Rate of Application: Ml./100 kg.	Rate of Application: Fl. oz./bu.	Dye Distribution (13 Examiners): Score	Dye Distribution (13 Examiners): Ranking No.
I	T_1	0.8	0.35	1.0	43.0	335	3	130	3
II	T_1	1.5	0.50	1.0	43.0	98	9/10	56	10
IIIa[a]	T_1	1.5	0.60	2.6	51.2	98	9/10	30	12
IIIb	T_1	1.5	0.60	2.6	51.2	98	9/10	15	13
IV	T_1	1.5	0.90	2.6	50.3	98	9/10	84	6
Va	T_1	3.0	1.4	3.2	51.0	70	2/3	104	5
Vb	T_1	3.0	1.4	3.2	51.0	70	2/3	61	9
VI	T_1	3.0	2.1	3.2	50.7	70	2/3	131	2
VII	T_1	4.5	2.4	3.2	50.3	40	3/8	51	11
VIII	T_1	4.5	3.3	3.6	50.2	40	3/8	110	4
IXa	T_3	1.6	0.53	6.0	56.9	98	9/10	82	7
IXb	T_3	1.6	0.53	6.0	56.9	98	9/10	72	8
X	T_4	4.7	3.4	17.8	53.4	33	3/10	176	1

[a] a and b denote duplicate tests.
T_1. Methanol-water 1:4.
T_3. Glycol-water 1:2.
T_4. Glycol-water 2:1.

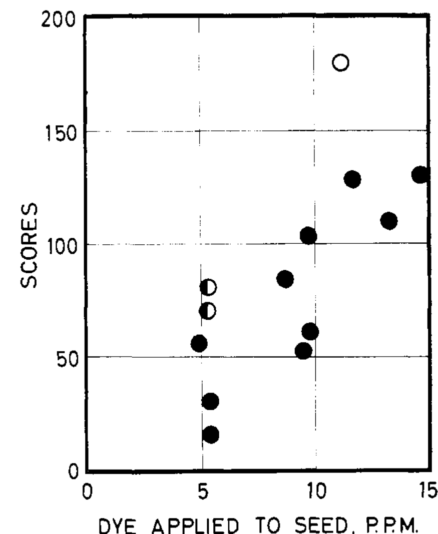

Figure 4. Score for dye distribution plotted as function of amount of dye applied to seed

Formulation types (Table IV)
● T_1 ◐ T_3 ○ T_4

Table V. Distribution Parameters for Wheat Seeds as a Function of Liquid Dosage

(Statistics based on whole kernel assay of 30 kernels. 95% confidence limits reported)

Formulation (Cf. Table IV)	Rate, Fl. Oz./Bu.	Check Analysis of Treated Seed, γ Hg/10 G.	Flame Analysis of Single Kernels, Av. Results: γ Hg/kernel	Flame Analysis of Single Kernels, Av. Results: P.p.m. Hg	R-Kernel Statistics: R	R-Kernel Statistics: S	R-Kernel Statistics: T	N-Kernel Statistics, Coefficients of Variation: γ Hg/kernel, A	N-Kernel Statistics: (Kernel weight)$^{2/3}$ $W^{2/3}$	N-Kernel Statistics: $A/W^{2/3}$	$\sqrt{A^2 - (W^{2/3})^2}$: W, act. sample	$\sqrt{A^2 - (W^{2/3})^2}$: W, large sample
I	3	285, 190	1.11 ± 0.19	28.0	1/28	0.05	2.4	0.41 ± 0.11	0.16 ± 0.04	0.36 ± 0.10	0.36	0.36
IIIa	9/10	152, 168	0.69 ± 0.12	16.2	3/30	0.13	2.3	0.32 ± 0.09	0.14 ± 0.04	0.32 ± 0.09	0.29	0.28
Va	2/3	205, 220	0.83 ± 0.14	21.9	2/30	0.10	2.6	0.21 ± 0.06	0.21 ± 0.05	0.24 ± 0.06	0.00	0.14
VII	3/8	197, 175	0.62 ± 0.06	18.0	0/30	0	...	0.26 ± 0.07	0.18 ± 0.05	0.24 ± 0.06	0.19	0.20
IXb	9/10	145, 162	0.71 ± 0.08	16.5	1/30	0.04	2.3	0.23 ± 0.06	0.13 ± 0.03	0.22 ± 0.06	0.19	0.17
X	3/10	236, 180	0.61 ± 0.07	15.4	0/30	0	...	0.32 ± 0.08	0.12 ± 0.03	0.33 ± 0.08	0.30	0.28
Av.		193		19.3	0.04	0.05	2.4	0.30	0.16	0.29	0.25	0.25

Table VI. Distribution Parameters for Wheat Seeds, 17.5% Moisture Content in Laboratory Treatment

(Statistics based on whole kernel assay of 30 kernels. 95% confidence limits reported)

Mode of Addition	Check Analysis of Treated Seed, γ Hg/10 G.	Flame Analysis of Single Kernels, Av. Results: γ Hg/kernel	Flame Analysis of Single Kernels, Av. Results: P.p.m. Hg	R-Kernel Statistics: R	R-Kernel Statistics: S	R-Kernel Statistics: T	N-Kernel Statistics, Coefficients of Variation: γ Hg/kernel, A	N-Kernel Statistics, Coefficients of Variation: (Kernel weight)$^{2/3}$ $W^{2/3}$	N-Kernel Statistics, Coefficients of Variation: $A/W^{2/3}$	N-Kernel Statistics, Coefficients of Variation: $\sqrt{A^2 - (W^{2/3})^2}$
On seed[a]										
a	145, 135	0.58 ± 0.08	13.1	1/30	0.05	2.5	0.28 ± 0.07	0.13 ± 0.03	0.23 ± 0.06	0.23
b		0.54 ± 0.04	11.3	0/30	0	...	0.21 ± 0.05	0.13 ± 0.03	0.17 ± 0.04	0.16
Via wall										
a	130, 125	0.63 ± 0.06	13.8	1/30	0.03	2.1	0.18 ± 0.05	0.13 ± 0.03	0.20 ± 0.05	0.14
b		0.58 ± 0.04	12.6	0/30	0	...	0.20 ± 0.05	0.12 ± 0.03	0.20 ± 0.05	0.16
Av.	134	0.58	12.7	0.02	0.02	2.3	0.22	0.13	0.20	0.17

[a] a and b are duplicates.

tor of 10. The wheat seeds, 12% moisture content, were not cleaned prior to treatment, in a further attempt to get larger differences between the tests. All treatments were made on the same occasion in a randomized order. Dye distribution was evaluated 1 week after treatment and mercurial distribution 3 to 4 weeks after treatment.

Results of the visual evaluation of dye distribution are reported in Table IV. Four groups are recognized, with maximum score for X followed by VI and I; a large group with mediocre score; and finally the poor IIIa and IIIb. Actual differences between the samples were, however, small (except between X and the poorest batches) as indicated, for example, by the differing scores for the duplicates, Va and Vb. No volume effect is recognized in Table IV in contrast to Table I. A most significant feature is, however, revealed in Figure 4, where the score is plotted as a function of the amount of dye applied to the seed in the treatment. Increase of the amount of dye apparently produces an impression of more uniformly colored seed. Some influence of the solvents used is also indicated.

Table V reports fungicide distribution parameters for some of the batches in Table IV. No agreement exists between dye and mercurial distribution: The best and the poorest formulations with respect to dye coverage exhibit about the same N-kernel distribution parameters. No conclusions can be made with respect to the R-kernel statistics, however, as larger samples are required for comparison in this case.

Reduction of the liquid dosage has apparently no adverse effects on the fungicide distribution, as shown by the data in Table V. There seems actually to be some improvement at lower dosages, though this trend is not statistically significant.

The use of a concentrated liquid disinfectant in a small dosage is the basic principle of the Panogen process. The present distribution studies, based on whole kernel assay as well as the earlier radioactive studies (*3*), show that satisfactory distribution patterns are obtained not only in the region of practical interest but also with much smaller volume dosages.

Table VII. Distribution Parameters for Commercially Disinfected Seeds Treated with Swedish Panogen 4925

(Statistics based on whole kernel assay of 30 kernels. 95% confidence limits)

Variety of Seed, Location of Treating Establishment	Moisture Content, %	Dosage, Ml./100 Kg.	Seed Treater	Distribution Parameters: Coeff. of variation (based on γ Hg/kernel)	Distribution Parameters: R	Distribution Parameters: S	Distribution Parameters: T
Spring wheat (Svenno), Hälsingborg, Sweden	17	200	Model JS	0.25 ± 0.06	0/30	0	...
Spring wheat, Skurup, Sweden	17	200	Model K	0.20 ± 0.05	2/30	0.11	2.6
Oats (Solhavre), Skara, Sweden	15	300	Model A	0.30 ± 0.08	2/30	0.08	2.2
Oats (Blenda), Håle-Täng, Sweden	18	300	Model J	0.29 ± 0.07	0/30	0	...

Comparison between Laboratory Treatments. Table VI reports a comparison between the two laboratory treatment procedures used in these studies: addition of disinfectant directly on the seed giving a less uniform initial coverage of the disinfectant than addition via the walls of the treatment bottle. Wheat seeds with a high moisture content (17.5%), which accelerates the resorption processes, were used to accentuate the differences between the two methods. In Sweden the moisture content of seed to be treated is frequently as high (sometimes even higher) as 17.5%, which is the present limit for seed of small grain to be sealed by the Swedish government. Because of the variability of the laboratory methods, representative samples made up of seven replicate treatments were prepared for the flame analysis. The analysis was run in duplicate with 30 kernels in each series. The seeds were stored 14 days prior to the chemical analysis. The checks run with the conventional analytical method compare very well with the averages from the flame analysis, as demonstrated in Table VI. In spite of the high moisture content, no significant differences are obtained between the two treatment procedures. This is in harmony with results of earlier radioactive studies (*3*).

Studies with Commercially Treated Seed. One important advantage of flame analysis is that it can be run on commercially treated seed received from the field. Distribution parameters in Table VII are of the same magnitude as obtained with seeds treated in the laboratory. A similar conclusion was reached by Arny in a biological study (*1*).

Actually the seed itself is likely to be an important source of variation in mercurial distribution. In a radioactive distribution study with a mixture of intact and damaged seed [Table XV, (*3*)] the final distribution was poorer than the initial one because of a very heterogeneous seed sample. The individual kernels in a lot of seed exhibit a large variation with respect to a number of properties, easily subjected to measurement, such as kernel weight, germination, and growth rate, and there is no reason why a similar variation should not be found with the factors governing the mercurial resorption.

Statistical Remarks

The large variation in kernel size in the seed samples studied is likely to contribute to the observed variability for whole kernel assays. To get an estimate of the importance of this source of variation, Tables III and VIII also contain the primary analytical results divided by the kernel weight, (kernel weight)$^{1/3}$, and (kernel weight)$^{2/3}$. Maximum coefficients of variation are obtained when the analytical results are divided by the kernel weight and minimum values when divided by the cube root of the kernel weight. A similar result was obtained by regression analysis on the analytical results underlying Table V. Rates varied in this experiment and therefore the primary figures for each test were converted to percentages, $p\%$, based on the averages (R-kernels excluded). A plot of $\log_{10} p$ *vs.* $\log_{10}$ kernel weight gives a scattered picture, but the larger kernels in general carry more mercury. The equation of the regression line is $y = (0.38 \pm 0.17)\,x + 1.38$. The 95% confidence limits for the slope indicate a significant correlation. For simplicity $y = \frac{1}{3}x +$ constant may be used with the mercury content proportional to the cube root of the kernel weight (a square root relation is, however, not excluded, as shown by the confidence limits). The correlation coefficient for p and (kernel weight)$^{1/3}$ is $r = 0.33$ with the 95% confidence limits 0.19 to 0.46.

In the earlier radioactive studies (*3*) it was assumed that under ideal conditions the mercury content should be proportional to the surface area of the kernel—i.e., proportional to (kernel weight)$^{2/3}$. The spreading error was then obtained by subtracting this surface variancy from the experimentally observed variancy. This procedure, exemplified in the column for $\sqrt{A^2 - (W^{2/3})^2}$ in Tables V and VI, is not justified in the light of the present findings, which indicate only a weak correlation between mercury content and kernel size. The best procedure is then to estimate the spreading error directly from the population of γ Hg/(kernel weight)$^{1/3}$, for example. This gives slightly higher coefficients of variation, as shown by Tables V and VI.

N-kernel statistics are derived assuming normal distributions. Distribu-

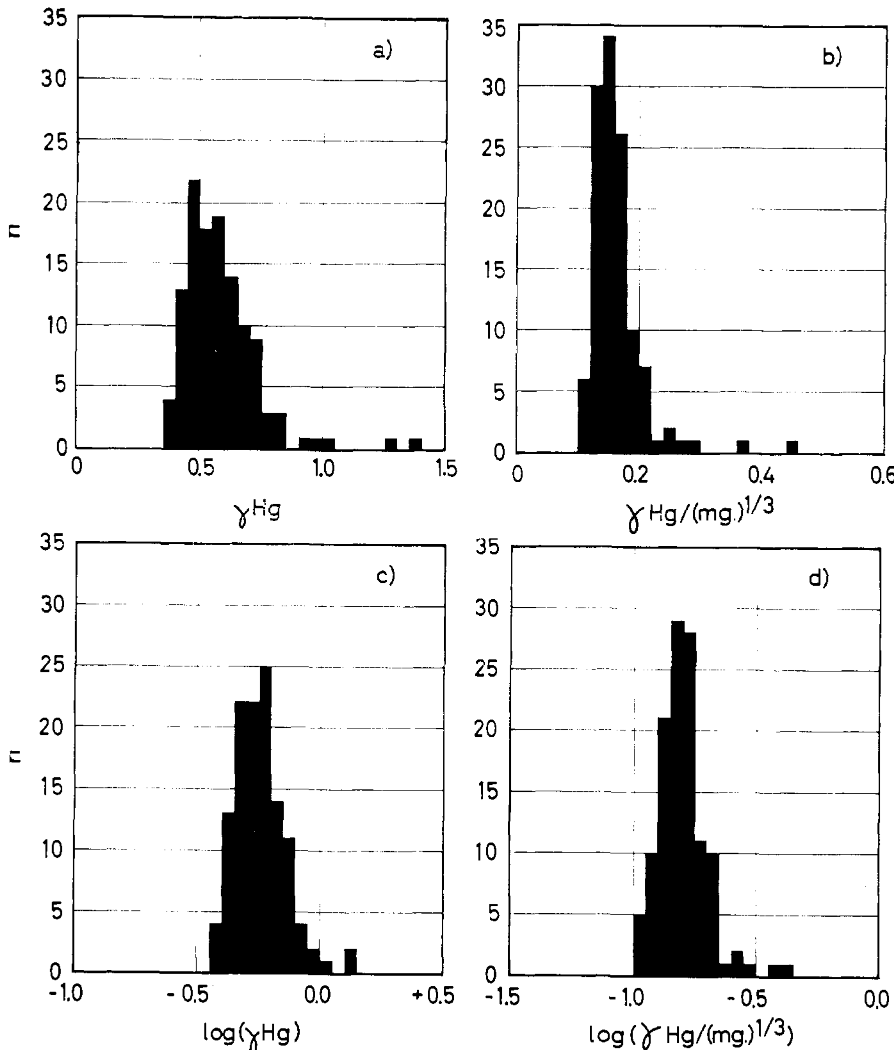

Figure 5. Histograms based on whole kernel assay

a. Primary data, A, γ Hg/kernel
b. $A/W^{1/3}$, γ Hg /(kernel weight)$^{1/3}$
c. $\log_{10} A$
d. $\log_{10} A/W^{1/3}$

Table VIII. Comparison between Coefficients of Variation Estimated in Different Ways

Reference Table V	*N-Kernels Only, Primary Data* A	$A/W^{1/3}$	$A/W^{2/3}$	A/W	*All Kernels (R + N), Primary Data* A	$A/W^{1/3}$	*All Kernels (R + N), Logarithms* Log A	Log $A/W^{1/3}$	*N-Kernels Only, Logarithms* Log A	Log $A/W^{1/3}$
I	0.409	0.374	0.356	0.361	0.464	0.433	0.613	0.543	0.565	0.497
IIIa	0.323	0.309	0.320	0.350	0.488	0.484	0.528	0.502	0.373	0.352
Va	0.210	0.199	0.237	0.326	0.449	0.420	0.397	0.366	0.236	0.224
VII	0.261	0.241	0.241	0.300	(0.261)	(0.241)	(0.311)	(0.272)	0.311	0.272
IXb	0.233	0.219	0.224	0.263	0.322	0.323	0.314	0.313	0.252	0.246
X	0.315	0.311	0.329	0.383	(0.315)	(0.311)	(0.385)	(0.377)	0.385	0.377
Table VI										
On seed										
a	0.282	0.248	0.228	0.234	0.374	0.395	0.348	0.345	0.279	0.254
b	0.206	0.174	0.166	0.183	(0.206)	(0.174)	(0.219)	(0.187)	0.219	0.187
Via wall										
a	0.179	0.183	0.199	0.243	0.254	0.266	0.249	0.248	0.197	0.187
b	0.201	0.194	0.201	0.247	(0.201)	(0.194)	(0.221)	(0.207)	0.221	0.207
Totals	2.62	2.44	2.51	2.88	3.37	3.23	3.58	3.36	3.23	2.77

Some samples contained no R-kernels, indicated by parenthesis in R + N columns.

tions are, however, slightly skewed with a deficit of low values, which also is apparent from the summarized data in Table XIV of (*3*).

The slightly nonsymmetrical frequency distributions observed in histograms *a* and *b* in Figure 5 are based on data underlying Table VI. An attempt is made to normalize the data by using logarithms, histograms *c* and *d*. The latter histograms are more symmetrical, which raises the question of whether normalization of primary data in this way would be a preferred route. Use of logarithms may, furthermore, diminish the differences between the R- and N-kernel populations to the extent that separation into two populations may even no longer be necessary in case of whole kernel assay, where differences between R- and N-kernels are moderate.

Table VIII illustrates these questions. The coefficients of variation reported here are based on: primary N-kernel data; primary (R+N)-kernel data; logarithms of N-kernel data; and logarithms of (R+N)-kernel data taken from the experiments in Tables V and VI. (The totals quoted in the bottom row of the table have no physical meaning but are introduced as a guide for comparison between the studied statistical procedures.) The primary N-kernel statistics show again minimum variation for $A/W^{1/3}$. Differences between statistics based on A, $A/W^{1/3}$, and $A/W^{2/3}$ are small, whereas A/W gives a higher variancy.

The practical conclusion of this is that although $A/W^{1/3}$ is the most correct choice, primary analytical data, A γ Hg/kernel, may be used as well in practice. Statistics based on A/W—e.g., p.p.m. figures—should, however, not be used. Logarithms give more symmetric distributions but do not reduce the coefficients of variation. The coefficient of variation reported in Table VIII for logarithims is the antilogarithm minus 1. (In this example an increase is actually observed due to the more sharply peaked distribution with a broad base indicated by the histograms *c* and *d* in Figure 5.) Statistics based on the combined (R + N)-kernel populations give much larger coefficients of variation, and consequently the separation into two populations seems justified also for populations based on whole kernel assay.

Conclusions

Distribution statistics based on whole kernel assay differs slightly from statistics based on the previous beta counting technique, but the same approach involving two populations, R- and N-kernels, should be used. Coefficients of variation for the N-kernel population, "spreading error," are about the same with the two methods, whereas R-kernel statistics exhibit larger differences with higher readings for beta counting R-kernels. Only a weak correlation exists between fungicide content and kernel size. The fungicide content is roughly proportional to the cube root of the kernel weight and not to the two thirds power as might be expected. In practice primary analytical results—e.g., γ Hg/kernel—may be used as well as data corrected for kernel size, in view of the weak correlation between size and mercurial uptake.

The results of the earlier radioactive distribution studies were confirmed by the present studies. Further proof is presented that the mercurial fungicide and the dye are distributed in different ways. The impression of dye uniformity is governed very much by the amount of dye used, which has no connection with the mercurial distribution. Whole kernel assay studies could also demonstrate that the small liquid dosages characteristic of the Panogen process can be reduced further, well below present volume rates, with no adverse effects on the mercurial distribution. This indicates a considerable margin of safety with the method. Laboratory treatment and large scale commercial treatment give similar distribution patterns because of the vapor action of these mercurial fungicides.

The spreading errors for the commercially treated seeds are of the order of 0.2 to 0.3, which indicates a sufficiently uniform distribution to produce but a negligible drop in disinfection efficiency (*4*). Estimates of the spreading error are furthermore conservative, as this statistics is actually mainly governed by kernels with larger amounts of mercurial. The distribution curves have no "tail" with deficient kernels.

Acknowledgment

The author expresses his most sincere thanks to Jörgen M. Blegvad, AB Casco, Stockholm 11, Sweden, and to Petrus Hellman, Panogen Co., Ringwood, Ill., for continuous support and the permission to publish this study. Thanks are also extended to Bengt Bergman, Åke Hedén, and Ulf Ulfvarson for valuable discussion and to Nunne Holtz, Kajsa Lundgren, Sven Bodin, John Carlsjö, and Erik Ljusberg for skillful assistance.

Literature Cited

(1) Arny, D. C., *Phytopathology* **46,** 342–4 (1956).
(2) Lindström, O., *Anal. Chem.* **31,** 461–7 (1959).
(3) Lindström, O., J. Agr. Food Chem. **6,** 283–98 (1958).
(4) *Ibid.*, **7,** 326–9 (1959).
(5) Westermark, T., Sjöstrand, B., accepted for publication in *Intern. J. Appl. Radiation and Isotopes.*

Received for review August 24, 1959. Accepted January 14, 1960.

FEED ADDITIVES

Determination of 3,5 Dinitro-*o*-toluamide (Zoalene) in Feed Concentrates

GRANT N. SMITH

The Dow Chemical Co., Midland, Mich.

Premix concentrates containing 25% 3,5-dinitro-*o*-toluamide (zoalene) with soybean meal are being used in the feed to aid in the prevention of cecal and intestinal coccidiosis in chickens. In the manufacture of such a premix concentrate it is essential to have a rapid easy-to-handle analytical method, with a high degree of accuracy, reproducibility, and specificity, for quality control.

The method which has been developed, using the colorimetric procedure previously described (*1*), is based on the extraction of the 3,5-dinitro-*o*-toluamide from the feed concentrate with dimethylformamide. This solution is then mixed with a solution of methylamine. The reaction results in the formation of a purple-colored complex, the absorbance of which can be read at wave length 550 mμ in a Beckman spectrophotometer. The intensity of the color is proportional to the concentra-