

**Procedure for Establishing Number of Seeds per Gram and  
Minimum Weight for Purity and Noxious Weed Exams  
Using the Purity Weight Calculator (*PW Calculator v3.xlsx*)**

In many cases, determinations of purity sample weights result in seed counts significantly less than 2,500, and in other cases in counts far above 2,500. The following method is designed to calculate purity and bulk/noxious exam weights with seed counts as close to 2,500 and 25,000 as possible, respectively, across a range of seed weights within the same species. This method also detects species with high variation in seed weight among lots, varieties, or production conditions, for which single purity and noxious exam weights cannot be recommended. The method includes the determination of number of seeds per gram, the minimum weight for a purity test, and the minimum weight for a bulk/noxious weed exam.

All single lot determinations, except for outlier detection, can be manually calculated, while determining a ‘Minimum purity weight’ and a ‘Minimum bulk/noxious weed exam weight’ require the use of the accompanying *PW Calculator v3.xlsx* (downloadable from AOSA’s website). In addition to its use in determining minimum purity and bulk/noxious weights, using the *PW Calculator* ensures that rounding is appropriately and uniformly applied across labs and analysts.

For a single seedlot, assuming proper sampling, an upper limit CV of 4 or 6% (non-chaffy or chaffy kinds, respectively), as in the current rules, is used to assess reliability. However, for the average of all seedlot samples, a CV of 10% and a 95% upper limit confidence interval are used to determine minimum purity and bulk/noxious weed weights, to avoid excessively high or very low seed counts. This method should be used for new species and to validate the minimum weights of current species in Table 2A, AOSA Rules, vol. 1 (2022 or current version).

***The PW Calculator is password-protected. To use the calculator, click on the ‘Read-Only’ option when prompted for a password. To save your data and output, save your working file under another name.***

**I. Number and scope of Seedlots**

A minimum of six seedlots representing different seed sizes, varieties, production environments, and years must be used. Including more than 6 seedlots is highly recommended. Chosen seed lots should be ‘market-ready,’ as appropriate for the kind under study. Field runs, uncleaned and unscreened seeds, as well as mixtures where the target species represents only a small proportion of the lot, should not be used. It is incumbent upon the study organizer to demonstrate that the chosen seed lots represent an acceptable scope of variation for the kind being investigated (see section VI.1 below).

## II. Determination of Purity and Bulk/Noxious Weed Exam Weights for a Single Seedlot

For each of the sampled seedlots, the following procedure must be followed:

- 1) Count eight replicates of 100 pure seed units each (sec. 13.3a of the Rules, vol. 1, 2022).
- 2) Weigh each replicate separately in grams to four significant figures.
- 3) After entering all seedlot identification numbers/names (part A of the *PW Calculator*), enter your replicate results in part B1 of the *PW Calculator*. The CV (B2 in the *PW Calculator*) and the unrounded mean (B3) are automatically calculated. Those parameters can also be manually calculated, as illustrated in Table 1.
- 4) If the CV is equal to or less than 6.0% and 4.0% for chaffy and non-chaffy seeds, respectively (B2 and *Example 1*), proceed to step 5 below. If the CV is above the acceptable limit, go directly to section III of this document.
- 5) Before calculating final purity and bulk/noxious weights for that seedlot, the unrounded average weight of the 8 replicates (B3 and *Example 1*) should be rounded using the same working samples criteria described in sec. 2.3a of the Rules, vol. 1 (2022): if the average weight of the replicates is less than 1.0000 g, round to 4 decimals; 1.000-9.999 g, round to 3 decimals; 10.00-99.99 g, round to 2 decimals; 100.0-999.9 g, round to 1 decimal; and 1000 g or more, round to a whole number. The CV is always rounded to one decimal place, while the variance and the SD values are not rounded (*Example 1*). The *PW Calculator* presents the same choices for rounding the mean replicate weight and requires the user to manually input the correctly rounded mean (part B4 of the *PW Calculator*) for subsequent determinations.
- 6) Use the rounded mean (*Example 1*) to calculate the 2,500 seed weight for purity analysis and 25,000 seed weight for bulk/noxious weed exams according to the formulas below. The *PW Calculator* automatically calculates these purity and bulk/noxious weights (B5) based on the rounded mean entered in B4.
  - Purity wt. (2,500 seeds)** = mean weight of 100 seeds x 25. *Do not round results.*
  - Bulk/noxious weed exam wt. (25,000 seeds)** = purity weight x 10. *Do not round results.*
- 7) Enter the minimum purity working weight (B5 of the *PW Calculator*) for that sample in the provided field in D1 the *PW Calculator*.
- 8) Repeat the above steps for each sampled seedlot when the CV does not exceed acceptable limits.

**Table 1.** Illustration of calculations for determining the mean, variance, standard deviation, and CV for a single seedlot (AOSA Rules, vol. 1, sec. 13.3b, 2022).

Rep.	$x$ (Wt. in g of 100 seeds)	$(x - \bar{x})^2$	$s^2 = \frac{\Sigma(x - \bar{x})^2}{n - 1}$	$SD = \sqrt{s^2}$	$CV(\%) = \frac{SD}{Mean} \times 100$
1	0.5321	$(0.5321 - 0.5078)^2 = 0.00059049$	$s^2 = \frac{0.00169114}{7}$ $= 0.00024159$	$SD =$ $\sqrt{0.00024159}$ $= 0.01554312$	$CV = \frac{0.01554321}{0.5078} \times 100$ $= 3.06089208$ $= 3.1\%$
2	0.5007	$(0.5007 - 0.5078)^2 = 0.00005041$			
3	0.4983	$(0.4983 - 0.5078)^2 = 0.00009025$			
4	0.4851	$(0.4851 - 0.5078)^2 = 0.00051529$			
5	0.5264	$(0.5264 - 0.5078)^2 = 0.00034596$			
6	0.5126	$(0.5126 - 0.5078)^2 = 0.00002304$			
7	0.4991	$(0.4991 - 0.5078)^2 = 0.00007569$			
8	0.5077	$(0.5077 - 0.5078)^2 = 0.00000001$			
	$\Sigma$ (sum) = 4.062	$\Sigma(x - \bar{x})^2$ = 0.00169114			
	Mean ( $\bar{x}$ ) $\bar{x} = \frac{4.062}{n}$ = 0.5078				

$n = 8$  (number of replicates);  $s^2$  = variance; SD (standard deviation) =  $s$ ; CV (%) = coefficient of variation. Note that hand calculations, as in the above example, may sometimes produce slightly different results compared to calculations using Excel's functions.

### III. Determination of Purity and Bulk/Noxious Weed Weights for a Single Seedlot Sample When the CV Exceeds the Acceptable Limit

- 1) If the CV of the first 8 replicates exceeds the acceptable limit of 6.0% or 4.0% for chaffy and non-chaffy seeds, respectively, count and weigh eight additional replicates from the same sample and enter the additional replicate weights in B1 (*Example 2*). The rank of each replicate weight, based on its absolute difference from the mean, is displayed to the right of the data. The two highest ranks (greatest absolute difference from the mean) are marked by corresponding red dots.
- 2) If the revised CV (C1 in the *PW Calculator*) is within acceptable limits (equal to or less than 4% or 6%) proceed to step 4 below without checking for outliers. If the CV exceeds the acceptable limit (*Example 2*), check for outliers among the 16 replicate weights. This should be done using the *PW Calculator* following the instructions under C2 and described in *Example 2*.
- 3) The generalized extreme studentized deviate (ESD) test (two-tailed test,  $\alpha = 0.05$ ) is used for identifying outliers. In part C2 of the *PW Calculator* this sequential outlier test is performed, first checking the replicate with the largest absolute difference from the mean (Rank 1), then checking the replicate with the second largest absolute difference (Rank 2) after removing

the first replicate weight from the data. A ‘YES’ (i.e., outlier) result for Rank 1 and a ‘NO’ (i.e., not an outlier) for Rank 2 is interpreted as the presence of a single outlier (Rank 1 result). Delete the corresponding Rank 1 replicate weight and proceed to C3 after confirming that the CV is now within acceptable limits. A ‘YES’ result for Rank 2 is interpreted as the presence of two outliers. When two out of 16 replicates are outliers, this set of replicate results should not be included when calculating purity and bulk/noxious working weights for inclusion in Table 2A. While variation among seedlots is to be expected, excessive variation among replicate weights from the same sub-sample, regardless of seedlot homogeneity, is a strong indication of methodological errors in dividing, sampling, counting and/or weighing, with possible errors in uniformly applying PSU criteria for the kind in question, rather than a problem with seedlot homogeneity. Dividing and re-sampling, with greater attention to counting, weighing and uniform application of PSU criteria should be considered.

- 4) Before calculating final results for that seedlot, the unrounded average weight of the remaining replicates (C3 and *Example 2*) should be rounded as described in II.5 above. The *PW Calculator* presents the same choices for correct rounding (C3) and requires the user to manually input the rounded mean (C4 of the *PW Calculator*) for subsequent determinations.
- 5) Use the rounded mean (*Example 2*) to calculate the 2,500 seed weight for purity analysis and 25,000 seed weight for bulk/noxious weed exams according to the formulas below. The *PW Calculator* automatically calculates these purity and bulk/noxious weights (C5) based on the rounded mean entered in C4.

**-Purity wt. (2,500 seeds)** = mean weight of 100 seeds x 25. *Do not round results.*

**-Bulk/noxious weed exam wt. (25,000 seeds)** = purity weight x 10. *Do not round results.*

- 6) Enter the minimum purity working weight (C5) for that sample in D1.
- 7) Repeat the above steps for each sampled seedlot when the CV exceeds the acceptable limit.

#### **IV. Determining Minimum Weights for Inclusion in Table 2A (Purity and Bulk/Noxious Weed Exams)**

- 1) To determine the minimum purity sample weight, enter the purity weight for each sampled seedlot, as calculated in sections II and III above, into part D1 of the *PW Calculator* (*Examples 3 and 4*).
- 2) Check the CV (part D2 of the *PW Calculator*). For the purposes of determining the minimum weight of a purity sample based on all sampled lots, the CV should not exceed 10% for either chaffy or non-chaffy kinds. Proceed to the next step only if the CV is acceptable. *If the CV is greater than 10%, consult the AOSA/SCST Statistics Committee before proceeding further.* By additional analysis of your results, the Statistics Committee may be able to suggest valid methods to deal with the excessive variation among seedlots. For some species, this will be due to natural and wide seed size variation among seedlots, and therefore single-value estimates for the species are not recommended. For others, where the CV is slightly above 10%, including additional seedlots may reduce the CV. In yet other cases, a single seedlot might be an outlier that does not reflect the true range of variation within lots of that species.
- 3) The mean purity weight from all lots is calculated to four decimal places (D3). *Do not use this value when proposing an addition/change to Table 2A of the Rules, vol. 1 (2022).* This mean is used to determine an unrounded minimum purity working weight (D4 of the *PW*

*Calculator*). Minimum purity weights are determined as the upper-tailed 95% confidence limits of the mean.

- 4) Round the minimum purity working weight (D4) as described in sec. 13.4b.1 of the rules, vol 1. (2022), before inclusion in Table 2A. If the minimum purity working weight is less than 1.000 g, round to 2 decimals; 1.000-4.999 g, round to 1 decimal; and more than 5.000 g, round to a whole number. The *PW Calculator* presents the same rounding choices (D4) and requires the user to manually input the correctly rounded value in D5. ***The value you enter in D5 of the PW Calculator is the proposed value for addition to or revision of Table 2A.***
- 5) The bulk/noxious minimum weight is then automatically determined by multiplying the rounded minimum purity weight by 10 (D6). ***The value found in D6 of the PW Calculator is the proposed value for addition to or revision of Table 2A.***

## V. Calculating Number of Seeds in a Sample Based on the Purity Weight Determination (Optional)

As noted in the introduction, the *PW Calculator* is designed to calculate purity and bulk/noxious exam weights with seed counts as close to 2,500 and 25,000 as possible, respectively. You can check your results and the range of seed numbers per seedlot in part E of the *PW Calculator*. This part would calculate actual seed numbers that would result from using the proposed minimum purity working weight, if seedlots of that species had the same range of seed weights as the ones used in your study. This range is provided so that both you and reviewers can assess the implications of adding the proposed values to Table 2A. The seed number (*Example 5*) can be calculated for any lot, L, according to the following formula:

$$\text{Expected seed number} = \frac{\text{Minimum purity weight (g)} \times 2500}{L \text{ purity weight}}$$

## VI. Information That Must Be Included in Rules Proposals to Establish Weight and Number of Seeds for Purity and Noxious Weed Exams

- 1) A separate section with a description of all seedlots, verifying that they encompassed a reasonable range of variation for the kind being investigated:
  - a. Genetic background information for each lot (e.g., variety name). If the kind tested is not sold under different variety names, this should be indicated. For kinds marketed under different variety names, a generic seedlot cannot be used. Multiple lots of the same variety cannot be used unless there are no other known varieties of that kind.
  - b. Differences in field production including location or country, production year, and production conditions (e.g., cultural practices and prevalent environmental conditions such as drought, heat stress, etc.).
  - c. Confirmation that tests were made on seeds from ‘market-ready’ seedlots, as appropriate for the kind being tested (i.e., the seedlot tested is in a condition that is commonly marketed to consumers). Field runs, uncleaned and unscreened seeds, as well as mixtures where the target species represents only a small proportion of the lot, should not be used.
- 2) For species that are not currently in the Rules, the PSU definition and PSU number, if applicable.

- 3) Final mean weight of 100-seed replicates for each lot (results of parts B4 or C4 of the *PW Calculator*).
- 4) Calculated purity (2,500 seeds) and bulk/noxious (25,000 seeds) weights for each seedlot (results of parts B5 or C5 of the *PW Calculator*).
- 5) CV and mean purity weight over all seedlots determined using the *PW Calculator* (results of parts D2 and D3 of the *PW Calculator*).
- 6) Calculated minimum purity weight determined using the *PW Calculator* (part D4).
- 7) The final (rounded) minimum purity and bulk/noxious sample weights proposed for inclusion in Table 2A of the Rules, Vol. 1 (parts D5 and D6 of the *PW Calculator*).

**In a separate appendix, include:**

- 1) All raw data (preferably as an Excel spreadsheet): single replicate 100-seed weights for each lot, replicate data used and discarded as outliers, as well as means and CVs.
- 2) **Optional.** The number of seeds in a purity working weight for each lot used in your study, calculated based on your proposed minimum purity weight (results of parts E of the *PW Calculator*).

**Example 1.** Calculations applied to a **single seedlot** sample of non-chaffy seed species when the CV **does not exceed** the acceptable limit (4.0%). The same calculations are applied to each seed lot sample with an acceptable CV.

Seedlot	Replicate	100-seed weight (g)
A	1	0.1434
	2	0.1403
	3	0.1472
	4	0.1519
	5	0.1488
	6	0.1346
	7	0.1469
	8	0.1407
Results before Rounding	Mean	0.144225
	Variance	0.0000309993
	SD	0.0055677
	CV (%)	3.86042657
Rounded Results	Mean	0.1442
	CV (%)	3.9 (below 4.0%) Mean replicate weight (0.1442) is used to determine weight of 2,500 seeds
<b>Purity weight</b> (2,500 seeds) = 0.1142 x 25 = 3.605 g (results are not rounded)		
<b>Bulk/Noxious weed exam wt.</b> (25,000 seeds) = 3.605 x 10 =36.05 g (results are not rounded)		

SD: standard deviation.

**Example 2.** Calculations applied to a **single seedlot** of non-chaffy seed species since the CV exceeds the acceptable limit (4.0%). The same calculations are applied to each seed lot with a CV exceeding the acceptable limit. In this example, the first 8 replicates resulted in a CV of 6.2%.

Seedlot	Replicates	100-seed weight (g)
<b>B Original 8 replicates</b>	<i>1</i>	0.1394
	<i>2</i>	0.1506
	<i>3</i>	0.1507
	<i>4</i>	0.1394
	<i>5</i>	<del>0.1623</del> ●
	<i>6</i>	0.1356 ●
	<i>7</i>	0.1399
	<i>8</i>	0.1407
<b>B Additional 8 replicates</b>	<i>9</i>	0.1455
	<i>10</i>	0.1454
	<i>11</i>	0.1473
	<i>12</i>	0.1449
	<i>13</i>	0.1453
	<i>14</i>	0.1443
	<i>15</i>	0.1495
	<i>16</i>	0.1491
	<b><i>Revised CV (%) of 16 replicates</i></b>	4.3
	<b><i>Mean of 16 replicates</i></b>	0.1456
<p>Replicates 5 and 6 are marked by the <i>PW Calculator</i> as potential outliers. An outlier check (C2) indicates that replicate 5, with the largest absolute difference from the mean, is an outlier while replicate 6 is not. Weight of replicate 5 is deleted and the mean re-calculated. The revised CV after deleting one replicate is now 3.2% (acceptable).</p>		
<b>Final mean</b>	0.1445 Rounded mean wt. after discarding one outlier weight (0.1623 g). Use this mean to determine purity/bulk/noxious weed exam weights for this lot.	
<b>Purity weight</b> (2,500 seeds) = 0.1445 x 25 = 3.6125 (results are not rounded)		
<b>Bulk/Noxious weed exam wt.</b> (25,000 seeds) = 3.6125 x 10 = 36.125 g (results are not rounded)		

**Example 3.** Two illustrations of data used in determining the minimum purity weight (MPW) and bulk/noxious weed weight (MBNW) for non-chaffy (species 1) and chaffy (species 2) kinds. The same data analysis for species 2 is illustrated in *Example 4* using the *PW Calculator*.

<b>Data entry into the <i>PW Calculator</i></b>		
<b>Seedlot</b>	<b>Purity Weight, Species 1</b> Low seed wt. variation across lots (uniform)	<b>Purity weight, Species 2</b> Moderate seed wt. variation across lots (less uniform)
<i>A</i>	3.105	0.6325
<i>B</i>	3.398	0.6158
<i>C</i>	3.302	0.6804
<i>D</i>	3.324	0.5961
<i>E</i>	3.335	0.6833
<i>F</i>	3.365	0.5499
<i>G</i>	3.405	0.6117
<i>H</i>	3.599	0.6239
<b>Output (using the <i>PW Calculator</i>)</b>		
<b>CV (%)</b>	4.1 (acceptable; less than 10%)	7.0 (acceptable; less than 10%)
<b>Mean</b>	3.3541	0.6242
<b>MPW (unrounded)</b>	3.44564	0.65335
<b>Rounded results for inclusion in Table 2A</b>		
<b>MPW</b>	3.4 g	0.65 g
<b>MBNW</b>	34 g	6.5 g



**Example 4.** Using the *PW Calculator* to analyze data presented in *Example 3* (species 2) above. Step by step instructions listed in Box D correspond to identically numbered parts of the calculator to the right of the box. The calculated purity weight is entered for each lot (D1), resulting in an unrounded minimum purity working weight (D4). The user is presented with all rounding options according to the rules and is required to manually input the correct value in D5. The rounded minimum purity weight (D5) and minimum bulk/noxious weed weight (D6) are then proposed for inclusion/revision of Table 2A, assuming that the CV (D2) is within acceptable limits.

D	D1. Average purity weight for each seed lot		
<p><b>D1.</b> For each seed lot, enter the average purity weight (2500 seeds) calculated in either B5 or C6 without further rounding.</p> <p><b>D2.</b> Check the CV. For the purposes of determining the minimum weight of a purity sample (or bulk/noxious weight), the CV should not exceed 10% for either chaffy or non-chaffy kinds. If the CV is greater than 10%, single reliable estimates of minimum purity and bulk/noxious weed weights cannot be calculated based on the sampled seed lots.</p> <p><b>D3.</b> The average purity weight from all lots is calculated. <i>Do not use this value when proposing an addition/change to Table 2A of the Rules, vol. 1 (2022).</i></p> <p><b>D4.</b> The <b>Minimum Purity Working Weight (g)</b>, derived from the value in D3, is calculated without rounding. This value is the upper limit 95% confidence interval for the mean (D3). Results must be rounded to the correct number of decimal places, as described in sec. 13.4b.1 of the rules, vol. 1 (2022), before inclusion in Table 2A of the rules.</p> <p><b>D5.</b> Manually enter the correctly rounded mean from D4 in the provided field. <i>This is the value to be proposed for addition to Table 2A of the rules.</i></p> <p><b>D6.</b> The minimum bulk/noxious weed weight for inclusion in Table 2A is automatically generated.</p> <p><i>Before a new analysis for a different species, make sure to clear the data entered under A1, B1, B4, C4, D1 and D5.</i></p>	Lot No.	ID	Calculated purity weight (g)
	1	A	0.6325
	2	B	0.6158
	3	C	0.6804
	4	D	0.5961
	5	E	0.6833
	6	F	0.5499
	7	G	0.6117
	8	H	0.6239
	9	✓	
	10	✓	
	11	✓	
	12	✓	
	13	✓	
	14	✓	
	15	✓	
	16	✓	
	17	✓	
	18	✓	
	19	✓	
20	✓		
<b>D2. CV (%):</b>		7.0	
<b>D3. Mean purity weight</b>		0.6242	
<b>D4. Unrounded Minimum Purity Working Weight (g):</b>		0.65335	
Minimum purity weight rounded to 2 decimals:		0.65	
Minimum purity weight rounded to 1 decimal:		0.7	
Minimum purity weight rounded to whole number:		1	
<b>D5. Minimum Purity Working Weight (g):</b>		0.65	
<b>D6. Minimum bulk/noxious weed weight (g):</b>		6.5	

**Example 5.** Actual seed number in a purity working weigh when the minimum purity weight from Table A2 (*PW Calculator* part D5) is used on seedlots with different purity weights (part D1 of the *PW Calculator*). Data in this example, automatically generated in part E of the *PW Calculator*, are from species 2 of Example 4 above, where the minimum purity weight for species 2 was calculated to be **0.65 g**.

<p><b>E (Optional)</b></p> <p><b>E1.</b> The minimum purity working weight entered in D5 is automatically imported.</p> <p><b>E2.</b> The purity weights for each lot are imported from D1. The estimated number of seeds for each lot is automatically calculated.</p> <p><i>Before a new analysis for a different species, make sure to clear the data entered under A1, B1, B4, C4, D1 and D5.</i></p>	<b>E1. Minimum purity working weight (g):</b>		<b>0.65</b>
	<b>E2. Estimated seed number</b>		
	<b>Lot ID</b>	<b>Purity weight</b>	<b>Seed number per lot</b>
	<b>A</b>	<b>0.6325</b>	<b>2569</b>
	<b>B</b>	<b>0.6158</b>	<b>2639</b>
	<b>C</b>	<b>0.6804</b>	<b>2388</b>
	<b>D</b>	<b>0.5961</b>	<b>2726</b>
	<b>E</b>	<b>0.6833</b>	<b>2378</b>
	<b>F</b>	<b>0.5499</b>	<b>2955</b>
	<b>G</b>	<b>0.6117</b>	<b>2657</b>
	<b>H</b>	<b>0.6239</b>	<b>2605</b>

**Interpretation example:** for seedlot A, the actual seed number would be:  $(0.65 \times 2500)/0.6325 = 2569.16996 = \mathbf{2569}$  after rounding.