I2I-SOP-43: Methods for Performing Calculations and Dilutions

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Prepared by

Name	Role	Institution
Jennie Gibson	Trainee Study Director	LITE
Annabel Murphy	Research Assistant	I2I
Katherine Gleave	PDRA	I2I

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Related documents

- I2I-SOP-029 Intrinsic toxicity testing

Contents

1. Purpose

The purpose of this SOP is to enable calculations to be carried out for various experimental techniques. These include but are not limited to:

- CDC Bottle bioassays
- Coating papers with insecticides
- Tarsal bioassay on glass plates
- Topical testing

2. Background

International System of Units

The International System of Units (SI Units) forms the basis of measurement within science. The system is built upon seven base units, a set of twenty prefixes and twenty-two derived units. For dilutions used within I2I only a few of these will be used.

Table 1: SI base units

Unit	Symbol	Description
Metre	m	Standard unit of length
Kilogram	kg	Standard unit of mass
Mole	Mol	Standard unit for amount of substance

Table 2: Prefix names and symbols used to indicate an increase or decrease against the SI base unit.

Prefix		Decimal
Name	**Symbol**	
Kilo	k	1000
		1
Centi	c	0.01
Milli	m	0.001
Micro	μ	0.000 001

Concentration and Units

Concentration is the abundance of a given substance within a specific volume. There are specific terms which are used to describe the substances involved in concentration. A solute is a substance (normally a solid) which is dissolved. A solvent is a substance that dissolves a solute resulting in a solution.

Concentration can be described qualitatively using the terms dilute and concentrated. A dilute substance is one which has a few molecules of solute in relation to the molecules of solvent. Conversely a concentrated substance is one in which there are a large number of molecules of solute in relation to the molecules of solvent.

There are several different units of concentration which may need to be used depending on the protocol.

Percentage Concentration

This is a standard unit of concentration with all other units of concentration are normally converted into a percentage. The formula for percentage concentration is as follows (note: units of mass is grams; units of volume are mL):

Percentage concentration= (Mass of solute/Volume of solution) x 100

Percentage concentration= (Volume of Solute/Volume of Solution) x 100

Note: A 10g solute dissolved in 10mL of solvent would give a 100% solution. With regards to percentage concentration mass and weight can be used interchangeably with weight being convention and mass being correct to SI standards.

Molar concentration

Formula for the calculation of molar concentration is as follows:

Concentration = Number of moles of solute/Volume of Solution

Number of Moles= Mass of substance/Relative Formula Mass

Note: Formula for the calculation of molar concentration. Units of mass is grams, units of volume are mL, unit of moles is mol, unit of concentration is mol/dm^3. Relative formula mass is calculated by adding the relative atomic mass of all atoms within a molecule or compound.

Mass concentration

Formula for the calculation of mass concentration is as follows:

Concentration = Mass of Solute/Volume of Solution

Note: Units of mass is grams, units of volume are mL, units of concentration are g/dm^3.

Mass/Area concentration

Mass/Area concentration is a:

Concentration = Mass of Solute/ Area of Application

Note: Units of mass is grams, units of area is cm^2, units of concentration are g/cm^2.

Conversion between different units of concentration

The standard unit of concentration used within I2I is percentage concentration. Conversion of units will be towards percentage concentration. As the standard unit of mass and volume given in percentage concentration are grams (g) and millilitres (mL) initial steps will involve conversion into these units before using the percentage concentration formula.

Mass concentration into Percentage Concentration

1. Presuming a volume of 1 the mass of solute used is equal to the concentration.
2. If necessary, convert the units for the mass of solute into grams.
3. If necessary, convert the units of volume in millilitres.
4. Use the percentage concentration formula to calculate the concentration.

A worked example of this is detailed below:

A solution has a concentration of 5mg/mL, convert this into percentage concentration as follows:

1. Presuming a volume of 1 the mass of solute is 5mg.
2. To convert mg into g divide by 1000 therefore the mass in g is 0.005g.
3. The units of volume are already mL, so no conversion is needed therefore the volume is 1mL.
4. Use the percentage concentration formula below: (0.005/1) x 100 = 0.5%

Percentage concentration= (Mass of solute/Volume of solution) x 100

Percentage concentration= (Volume of Solute/Volume of Solution) x 100

3. Materials and equipment

- Balance, spatulas and weighing boats for weighing insecticide
- Appropriate size pipettes and filtered tips
- Glass bottles and caps
- Fume hood for all insecticide work
- Paper support rack for impregnation of filter papers
- Sharps bin
- Permanent marker
- Blue roll
- Acetone (or alternative suitable solvent)
- Rack for dilution bottles
- Plastic beakers
- Glass pen

4. Health, safety, and environmental protection

- Refer to Material Safety Data Sheets (MSDS) for chemical hazard information for each chemical used.
- Refer to Control of Substances Hazardous to Health (COSHH) Assessment for each chemical used.
- All staff working in laboratories must have received laboratory induction training.
- All staff using this procedure must be trained in safe operation of chemical fume hoods.
- Appropriate personal protective equipment (PPE) must be worn when handling insecticides. Including, laboratory coat, gloves, safety glasses and a face mask when weighing out chemicals.
- Dispose of all waste materials quickly and appropriately. Chemically hazardous waste materials should be disposed of according to laboratory procedure.

5. Procedure

Dilution

In order to find the actual amount of active required use the following formula:

C2 x V2/C1= V1

- **C2**= Final Concentration
- **V2**=Volume of Solvent required (mL)
- **C1**= Initial Concentration
- **V1**- Initial mass required (g)

Calculations from µg/test item or mg/test item into percentage (%)

When carrying out calculations for a bioassay test it is calculated in percentage (%). The amount of insecticide required may be given in µg/test item or mg/test and not the required % concentration from a stock. To use the standard record sheets a conversion from µg/test item to % stock concentration a conversion is required.

Follow these steps to do the conversion:

1. Take your value for µg/test item and divide by 1000 to get mg/test item (as there are 1000µg in one mg).
2. Divide the value of mg/test item by how many mL are put into the test item to get mg/mL.
3. 10mg/mL is equal to a 1% stock solution, so divide your value for mg/mL by 10 to get your required % stock solution.

Refer to Appendix 1 for CDC bottle assay examples and Appendix 2 for tarsal plate assay examples.

Calculation for conversion from mg/m^2 to percentage (%)

When carrying out calculations for a bioassay test it is calculated in a percentage (%). If the amount required is given in mg/m^2 and not the required % concentration a conversion is required.

Follow these steps to do the conversion:

1. Take mg/m^2 and multiply it by the area, then divide by 10000 ($1m^2$=10000 sq cm), to calculate mg.
2. Divide mg by volume in mL to calculate mg/mL.
3. Divide mg/mL by 10 to get concentration % (1 % = 10mg/mL)

Refer to Appendix 2 for a tarsal plate assay example and Appendix 3 for a CDC bottle bioassay assay example.

Dilution calculations from percentage (%)

Once the % stock solution is calculated/obtained use the equation below to make up your stock solutions:

C2 x V2/ C1= V1

Starting with a baseline solution, in general this is a 1% solution of active ingredient (this may change depending on the dilution requirements).

- **C2**= Final concentration of active
- **V2**=Volume of Solute required (mL)
- **C1**= Stock concentration (taken from the active ingredient bottle)
- **V1**- Amount of active required (g)

To determine the actual amount of active required use the following formula:

C2 x V2/C1=V1

- Take your final concentration of active (C2) and multiply it by the amount of solute required (mL) (V2), and then divide it by the stock concentration taken from the active ingredient bottle (C1), to calculate the amount of active required (V1)
- e.g. 1 x 1 / 99= 0.0101g as shown in Table 3 (the blue columns are filled out once the active ingredient has been weighed out.)

Table 3: Dilution calculation example

Active Ingredient (Name and concn)	C2= Final concn (%)	V2= Volume of final concn required (mL)	C1= Stock concn (%)	V1= Amount required (g or mL)	V1= Actual amount of active weighed (g)	Adjustment required to V1? Yes/No	V2= Actual solvent amount added for final concn (mL)
Example	1	1	99	0.0101g	0.0101g	No	1.000mL

Weighing out the Active Ingredient for a solid

Note: Before the dilution calculation table in the record sheet template is completed, consider the required concentration, and decide if a serial dilution will be required to achieve the desired concentration. For example, if the stock is 95% and you require 0.001% concentration, good practice would be to make up a 1% stock dilution first from the 95% stock and then a 0.05% followed by the final 0.001% dilution. Come down the units if necessary and not skip straight from 1%-0.001%.

In the fume hood, obtain the glass bottle for the stock, label and tare it on the balance. Using a pipette if the active ingredient is a liquid or a spatula if it is a powder, carefully weigh out the amount required (V1=Amount of active required). Record in the Dilutions Calculation table, (Table 7) available in the record sheet template.

The fume hood fan should be switched off when weighing as this can interfere with the reading. Switch the fume hood fan back on once the reading is obtained.

If the actual weight is not achieved, recalculation is required as shown in Table 4 and 5.

From the information calculated in the dilution calculation sheet, pipette the required amount of solvent into the stock bottle, along with the active and vortex.

- Using the equation **C1** (Actual stock concentration) x **V1** (Actual amount of active weighted)/ **C2** (Final concentration required) = **V2** (Actual solute amount added for final concentration)
- E.g. 99 x 0.0105g / 1 = 1.040mL shown in Table 4.

Add the required amount of acetone to the empty dilution bottles following the dilutions calculation table available in the record sheet template (Table 7).

Perform the serial dilution/s by pipetting from the first bottle into the second and vortexing. Next, pipette the required volume from the second bottle into the third and vortex, repeat this process for all the required serial dilutions. More than one dilution can be taken from a stock bottle, e.g. 1% might be used to make a 0.1% and a 0.01% solution.

Note: Do not pipette directly from a solvent container. Pour some of the solvent in a separate beaker beforehand. If the solvent container is large, use a titration pipette to decant some into a separate beaker.

Table 4: Dilution calculation example. Worked example of an adjustment.

Active Ingredient (Name and concn)	C1= Stock concn (%)	V1= Actual amount weighed (g)	C2= Final concn (%)	V2= Actual solvent amount added for final concn (mL)
Example	99	0.0105	1	1.040mL

Table 5: Worked example of a 1% solution with an adjustment required.

Active Ingredient (Name and concn)	C2 = Final concn (%)	V2= Volume of final concn required (mL)	C1= Stock concn (%)	V1= Amount required (g or mL)	V1= Actual amount of active weighed (g)	Adjustment required to V1? Yes/No	V2= Actual solvent amount added for final concn (mL)
Example	1	1	99	0.0101g	0.0105g	Yes	1.040mL

Calculating the volume needed when dealing with a liquid compound

Note: If the active ingredient is a liquid, always add this into the stock bottle already containing the solvent. Take the required amount from the active, then place the pipette tip into the bottle containing the solvent just slightly underneath the surface. Press the pipette plunger to the first stop, then slowly release up. Do this in a continual motion three times, then press the pipette to the second stop, hold, remove the pipette from the bottle and release and eject the plunger. This will ensure the whole amount of the active ingredient is transferred from the pipette tip into the solvent.

A record sheet template for liquid dilutions is available in Table 8 in the record sheet template. When dealing with an active ingredient which is a liquid, the density of the liquid needs to be considered to calculate the volume of the active ingredient needed to make a 1% solution. If the density is supplied with the chemical, then the equation **V1/Density=Amount** required is used. If the density is not supplied with the compound use the following steps to calculate the density. Use Table 6 in the record sheet section to record the following steps.

1. Place a minimum of 1mL of deionized water into a 7mL bottle with a lid, place onto the balance and press tare.

2. Remove from the balance and add 0.020mL of active ingredient, place onto the balance and weigh.
3. Repeat step 2 a further two times.
4. To calculate the density add all weights together and get a total weight, divide the total weight by the number of readings (total weight/ 3=average), divide the average weight by the volume used to give the density (average/0.02=density). Available in Table 6 in the record sheet template.

6. Cleaning and maintenance

Cleaning and consumable preparation

Wipe down all pipettes, tip boxes, balance and sharps bin with 5% Decon 90 followed by a water and an ethanol wipe. Clean spatulas by soaking in a 5% Decon 90 solution for 2 hours then rinse twice with tap water and once with deionized water.

Dispose of remaining dilutions, spare solvent and contaminated glassware as chemical waste.

7. Glossary of terms

- CDC Centres for Disease Control and Prevention
- COSHH Control of Substances Hazardous to Health
- C Centi
- Cm^2 Centimetre squared
- Concn Concentration
- Decon Decontamination
- Dm Decimetre
- G Gram
- I2I Innovation to Impact
- K Kilo
- Kg Kilogram
- LSTM Liverpool School of Tropical Medicine
- M^2 Metre squared
- µ Micro
- Mg Miligram
- MSDS Material Safety Data Sheets
- mL Mililitre
- Mol Mole
- % Percentage
- PPE Personal Protective Equipment
- SI Internation System of Units
- SOP Standard Operating Procedure
- Sq Square

8. References

9. Appendices

Appendix 1: CDC Bottle assay example calculations from µg/test item or mg/test item into percentage %

When coating bottles 1.6mL of the stock concentration is put into a 250mL bottle with a surface area of 280sq cm.

1. Take your value for µg/bottle and divide by 1000 to get mg/bottle (as there are 1000µg in one mg). E.g. 7.5/1000=0.0075mg/bottle.
2. Divide the value of mg/bottle by how many mL are put into the bottle to get mg/mL (i.e. divide by 1.6 if 1.6mLs are added to the bottle). E.g. 0.0075/1.6=0.004688

10mg/mL is equal to a 1% stock solution, so divide your value for mg/mL by 10 to get your required % stock solution. E.g. 0.004688/10=0.0004688%

µg/bottle	Mg/bottle = (µg/bottle)/1000	Mg/mL=(mg/bottle)/1.6	Concn % =(mg/mL)/10
7.5	0.0075	0.004688	0.0004688

Appendix 2: Tarsal assay example calculations from µg/test item or mg/test item into percentage %

When coating the tarsal plate 0.5mL of the stock concentration is put onto a tarsal plate with a surface area of 19.6sq cm.

1. Take your value for µg/plate and divide by 1000 to get mg/plate (as there are 1000µg in one mg). E.g. 7.5/1000=0.0075mg/plate
2. Divide the value of mg/plate by how many mL are put onto the plate to get mg/mL (i.e. divide by 0.5 if 0.5mLs are added to the tarsal plate) e.g. 0.0075/0.5=0.015mg/mL
3. 10mg/mL is equal to a 1% stock solution, so divide your value for mg/mL by 10 to get your required % stock solution. E.g. 0.015/10=0.0015%

µg/plate	Mg/plate = (µg/plate)/1000	Mg/mL=(mg/plate)/0.5	Concn % =(mg/mL)/10
7.5	0.0075	0.015	0.0015

Appendix 3: CDC bottle assay example calculation for conversion from mg/m^2 to percentage %.

The amount of RME required on a bottle is $100mg/m^2$ (this may change depending on requirements). When coating the glass bottle 1.6mL of stock concentration is a standard required amount added to a 250mL glass bottle with a surface area of 280 sq cm. To achieve a % stock concentration a conversion from mg/m^2 is required.

1. Take mg/m^2 and multiply it by the area, then divide by 10000 ($1m^2$=10000 sq cm), to calculate mg. E.g. (100x280)/10000=2.8mg.
2. Divide mg by volume in mL to calculate mg/mL. E.g. 2.8/1.6=1.75mg/mL
3. Divide mg/mL by 10 to get concentration % (1%= 10mg/mL). E.g. 1.75/10=0.175%

Use this to get from mg/m^2 to % concentration

mg/m^2	area (sq cm)	mg	volume mL	mg/mL	Concn %
100	280	2.8	1.6	1.75	0.175

The amount of RME required on a tarsal plate is $100mg/m^2$ (this may change depending on requirements). When coating a tarsal plate 0.5mL of stock concentration is a standard required amount added to a tarsal plate with a surface area of 19.6 sq cm. To achieve a % stock concentration a conversion from mg/m^2 is required.

Follow these steps to do the conversion:

1. Take mg/m^2 and multiply it by the area, then divide by 10000 ($1m^2$ =10000sq cm), to calculate mg. E.g. (100x19.6)/10000=0.196mg
2. Divide mg by volume in mL to calculate mg/mL. E.g. 0.196/0.5=0.392 mg/mL

3. Divide mg/mL by 10 to get concentration % (1%= 10mg/mL) E.g. 0.392/10=0.0392%

Use this to get from mg/m^2 to % concentration:

mg/m^2	area (sq cm)	mg	volume mL	mg/mL	Concn %
100	19.6	0.196	0.5	0.392	0.0392

Appendix 4: Example table for CDC bottle assay example to calculate solvent and weight requirements for a dilution series

A	B	C	D	E	F	G	H	I
Desired concentration (µg/bottle)	**Dilution Series**	**Final Concentration**	**Final volume**	**C2V2**	**Purity**	**Desired insecticide to weigh (g)**	**Solvent to add**	**µg per bottle**
	Dilution 1	1.00	1000	1000	91.8	0.010893	1000	10000.00 µg/bottle
	Dilution series	C2 (final concentration) (**A** / 10000)	C2 (final volume)	C2V2 (**C x D**)	C1 (previous concentration)	c2v2/c1=v1 **(E/F)**	Solvent to add **(D-G)**	µg/bottle
1000.00	Dilution 2	0.1000000	1000	100	1.0000000	100	900	1000.00 µg/bottle
500	Dilution 3	0.0500000	1000	50	0.1000000	500	500	500.00 µg/bottle
100	Dilution 4	0.0100000	1000	10	0.0500000	200	800	100.00 µg/bottle
50	Dilution 5	0.0050000	1500	7.5	0.0100000	750	750	50.00 µg/bottle
40	Dilution 6	0.0040000	1500	6	0.0050000	1200	300	40.00 µg/bottle
30	Dilution 7	0.0030000	1500	4.5	0.0040000	1125	375	30.00 µg/bottle
20	Dilution 8	0.0020000	2500	5	0.0030000	1667	833	20.00 µg/bottle
10	Dilution 9	0.0010000	2500	2.5	0.0020000	1250	1250	10.00 µg/bottle
5	Dilution 10	0.0005000	1500	0.75	0.0010000	750	750	5.00 µg/bottle

Record sheet templates

Table to record density of a compound

Table 6: Table used to calculate density of a compound.

Active Ingredient Details (Name and concn)	Run	Weight (g)	Average = Total / 3	Density= Average/0.020
	1.			
	2.			
	3.			
	Total:			

Dilutions Calculation Table for a solid

To calculate the amount of active ingredient required C2 X V2 / C1= V1 Amount required. The blue columns are filled out once the active ingredient has been weighed out.

Table 7: Dilutions calculation table for a solid

Active ingredient details (Name and concentration)	C2= Final concn (%)	V2= Volume of final concn required (mL)	C1= Stock concn (%)	V1= Amount required (g or mL)	V1= Actual amount weighed (g)	Adjustment required to V1? Yes/No	V2=Actual Solvent amount added for final concn (mL)

Dilutions Calculation Table for a liquid

The blue columns are filled out once the active ingredient has been weighed out.

Table 8: Dilutions calculation table for a liquid

Active ingredient details (Name and concentration)	Density	C2= Final concn (%)	V2= Volume of final concn (mL)	C1= Stock concn (%)	V1= Amount required (g or mL)	V1/Density= Actual amount required (mL)	Adjustment required to V1? Yes/No	V2=Actual solute amount added for final

V2 Adjustment calculation for Active Ingredient

If the actual amount of the active ingredient is not achieved, a recalculation of the actual solvent will be required using the following calculation:

C1 Actual stock concentration x **V1** actual active ingredient added/ **C2** final concentration required = **V2** Actual solvent amount added for final concentration.

Table 9: Table for adjustment calculation for an active ingredient

Active ingredient details (Name and concentration)	C1=Stock Concn (%)	V1= Actual amount weighed	C2= Final concn (%)	V2= Actual Solvent amount added for final concn (mL)

V2 Control - Adjustment calculation for Control

If the actual amount of the control is not achieved, a recalculation of the actual solvent will be required using the following calculation **C1** Actual stock concentration **x V1** actual active ingredient added/ **C2** final concentration required **V2** Actual Solvent amount added for final concentration.

Table 10: Table for adjustment calculation for control

Active ingredient details (Name and concentration)	C1=Stock Concn (%)	V1= Actual amount weighed	C2= Final concn (%)	V2= Actual Solvent amount added for final concn (mL)