Practical Skills in Food Science, Nutrition and Dietetics

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Box 23.1 Applying for ethics approval for your research from an ethics committee

The exact procedures and processes for applying for ethical approval will vary between organisations, but the following questions are likely to be asked (ESRC, 2010). All of the information submitted to the committee should be explained in a way that would be understandable to someone with limited scientific knowledge of the subject.

1. **Aims of the research**. You need to make it clear what you are going to achieve in the project so that the committee can judge whether your proposal is likely to achieve these aims.
2. **Scientific background of the research.** All research proposals are supported by existing research. Review of existing literature will allow you to ensure that this research has not already been done.
3. **Study design**. How do you propose to do the study?
4. **Participants**. Who will be taking part in the study and also who is not eligible to take part? How will these people be identified and how many participants do you need to achieve your aims?
5. **Vulnerable groups.** Does your research involve children or those with a learning disability or cognitive impairment?
6. **Methods of data collection.** What methods do you intend to use? The committee will consider the appropriateness of the methods to meet the aims of the project.
7. **Methods of data analysis.** It is important that you consider how you will analyse the data you collect. If you are not clear how you will do this you may need to reconsider your methods or study design.
8. **Risks and benefits to research participants or third parties**. It is generally not considered ethical to offer any kind of inducement to take part in a research project as this may be seen as coercion. Risks to participants may include physical or psychological harm.
9. **Risks to researchers**. It is important that you consider your own safety, whether you are using harmful chemicals in a laboratory or are collecting data outside of the university.
10. **Procedures for informed consent**. Participants must be fully aware of what is expected of them, what you will do with the data you collect and what will be the impact of taking part in this research. Informed consent must be collected for all aspects of the project, so if you plan to use data or samples collected at a later date you must make this clear to participants.
11. **Expected outcomes, impacts and benefits of research**. How will this research project add to existing knowledge?
12. **Dissemination of findings**. How will your results be shared with a wider audience and how will participants be able to find out the outcomes of the research? It is not usually appropriate to provide individual feedback to participants.
13. **Measures taken to ensure confidentiality, privacy and data protection.** This could involve using only numbers to identify data and ensuring that any personal information is stored appropriately, usually by your supervisor. Information would be destroyed at the end of the project.

Text references

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Using chemicals responsibly – be considerate to others: always return storeroom chemicals promptly to the correct place. Report when supplies are getting low to the person who looks after storage/ordering. If you empty an aspirator or wash bottle, fill it up from the appropriate source.

Finding out about chemicals – *The Merck Index* (O'Neil *et al.*, 2006) and the *CRC Handbook of Chemistry and Physics* (Lide, 2008) are useful sources of information on the physical and biological properties of chemicals, including melting and boiling points, solubility, toxicity, etc. (see Fig. 24.1).

8599. Sodium Chloride. [7647-14-5] Salt; common salt. ClNa; mol wt 58.44. Cl 60.67%, Na 39.34%. NaCl. The article of commerce is also known as ***table salt***, ***rock salt*** or ***sea salt***. Occurs in nature as the mineral ***halite***. Produced by mining (rock salt), by evaporation of brine from underground salt deposits and from sea water by solar evaporation: *Faith, Keyes & Clark's Industrial Chemicals*, F. A. Lowenheim, M. K. Moran, Eds. (Wiley-Interscience, New York, 4th ed., 1975) pp 722-730. Toxicity studies: E. M. Boyd, M. N. Shanas, *Arch. Int. Pharmacodyn.* **144,** 86 (1963). Comprehensive monograph: D. W. Kaufmann, *Sodium Chloride*, ACS Monograph Series no. **145** (Reinhold, New York, 1960) 743 pp.

Cubic, white crystals, granules, or powder; colorless and transparent or translucent when in large crystals. d 2.17. The salt of commerce usually contains some calcium and magnesium chlorides which absorb moisture and make it cake. mp 804° and begins to volatilize at a little above this temp. One gram dissolves in 2.8 ml water at 25°, in 2.6 ml boiling water, in 10 ml glycerol; very slightly sol in alcohol. Its soly in water is decreased by HCl. Almost insol in concd HCl. Its aq soln is neutral. pH: 6.7-7.3. d of satd aq soln at 25° is 1.202. A 23% aq soln of sodium chloride freezes at −20.5°C (5°F). LD_{50} orally in rats: 3.75 ±0.43 g/kg (Boyd, Shanas).

Note: ***Blusalt***, a brand of sodium chloride contg trace amounts of cobalt, iodine, iron, copper, manganese, zinc is used in farm animals.

USE: Natural salt is the source of chlorine and of sodium as well as of all, or practically all, their compds, e.g., hydrochloric acid, chlorates, sodium carbonate, hydroxide, etc.; for preserving foods; manuf soap, to salt out dyes; in freezing mixtures; for dyeing and printing fabrics, glazing pottery, curing hides; metallurgy of tin and other metals.

THERAP CAT: Electrolyte replenisher; emetic; topical anti-inflammatory.

THERAP CAT (VET): Essential nutrient factor. May be given orally as emetic, stomachic, laxative or to stimulate thirst (prevention of calculi). Intravenously as isotonic solution to raise blood volume, to combat dehydration. Locally as wound irrigant, rectal douche.

Fig. 24.1 Example of typical *Merck Index* entry showing type of information given for each chemical. From *The Merck Index: An Encyclopedia of Chemicals, Drugs, and Biologicals,* Fourteenth Edition, Maryadele J. O'Neil, Patricia E. Heckelman, Cherie B. Koch, Kristin J. Roman, Eds. (Merck & Co., Inc., Whitehouse Station, NJ, USA, 2006). Reproduced with permission from *The Merck Index*, Fourteenth Edition.

Using chemicals

Safety aspects

In practical classes, the person in charge has a responsibility to inform you of any hazards associated with the use of chemicals. For routine practical procedures, a risk assessment (p. 133) will have been carried out by a member of staff and relevant safety information will be included in the practical schedule: an example is shown in Table 24.1. This information is part of basic health and safety requirements (Chapter 22, p. 133).

In project work, your first duty when using an unfamiliar chemical is to find out about its properties, especially those relating to safety. Your department must provide the relevant information to allow you to do this. If your supervisor has filled out the form, read it carefully before signing. Box 24.1 gives further advice.

KEY POINT Before you use any chemical you must find out whether safety precautions need to be taken and complete the appropriate forms confirming that you appreciate the risks involved.

Selection

Chemicals are supplied in various degrees of purity and this is always stated on the manufacturers' containers. Suppliers differ in the names given to the grades and there is no conformity in purity standards. Very pure chemicals cost more, sometimes a lot more, and should only be used if the situation demands. If you need to order a chemical, your department will have a defined procedure for doing this.

Preparing solutions

Solutions are usually prepared with respect to their molar concentrations (e.g. mmol L^{-1}, or mol m^{-3}), or mass concentrations (e.g. g L^{-1}, or kg m^{-3}):

Table 24.1 Representative risk-assessment information for a practical exercise in molecular biology, involving the isolation of DNA

Substance	Hazards	Comments
Sodium dodecyl sulphate (SDS)	Irritant Toxic	Wear gloves
Sodium hydroxide (NaOH)	Highly corrosive Severe irritant	Wear gloves
Isopropanol	Highly flammable Irritant/corrosive Potential carcinogen	No naked flames Wear gloves
Phenol	Highly toxic Causes skin burns Potential carcinogen	Use in fume hood Wear gloves
Chloroform	Volatile and toxic Irritant/corrosive Potential carcinogen	Use in fume hood Wear gloves

Box 24.1 Safe working with chemicals

You should always treat chemicals as potentially dangerous, following these general precautions:

- **Do not use any chemical until you have considered the risks involved** – for lab classes, you should carefully read all hazard and risk information provided *before* you start work. In project work, you may need to be involved in the risk-assessment process with your supervisor.
- **Wear a laboratory coat at all times** – the coat should be fully fastened and cleaned appropriately, should any chemical compound be spilled on it. Closed-toe footwear will protect your feet should any spillages occur.
- **Make sure you know where the safety apparatus is kept before you begin working** – this apparatus includes eye bath, fire extinguishers and blanket, first aid kit.
- **Wear safety glasses and gloves when working with toxic, irritant or corrosive chemicals, and for any substances where the hazards are not yet fully characterised** – make sure you understand the hazard warning signs (p. 134) along with any specific hazard-coding system used in your department. Carry out procedures with solid material in a fume cupboard.
- **Use aids such as pipette fillers to minimise the risk of contact with hazardous solutions** – these aids are further detailed on pp. 154–155.
- **Never smoke, eat, drink or chew gum in a lab where chemicals are handled** – this will minimise the risk of ingestion.
- **Label all solutions appropriately** – use the appropriate hazard warning information (see pp. 134, 150).
- **Report all spillages of chemicals/solutions** – make sure that spillages are cleaned up properly.
- **Store hazardous chemicals only in the appropriate locations** – for example, a spark-proof fridge is required for flammable liquids; acids and solvents should not be stored together.
- **Dispose of chemicals in the correct manner** – if unsure, ask a member of staff (do not assume that it is safe to use the lab waste bin or the sink for disposal).
- **Wash hands after any direct contact with chemicals or biochemical material** – always wash your hands at the end of a lab session.

Examples Using eqn [24.1], 25 g of a substance dissolved in 400 ml of water would have a mass concentration (p. 154) of $25 \div 400 = 0.0625$ g mL^{-1} ($\equiv 62.5$ mg mL^{-1} $\equiv 62.5$ g L^{-1})

Using eqn [24.1], 0.4 mol of a substance dissolved in 0.5 litres of water would have a molar concentration of $0.4 \div 0.5 = 0.8$ mol L^{-1} ($\equiv 800$ mmol L^{-1}).

Solving solubility problems – if your chemical does not dissolve after a reasonable time:

- check the limits of solubility for your compound (see *Merck Index*, O'Neil *et al.*, 2006),
- check the pH of the solution – solubility often changes with pH, e.g. you may be able to dissolve the compound in an acidic or basic solution.

both can be regarded as an amount of *substance* per unit volume of *solution*, in accordance with the relationship:

$$\text{Concentration} = \frac{\text{amount}}{\text{volume}} \qquad [24.1]$$

The most important aspect of eqn [24.1] is to recognise clearly the units involved, and to prepare the solution accordingly: for molar concentrations, you will need the relative molecular mass of the compound, so that you can determine the mass of substance required. Further advice on concentrations and interconversion of units is given on p. 161.

Box 24.2 shows the steps involved in making up a solution. The concentration you require is likely to be defined by a protocol you are following and the grade of chemical and supplier may also be specified. Success may depend on using the same source and quality, e.g. with enzyme work. To avoid waste, think carefully about the volume of solution you require, although it is always a good idea to err on the high side because you may spill some or make a mistake when dispensing it. Try to choose one of the standard volumes for vessels, as this will make measuring-out easier.

Use distilled or deionised water to make up aqueous solutions and stir to make sure all the chemical is dissolved. Magnetic stirrers are the most convenient means of doing this: carefully drop a clean magnetic stirrer bar ('flea') in the beaker, avoiding splashing; place the beaker centrally on the stirrer plate, switch on the stirrer and gradually increase the speed of stirring. When the crystals or powder have completely dissolved, switch off and

Box 24.2 How to make up an aqueous solution of known concentration from solid material

1. **Find out or decide the concentration of chemical required** and the degree of purity necessary.
2. **Decide on the volume of solution required.**
3. **Find out the relative molecular mass of the chemical (M_r).** This is the sum of the atomic (elemental) masses of the component elements and can be found on the container. If the chemical is hydrated, i.e. has water molecules associated with it, these must be included when calculating the mass required.
4. **Work out the mass of chemical that will give the concentration desired in the volume required.**

   Suppose your procedure requires you to prepare 250 ml of 0.1 mol L^{-1} NaCl.

   (a) Begin by expressing all volumes in the same units, either millilitres or litres (e.g. 250 mL as 0.25 litres).

   (b) Calculate the number of moles required from eqn [24.1]: 0.1 = amount (mol) ÷ 0.25. By rearrangement, the required number of moles is thus 0.1 × 0.25 = 0.025 mol.

   (c) Convert from mol to g by multiplying by the relative molecular mass (**M_r for** NaCl = 58.44)

   (d) Therefore, you need to make up 0.025 × 58.44 = 1.461 g to 250 mL of solution, using distilled water.

   In some instances, it may be easier to work in SI units, though you must be careful when using exponential numbers (p. 243).

   Suppose your protocol states that you need 100 ml of 10 mmol L^{-1} KCl.

   (a) Start by converting this to 100×10^{-6} m^3 of 10 mol m^{-3} KCl.

   (b) The required number of mol is thus $(100 \times 10^{-6}) \times (10) = 10^3$.

   (c) Each mol of KCl weighs 72.56 g (**M_r, the relative molecular mass).**

   (d) Therefore you need to make up 72.56×10^{-3} g = 72.56 mg KCl to 100×10^{-6} m^3 (100 mL) with distilled water.

   See Box 26.1 for additional information.
5. **Weigh out the required mass of chemical to an appropriate accuracy.** If the mass is too small to weigh to the desired degree of accuracy, consider the following options:

   (a) Make up a greater volume of solution.

   (b) Make up a stock solution that can be diluted at a later stage (see below).

   (c) Weigh the mass first, and calculate what volume to make the solution up to afterwards using eqn [24.1].
6. **Add the chemical to a beaker or conical flask then add a little less water than the final amount required.** If some of the chemical sticks to the paper, foil or weighing boat, use some of the water to wash it off.
7. **Stir and, if necessary, heat the solution to ensure all the chemical dissolves.** You can visually determine when this has happened by observing the disappearance of the crystals or powder.
8. **If required, check and adjust the pH of the solution when cool** (see p. 173).
9. **Make up the solution to the desired volume.** If the concentration needs to be accurate, use a class A volumetric flask; if a high degree of accuracy is not required, use a measuring cylinder (class B).

   (a) Pour the solution from the beaker into the measuring vessel using a funnel to avoid spillage.

   (b) Make up the volume so that the meniscus comes up to the appropriate measurement line (p. 154). For accurate work, rinse out the original vessel and use this liquid to make up the volume.
10. **Transfer the solution to a reagent bottle or a conical flask and label the vessel clearly.**

retrieve the flea with a magnet or another flea. Take care not to contaminate your solution when you do this and rinse the flea with distilled water.

'Obstinate' solutions may require heating, but do this only if you know that the chemical will not be damaged at the temperature used. Use a stirrer-heater to keep the solution mixed as you heat it. Allow the solution to cool before you measure volume or pH as these are affected by temperature.

Stock solutions

Stock solutions are valuable when making up a range of solutions containing different concentrations of a reagent or if the solutions have

Table 24.2 Use of stock solutions. Suppose you need a set of solutions 10 mL in volume containing differing concentrations of KCl, with and without reagent Q. You decide to make up a stock of KCl at twice the maximum required concentration (50 mmoL l^{-1} = 50 mol m^{-3}) and a stock of reagent Q at twice its required concentration. The table shows how you might use these stocks to make up the media you require, based on eqn [24.1]. Note that the total volumes of stock you require can be calculated from the table (last column).

Stock solutions	Volume of stock required to make required solutions (ml)						Total volume of stock required (ml)
	No KCl plus Q	No KCl minus Q	15 mmoL l^{-1} KCl plus Q	15 mmoL l^{-1} KCl minus Q	25 mmoL l^{-1} KCl plus Q	25 mmoL l^{-1} KCl minus Q	
50 mmoL l^{-1} KCl	0	0	3	3	5	5	16
[reagent Q] × 2	5	0	5	0	5	0	15
Water	5	10	2	7	0	5	29
Total	10	10	10	10	10	10	60

some common ingredients. They also save work if the same solution is used over a prolonged period (e.g. a nutrient solution). The stock solution is more concentrated than the final requirement and is diluted as appropriate when the final solutions are made up. The principle is best illustrated with an example (Table 24.2).

Preparing dilutions

Making a single dilution

You may need to dilute a stock solution to give a particular mass concentration or molar concentration. Use the following procedure:

1. Transfer an accurate volume of stock solution to a volumetric flask, using appropriate equipment (Table 25.1).
2. Make up to the calibration mark with solvent – add the last few drops from a pipette or solvent bottle, until the meniscus is level with the calibration mark.
3. Mix thoroughly, either by repeated inversion (holding the stopper firmly) or by prolonged stirring, using a magnetic stirrer. Make sure you add the magnetic flea *after* the volume adjustment step.

For routine work using dilute aqueous solutions where the highest degree of accuracy is not required, it may be acceptable to substitute test tubes or conical flasks for volumetric flasks. In such cases, you would calculate the volumes of stock solution and diluent required, with the assumption that the final volume is determined by the sum of the individual volumes of stock and diluent used (e.g. Table 24.2). Thus, a two-fold dilution would be prepared using 1 volume of stock solution and 1 volume of diluent. The dilution factor is obtained from the ratio of the initial concentration of the stock solution and the final concentration of the diluted solution. The dilution factor can be used to determine the volumes of stock and diluent required in a particular instance. For example, suppose you wanted to prepare 100 ml of a solution of NaCl at 0.2 mol L^{-1}. Using a stock solution containing 4.0 mol L^{-1} NaCl, the dilution factor is $0.2 \div 4.0 = 0.05 = 1/20$ (a 20-fold dilution). Therefore, the amount of stock solution required is 1/20th of 100 mL = 5 mL and the amount of diluent needed is 19/20th of 100 mL = 95 mL.

Making a dilution – use the relationship $[C_1]V_1 = [C_2]V_2$ to determine volume or concentration (see p. 161).

Removing a magnetic flea from a volumetric flask – use a strong magnet to bring the flea to the top of the flask, to avoid contamination during removal.

Using the correct volumes for dilutions – it is important to distinguish between the volumes of the various liquids: a one-in-ten dilution is obtained using 1 volume of stock solution plus 9 volumes of diluent (1 + 9 = 10). Note that when this is shown as a ratio, it may represent the initial and final volumes (e.g. 1 : 10) or, sometimes, the volumes of stock solution and diluent (e.g. 1 : 9).