SSERC Bulletin



Ideas and inspiration supporting science and technology for all Local Authorities

No. 242 - Spring 2013

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Beyond the visible: Explorations with a

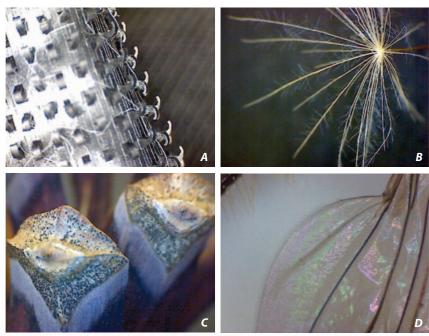


Figure 1 - Four images.

Can you identify the images shown in Figure 1? (Answers are given at the end of the article). As you have possibly guessed from the title of this article, each of the pictures represents an aspect of an object viewed using a microscope.

Each of the images has been captured using a Veho VMS-001 -200x USB digital microscope (Figure 2). This microscope costs in the region of £35 and is available from a wide range of outlets including Misco [1] and Amazon [2]. The microscope comes with software that allows images to be viewed, saved and manipulated on-screen.

This simple-to-use digital microscope offers easy access to a world that is usually invisible and its use can reveal all sorts of its fascinating secrets. Who can fail to be moved by the beauty of the microscopic algae (Figure 3a) that appear when a drop of 'green sludge' (Figure 3b) is magnified around 200x, and the fascinating shape of ammonium sulfate crystals as they appear from a warm saturated solution as it cools (Figure 4). It is little wonder that 'wow' is the most common utterance in our microscope workshops! In SSERC, we believe that through discovering a previously hidden microscopic world, learners will be motivated and that this Veho VMS-001 microscope can also be used as a vehicle to develop a range of the science inquiry skills such as those described in the Sciences Principles and Practice paper. [3]

Close observation of the images in Figure 1 offers clues to the identity of each object but additional information might help. For example, it is useful to know by how many times the object has been magnified. Take a look at Figure 5 - we might think at first glance that it looks like a picture of a cucumber, but would we come to the same conclusion when told that the object has been magnified 25 times? (It is, in fact, an image of a bamboo leaf, taken with the Veho VMS-001, magnification x25). The significance and importance of full and accurate recording becomes apparent. The Veho VMS-001 software is uncomplicated to use, allowing full labelling of the images, making recording the name of the sample and the magnification on to the image itself very straightforward. The time that the image was taken can also be automatically recorded. The Veho VMS-001 software comes with a short, electronic manual which provides comprehensive, easy-to-use instructions for using each of its facilities.



Figure 2 - Veho VMS-001 - 200x USB Digital Microscope and protective cap.

simple, inexpensive digital microscope

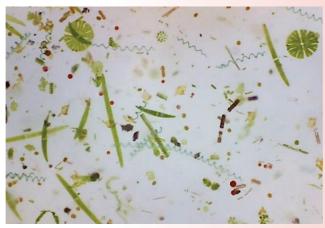




Figure 3a - Microscopic algae x220.

Another useful feature of the Veho VMS-001 is its facility to yield a measurement. By entering the magnification (read from the ruler on the microscope) into the box on the preview screen as shown in Figure 6, then drawing a line between two points, the software will provide a measurement of the distance between the two points. A variety of units is provided. Figure 6 shows cells from red onion epidermis, magnified x200 times in which the length and breadth of three cells have been measured. Clearly, this feature could be used

to highlight the importance of accuracy of manipulation skills when taking the measurements. There is also the opportunity to explore the meanings of, and highlight the differences between, accuracy and precision. Measurements made using the Veho VMS-001 are unlikely to be as accurate as those using a scale micrometer. A given Veho VMS-001 microscope will measure a fixed distance to be the same each time and so it measures precisely, although not necessarily completely accurately.

By taking images at short, regular time intervals and then inserting the images into a PowerPoint and running them together, a time lapse movie can be made. This technique was used to record the crystallisation of ammonium sulfate from a warm saturated solution as it cooled (www.sserc.org.uk). In addition to providing still images, the Veho VMS-001 software can also take short videos. We at SSERC have used the Veho VMS-001 to record plasmolysis in red onion cells in real time (www.sserc.org.uk).

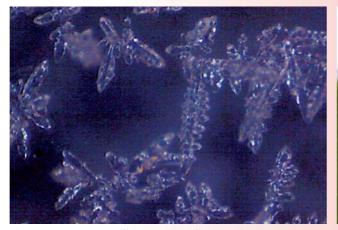


Figure 4 - Ammonium sulfate crystallising from a cooling saturated solution x200.



Figure 5 - Bamboo leaf x25.

The software allows for only short videos of up to 30 seconds to be recorded. If a longer video is required, then a number of shorter ones can be linked together using Windows Moviemaker. The set-up and method used for that will appear in a future article. To record good quality, sharp images, either as a series of stills or video calls for careful planning and setting up of the microscope and the subject to be photographed, skills included in the Principles and Practice document [1]. Clearly, opportunities exist for learners to bring together their skills from different subject areas - for example, the science disciplines, technology, art.

The Veho VMS-001 microscope does have limitations. Its maximum effective magnification is x220. While this magnification is great enough to view onion cells, a magnification of x400 is required to view most animal cells. (Veho do produce a microscope that magnifies to x400, the VMS-004D - 400x USB Microscope. The additional x200 over the Veho VMS-001 is digital rather than optical magnification and, in our experience, images produced from the x400 microscope are significantly less clear and are of poorer quality than those produced by the Veho VMS-001). Lighting is only from above and so it is not easy to view organisms that are largely colourless such as protozoa. That said, we believe that the positive aspects of the Veho VMS-001 far outweigh those disadvantages. The microscope simply has to be connected via a USB to a computer, focussing is straightforward and the image appears on the screen so you can be sure that your learner is observing the desired image. The Veho VMS-001 works extremely well within its limitations and we consider that it provides an engaging and exciting introduction to microscopy that can be used across a range of subject areas.

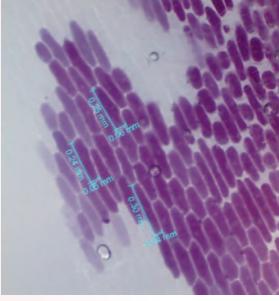


Figure 6 - Red onion epidermis, x220.

Answers to guiz:

- A: Velcro x25
- B: Dandelion seed x20
- C: Cone from Scots Pine Tree x17
- D: Mosquito wing x195

References

- [1] www.misco.co.uk/ (accessed 26th October 2012).
- [2] www.amazon.co.uk (accessed 26th October 2012).
- [3] Education Scotland, Curriculum for Excellence: Sciences Principles and Practice, www.educationscotland.gov.uk/Images/sciences_principles_practice_tcm4-540396.pdfl (accessed 26th October 2012).

Doing it outdoors - Why would you want to take your class outside?

Duncan MacLean, Senior Tutor at the Field Studies Council centre at Kindrogan, offers us his insight into why outdoor learning should be encouraged.

The real world is all around us. Look out of the window and check it out. Seriously; count how many scientific processes you can immediately jot off in your head. Nearly everything that is being taught inside of the classroom is going about its day-to-day business on the other side of that glass pane.

There's biology, physics, chemistry and a bundle load of additional holistic outcomes to be achieved by having a good gander outside.

Outdoor learning is not complicated. You and your class can enjoy the glory of the winter sun or the summer rain and go about the teaching and learning process outdoors. Not exclusively, unless perhaps a science experiment has gone seriously wrong and it's only you and the last oak tree left in the school grounds, but regularly and meaningfully.

In this article I hope to express at least a little bit of my personal joy for taking kids into the outdoor classroom. We can discuss the objectives, learning domains and practical effectiveness then, having justified our curriculum links, have a look at the further learning outcomes that are met. Essentially, my stance is that the laboratory is only a reflection of the dynamics of the real planet. It is a controlled means to explain the world-motions to your students. The classroom is, of course, the ideal place to conduct this lesson in. But the outdoor world is the rich, diverse and inter-disciplinary end-game of everything that you are teaching. Once in a while it will pay dividends to get your young learners interacting with it.



Outdoor learning is good pedagogy. It is certainly not the be-all and end-all but it does have an important role in any teacher's ammunition belt of teaching strategies. Before cracking on with the justifications though, there are a few conceptions of outdoor learning that should be challenged. The first is that it involves a residential trip, is costly and needs a coach to be organised. While this may be true and is one end of the spectrum for field work - only to be trumped by the overseas trip - there is another end of the scope. Learning outdoors begins with an activity that has been planned for the school grounds. True, this probably isn't as grand as the Cairngorm plateau or the Falkirk Wheel, but it can be done

in a jiffy. The second misconception concerns risk assessments. The risk assessment needed for a practical activity in the school grounds need not be more complicated that for an activity in the classroom. Again, these are certainly required for the moment that you leave the school gates with a duty of care for students, but there is plenty to be done within the playground. Unless you're climbing trees to' do a Galileo' then you should not need one. Third is that an outdoor learning lesson is time consuming and can only just be squeezed into a double period. This depends on what your intentions are. Learning outdoors can be an introduction, a consolidation or a full activity.



Yes, there is the faff of taking jackets on and off and trooping down the corridor but the more regularly you do it the speedier the students will become.

The flip side is the justifications for leading a lesson outdoors. Curriculum for Excellence promotes it to the hilt. Right through from primary levels and on into the outcomes of National 4 and 5 and the in-coming Higher and Advanced Higher, opportunities for outdoor education are endorsed. Primary practitioners and leaders are already taking it to heart and incorporating the woodlands and local streams into their timetables. They are already setting the attitude for learning experiences outside of the classroom. The need at secondary level in Biology for assignments, sampling experience and ecological procedures are mandatory aims of the 'Life on Earth' course while ecosystems and biodiversity in the higher 'Sustainability and Interdependence' unit lend themselves to a fieldwork investigation. All of these are outside techniques.

It isn't just Biology! Teachers of Chemistry and Physics can also take a class outdoors. The learning objectives are not as neat, but the changes of pace for a lesson are the same. Chemical changes (*SCN 4-18a*), Forces (*SCN 4-07a & b*) and Processes of the Planet (*SCN 4-05b*) can be demonstrated outside. Sustainable learning is a goal that is reflected throughout the science curriculum. Taking this theme outdoors requires a bit of effort to plan for meaningful outcomes, but the reward of transforming the lesson into a tangible interpretation of natural events is massive.

An example is to measure a student's carbon footprint, itself an ephemeral quantity of carbon dioxide creation. To help make sense of this number, take the students outdoors to measure trees using trigonometry. Convert this to the biomass and then to the carbon consumption and then the students can count how many trees are needed to sustain their lifestyle (it's usually about 20 mature oaks with a combined age of around 2,000 years). Alongside the scientific outcomes that are met wider links such as of numeracy, team-work,

health and well-being, positive behaviours towards the natural world and independent project work.

Even in the school yard that has been crossed a million times, there is plenty to investigate. With the fresh eyes of a learning objective, you can ask your students to interrogate their surroundings with a new keenness. The true beauty of the outdoor facility as an educational arena is that, in a young learner's eyes, this is the domain of play and of recreation. With the exception of rainy days, outdoors is fun and indoors is controlled. So take your lessons outdoors once in a while; capitalise upon the feel-good element, appropriate the generic playground for education, make indoor learning meaningful in a real-world context. And, if you're wondering what to begin with, take any one of the scientific processes you can see out of the window and take the class out to see what they make of it. It might just be a breath of fresh air.

Signposting Outdoor Resources

In the next Bulletin we will include details of resources that have been developed to link the great outdoors with specific learning aims in the national 4 and 5 curriculum.

In the meantime, however, here are some addresses that might get you started:

www.opalexplorenature.org

- Resources to give young learners techniques, recording sheets, ID cards and activities to sample for flora and fauna in their neighbourhood.
- www.field-studies-council.org
 - Residential field work trips (FSC Kindrogan at Pitlochry is your closest) and an excellent range of hard-wearing ID charts.
- www.jmt.org
- The John Muir Award provides a framework and award system for young learners and teachers to discover the outdoors with.
- www.educationscotland.gov.uk/outdoorlearning
 - An umbrella of information to get you started. The interactive map helps to show you where to go and who can help.

Demonstration corner

The elephant's toothpaste

This is a fun, yet simple demonstration showing the exothermic catalytic breakdown of hydrogen peroxide.

Preparation

You will need:

- A 250 or 500 cm³ measuring cylinder.
- A bowl of some sort to stand it in (a washing up bowl is fine).
- 1.4 g of potassium iodide crystals
- 60 cm³ of hydrogen peroxide (100 vol).
- 1 cm³ (or so) of washing-up liquid.
- A few drops of food colouring.

Carrying out

- 1) Wear eye protection (demonstrator).
- 2) Make sure the audience is standing at least 1 m back.
- 3) Place the measuring cylinder in the bowl.
- 4) Pour the hydrogen peroxide into the measuring cylinder and add the washing-up liquid and food colouring.
- 5) Drop the potassium iodide crystals into the liquid at the bottom of the cylinder.

After a second or two's delay, you will see foam moving rapidly up the measuring cylinder, coloured by whatever colouring you have used. It will rapidly start to pour out of the top and pile up in the bowl (assuming you remembered to put one there!) like toothpaste being squeezed from a tube.

You will also notice steam rising from the foam as the reaction is highly exothermic.



It is possible to use less concentrated hydrogen peroxide but the reaction is slower and less spectacular which in part defeats the object.

If you put the food colouring down the side of the measuring cylinder, you can use more than one colour and get 'toothpaste' with stripes.

An interesting variation is to have two experiments side by side. Add the potassium iodide to one and at the same time add a piece of liver to the other. Liver, as you will probably know, contains the enzyme catalase which catalyses the breakdown of hydrogen peroxide. You will see the foam in the tube with the liver starting off faster but stopping, sometimes before it gets out of the top of the tube. The reason is that, as a protein, the catalase is denatured by the heat of the decomposition and thus the reaction stops. A useful comparison between enzymes and inorganic catalysts.

Dopa oxidase -

The advent of the Revised and *CfE* Highers in Biology and Human Biology, and Revised and *CfE* Advanced Higher in Biology [1-4] has prompted the Biology Team in SSERC to consider different enzyme systems for use in practical work and/or investigations.

At Higher/Advanced Higher levels, enzyme practical work should meet a number of criteria. Our belief is that practical work, as far as is possible, should in no order of priority:

- be robust;
- involve affordable (and readily available) substrates and enzymes;
- be versatile and offer opportunities for investigative work;
- · be reliable;
- allow students to extract enzymes from 'living things';
- incorporate an assay that is simple to follow;
- produce results in short timescales;
- offer opportunities for kinetic studies.

We hope to show you that the enzyme-catalysed conversion of L-dopa to dopaquinone followed by conversion to dopachrome meets most if not all of the criteria in the bulleted list above.

The enzyme system

The enzyme studied here is called dopa oxidase although it is known by a myriad of other names (see

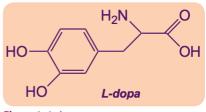


Figure 1 - L-dopa.

- N-acetyl-6-hydroxytryptophan oxidase
- o-diphenol oxidase
- o-diphenol oxidoreductase
- monophenol dihydroxyphenylalanine: oxygen oxidoreductase
- o-diphenol: oxygen oxidoreductase
- o-diphenolase
- catechol oxidase
- catecholase
- chlorogenic acid oxidase
- chlorogenic oxidase
- cresolase
- diphenol oxidase
- dopa oxidase

- o-diphenol: O₂ oxidoreductase
- monophenol monooxidase
- monophenol oxidase
- monophenol,
- dihydroxy-L-phenylalanine oxygen oxidoreductase
- monophenolase
- phenol oxidase
- phenolase
- polyaromatic oxidase
- polyphenol oxidase
- polyphenolase
- pyrocatechol oxidase
- tyrosinase

 Table 1 - Alternative names for dopa oxidase [Enzyme Commission Number 1.14.18.1].

 Common names are highlighted; information taken from [5].

Table 1 for a comprehensive list) including tyrosinase and catechol oxidase. Dopa oxidase, a copper containing metalloenzyme [5], is involved in a number of metabolic reactions including melanin biosynthesis.

A key substrate for the enzyme is L-dopa (alternatively known as 3,4-dihydroxyphenylalanine) (Figure 1) and it is closely related to the amino acid tyrosine (Figure 2) from which it is snythesised in humans.

HO HO Tyrosine Figure 2 - Tyrosine. In humans a related product of tyrosine metabolism is dopamine (Figure 3) which is released at a number of sites in the brain and is reported to exhibit a range of effects on sleep, mood, attention and learning [6].

It appears that L-dopa can cross the blood-brain barrier and be converted to dopamine and this has led to L-dopa being used in the treatment of Parkinson's disease.



a perfect enzyme?

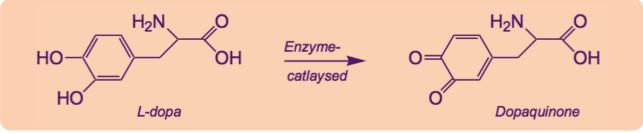


Figure 4.

A key reaction in melanin biosynthesis is the oxidation of L-dopa to dopaquinone (Figure 4).

Both substrate and product of the above reaction are colourless and so monitoring the conversion of L dopa to dopaquinone is not possible using conventional colorimetry. However, dopaquinone is spontaneously converted to dopachrome which is orange/red in colour with an absorption peak at about 470 nm (Figure 5).

Sources of tyrosinase for experimental work

Dopa oxidase can be conveniently extracted from animals, plants and fungi. Readily available sources include bananas and potato peelings.

The enzyme assay

The following is an adaptation of the method available on the Mystrica website [7]; a more detailed protocol will be published shortly on the SSERC website [8].

Extracting the enzyme

1) Weigh out about 2.5 g of banana.

- 2) Add 5 cm³ of **cold** distilled water and crush the banana to a pulp.
- The enzyme mixture can be filtered through muslin or (preferably) centrifuged to remove cellular debris. The filtrate will contain dopa oxidase.

Following the reaction

- 4) The formation of dopachrome can be conveniently followed using a colorimeter. For a 'standard assay' we find the following mixture is convenient:
 - L-dopa (we used L-dopa from Sigma-Aldrich) in distilled water at a concentration of 2.5 x 10⁻² mol dm⁻³ (2 cm³)
 - dopa oxidase extract from step 3 (0.5 cm³)
 - distilled water (0.5 cm³)

Both substrate and enzyme extract will slowly degrade

at room temperature and so it is best to keep these solutions on ice when they are not being used in the assay. (It is worth noting that solid L-dopa is relatively stable and has a long 'shelf-life'.)

5) If the above mixture is prepared in an absorption cuvette the reaction can be followed at 470 nm. Over a 6 minute period we typically find that the absorbance of the mixture changes from 0.0 to 0.7 (see Figure 6). Using a colorimeter such as that produced by Mystrica (www.mystrica.com) allows continuous monitoring of absorbance changes coupled with potential kinetic analysis.

It is worth noting that the effect of both substrate and enzyme concentration on the rate of dopachrome production can be easily studied [7].

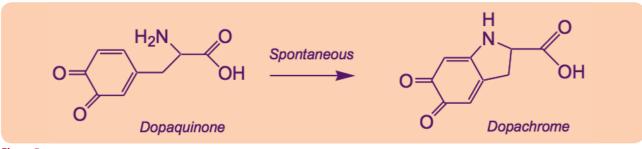


Figure 5.

Conclusion

So, does dopa oxidase live up to the title of this article and meet the criteria in terms of the desirable characteristics for an enzyme assay? We acknowledge that the substrate, L-dopa, is not cheap; for example the current cost from Sigma-Aldrich [catalogue number D9628-5G] is £27.20 for 5 g. That said, only small amounts are used in each assay and an investment of £35 (to include postage and packing) will last a significant proportion of us to retirement age and beyond....

Whilst the substrate is not cheap from 'conventional' suppliers an on-line search reveals a number of possible other sources although we have not explored these in any depth. Against the other criteria, the enzyme performs pretty well.

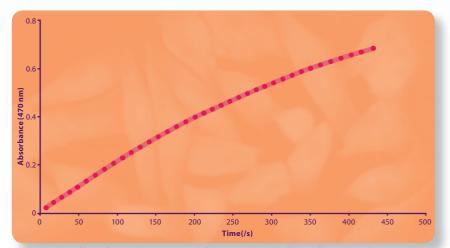


Figure 6 -The effect of a crude preparation of dopa oxidase (produced by crushing banana in distilled water) on the absorbance of a solution of L-dopa (final concentration 1.67 x 10⁻² mol dm⁻³) at 470 nm. The sample was contained in a cuvette of pathlength 1 cm.

There would seem to be lots of scope for investigative work given the ubiquitous nature of the enzyme in different plant materials.

References

- [1] SQA (2010) Biology (revised) Higher the Arrangements Document can be downloaded at www.sqa.org.uk/sqa/files_ccc/HigherBiologyCourseSpec.pdf
- [2] SQA (2012) Higher Biology Course Support Notes can be downloaded at www.sqa.org.uk/files_ccc/CfE_CourseUnitSupportNotes_Higher_Sciences_Biology.pdf
- [3] SQA (2010) Human Biology (revised) Higher the Arrangements Document can be downloaded at www.sqa.org.uk/sqa/files_ccc/HigherHumanBiologyCourseSpec.pdf
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- [6] Reece, J.B., Minorsky, P.V., Cain, M.L., Wasserman, S.A., Jackson, R.B. and Urry, L.A. (2011) Campbell Biology (9th edition), Pearson Education.
- [7] Dopa oxidase available at www.mystrica.com/Experiment.aspx?PageId=59 and www.mystrica.com/files/Dopa%20oxidase.pdf
- [8] The SSERC website is available at www.sserc.org.uk Please note that to access all resources on the website you will need a log-on ID and password.

Health & Safety

Eye dissection - using deer eyes

SSERC has recently received a number of enquiries about whether deer eyes can be used for dissection. Although not mentioned specifically in the Code of Practice *Materials of Living Origin* [1] the guidance within the code is also relevant for use with deer eyes. Further more general advice on eye dissection can be found in the SSERC Bulletin articles *Dissecting eyes - hints and tips* [2] and *Dissecting bulls' eyes* [3].

The brain and spinal chord (including eyes) can potentially be a source of transmissible spongiform encephalopathies (TSEs). Concern in recent years has centred particularly on bovine spongiform encephalopathy (BSE) which is linked to new variant Creutzfeldt-Jakob disease (nvCJD), a human form of TSE. Legislation is designed to prevent such materials entering both the human and animal food chain.

Deer are susceptible to a TSE known as chronic wasting disease (CWD). Although endemic in some parts of the USA, there is no evidence of CWD (or other TSEs) in deer in the UK. Deer keepers in the UK are legally obliged to report any suspected cases of TSE [4]. Laboratory and epidemiological studies in the USA [5] have not demonstrated a causal link between CWD and nvCJD or TSEs in other species.

Consequently teachers can be confident that the advice and guidance in Materials of Living Origin forms a suitable and sufficient basis on which to base control measures to control the low level of risk associated with deer eyes. That is the eyes should be sourced from animals fit for human consumption and the guidance in the Code of Practice followed with respect to their dissection and disposal. This demonstrates the robust nature of SSERC's approach to sensible and proportionate risk assessment in that the control measures in the Code of Practice can be reviewed and adopted for a new situation as part of ongoing risk assessment. It is worth emphasising that under the guidance provided (paragraph 3.5) in the Code of Practice [1] no native, wild birds or mammals living or dead (including road kill), may be brought into school.



References

- [1] Materials of Living Origin (2012), www.sserc.org.uk
- [2] Dissecting eyes hints and tips (2012) *SSERC Bulletin*, 241, 4-7.
- [3] Dissecting bulls' eyes (2012) SSERC Bulletin, 240, 11
- [4] Transmissible Spongiform Encephalopathies (TSEs) in Deer - Advisory Notes for Farmers (2010), www.defra.gov.uk
- [5] Ermias D. Belay, Ryan A. Maddox, Elizabeth S. Williams, Michael W. Miller, Pierluigi Gambetti, and Lawrence B. Schonberger (2004), Chronic Wasting Disease and Potential Transmission to Humans, Emerging Infectious Diseases, Vol. 10, No. 6.

Health & Safety

Citric acid

When carrying out any quantitative work in chemistry, it is important to know the concentration of any solutions you use. Too great a concentration and some reactions will become dangerous, too low a concentration and some reactions will not work.

An eagle-eyed SSERC member has spotted that different containers of citric acid which her school has bought recently from the same supplier have different hazard symbols on them and is, understandably, a bit confused.

The issue is that we are in the middle of the process of switching from CHIP to GHS registration. All chemical manufacturers have to submit their classifications of chemicals to ECHA (the European Chemicals Agency) and ECHA will eventually come up with an agreed standard classification for the EU.

Unfortunately, manufacturers don't have to register chemicals until June 2018 and there will then be more delays before all substances have a harmonised classification. It's not quite as bad as it sounds. About 2 thirds of the chemicals on our database have a harmonised classification already but citric acid is not one of them.

As for citric acid itself, we have looked at the ECHA database, where the manufacturers register their proposed classifications. Of the 650 registrations, 490 rate it as a Cat 2 Irritant (skin or eye), 107 as having no hazard at all and only 65 as Cat 1 Eye Damage (which rates a Danger signal word and a corrosive symbol).

Whenever a supplier gets a batch of a chemical, such as citric acid, they pass on the assessment of the hazard given to them by their supplier. Unfortunately, different manufacturers classify it differently - hence the different labels on different tubs. The assessment of irritant/ corrosive is not an exact science and we suspect that citric acid is relatively close to the dividing line, hence the different classifications.

On a related topic, the same contributor looked at the manufacturer's Safety Data Sheet (MSDS) and was a little alarmed to see that although it gave no hazard, it said that gloves should be worn to handle it.



These data sheets need to be read with an educated eye. The safety information on the MSDS is drawn up considering industrial processes, using large quantities for long periods of time. This can lead to advice that might seem rather over the top for the sort of small-scale laboratory use that is the norm in schools. For instance, here is some information from a MSDS.

Personal protection

- Splash goggles.
- Lab coat.
- Dust respirator.
- Be sure to use an approved/certified respirator or equivalent.
- Gloves.

Personal protection in case of a large spill

- Splash goggles.
- Full suit.
- Dust respirator.
- Boots.
- Gloves.
- A self contained breathing apparatus should be used to avoid inhalation of the product.
- Suggested protective clothing might not be sufficient; consult a specialist BEFORE handling this product.

The chemical involved? Sodium chloride - salt!

The precautions taken when handling a chemical should be determined by a risk assessment that uses the best available advice. We would suggest, not surprisingly perhaps, that the advice of SSERC trumps the 'raw' MSDS as ours is drawn up considering the sorts of uses and exposure encountered in schools.

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