Behaviour-changing ingredients in soft drinks: an experiment developed by school children in partnership with a research scientist

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ABSTRACT
A team of six children (13–14 years old) developed and conducted an experiment to assess the behaviour of the planarian flatworm, an invertebrate animal model, before, during and after exposure to chemicals. The aim of the project was to engage children in pharmacology and toxicology research. First, the concept that exposure to chemicals can affect our nervous systems and alter our behaviour was introduced by the scientist. Then pupils were asked to conduct independent online research on chemicals we consume and used this information to select chemicals of interest. Caffeine and the artificial sweetener aspartame were chosen. As aspartame is broken down in the body, the aspartame metabolites phenylalanine, methanol and aspartic acid were also tested. Pupils assessed flatworm behaviour and determined that phenylalanine and methanol altered behaviour. The experiment demonstrated that chemicals can affect the nervous system and can be used in classrooms to illustrate that chemicals, such as recreational drugs, can alter behaviour. Students found the experiential nature of the programme valuable, appreciated the active components and considered the scientist as an 'expert'. This partnership contributes to academic knowledge in children and provides insight into teaching and raises awareness of the curriculum as a tool of social change.

Introduction
On a daily basis we are exposed to chemicals such as pollutants and also to chemicals we deliberately consume in our diets such as caffeine and alcohol. Some chemicals may affect the nervous system, for example stimulating or depressing activity in the brain. Understanding how chemicals can affect brain function (neuropharmacology and neurotoxicology) provides professionals and policy-makers with the knowledge to improve health protection. However, it is equally important that the public understand how chemicals purchased in shops or off the internet, such as 'legal highs', can affect their bodies. In line with this, the effects of recreational drugs on behaviour, health and life processes, is included in

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the key stage 3 National curriculum science programme (Department of Education 2013). However, the impact of behaviour-changing chemicals extends beyond the curriculum. There is increasing concern amongst teachers about the effects of caffeine in soft drinks on the behaviour of pupils. The teachers union NASUWT stated that ‘they [caffeine] are readily available legal highs’ and that ‘Parents and young people need to be made aware of what these drinks contain and their potential impact on behaviour’ (Nasuwt 2015).

The Medical Toxicology Centre at Newcastle University is comprised of a team of clinicians and scientists who work together to improve health through research on the health consequences of chemical exposure and the development of treatments and preventive measures. It is the responsibility of public-funded researchers to engage with the public. This is most often through educational activities such as public lectures or workshops to improve public understanding of science (Grand et al. 2015). However, it is recognised that the public do not just want to be told about science, they want communication to be two-way. In a recent survey on public attitudes to science, 66% agreed ‘scientists should listen more to what ordinary people think’ (Ipsosmori 2011). In the same survey, many people who felt disengaged with science indicated that they were put off by their experience of science in school. In the light of this, the Medical Toxicology Centre took part in Leading Edge at Newcastle University; a scheme to partner small groups of Year 9 school children (13–14 years old) with research scientists on a science project. In this case, the project was to investigate the effect of chemicals on behaviour and expose these pupils to scientific inquiry.

Behaviour is controlled by the nervous system and so alterations in behaviour can indicate that a chemical has affected the nervous system and/or brain. Behavioural assessments are therefore frequently used in neuropharmacology and neurotoxicology to evaluate the effect of a chemical on the nervous system. These assessments range from observing spontaneous behaviours such as movement through to learned behaviours requiring training. Traditionally, rodents are used for behavioural assessments to predict human outcomes as there are similarities between our nervous systems. However, experiments using alternative species are encouraged to reduce, replace and refine the scientific use of vertebrate animals (lesson notes on animal use in research and the 3Rs can be found at http://www.understandinganimalresearch.org.uk/schoolzone/). The nervous system of the invertebrate planarian flatworm shares cellular features and many of the neurochemicals present in vertebrate nervous system (Buttarelli, Pellicano, and Pontieri 2008). Therefore, flatworms have been used as an animal model in neuropharmacological and neurotoxicological studies (Raffa, Cavallo, and Capasso 2007; Rawls et al. 2009; Sacavage et al. 2008).

Under the guidance of the research scientist, the team of six children developed and conducted experiments in a university laboratory to assess the effects of commonly consumed chemicals on flatworm behaviour. The objectives of this partnership were to engage school children with hands-on research in pharmacology and toxicology and provide them with the opportunity to develop their own line of scientific enquiry. The pupils chose to investigate caffeine and aspartame. Consumer concerns about aspartame have recently led the manufacturers of Pepsi cola to stop using it in their US products (Pepsico 2015). The outcome was a novel experiment that can be transferred to a classroom and clearly illustrates the effects of chemicals on behaviour and is based on the interests of school children.

**Methods**

**Pupils**

Secondary schools in the vicinity of Newcastle University were invited to participate in Leading Edge at Newcastle University; a scheme to partner groups of six Year 9 school children (13–14 years old) with a research scientist on a science project. Dr Sarah Judge was partnered with the team from St Mary’s Catholic School. This is a voluntary aided secondary school in the city of Newcastle upon Tyne with pupils coming from a very wide range of backgrounds. The proportion of pupils eligible for free school meals is above average. In the 2014/15 academic year, Year 9 students at St Mary’s Catholic School were
invited by their Head of Science, Dr Jankowski, to apply to be part of the Leading Edge Scheme team. Dr Jankowski asked interested pupils to prepare a 2-min presentation on an area of science which had caught their imagination recently. Six pupils (three girls and three boys) were selected based on their performance and enthusiasm by a panel of school science staff.

Independent information-gathering (Sessions 1 and 2)

The team of pupils visited the University with their school teacher and were given a short introductory presentation. Images of products containing chemicals we are exposed to (e.g. pollutants, drugs, household chemicals and soft drinks) were shown to highlight the relevance of pharmacological and toxicological research to their lives. The use of animals for behavioural assessments was introduced and the need to reduce, replace and refine animal use in research. On a second visit they met the research scientist, were given a tour of the laboratories at Medical Toxicology Centre and given a task to conduct at home. They were asked to conduct independent research to find information on chemicals we commonly consume.

Using information from books and reliable sources on the internet try to answer the following questions

1. Which behaviour-changing chemicals can be found in products in the supermarket?
2. Which products contain the chemicals? Compare the amount of chemical found in each product.

How does each of these chemicals alter behaviour in humans? For example, would they make you sleepy, confused, alert and hyperactive?

Development of the experimental design (Session 3)

The scientist visited the school and the pupils presented the findings of their independent online research. This started the basis of a discussion about which chemicals they were most interested in investigating. The pupils were also given a journal publication describing the methodology of using flatworms in a behavioural pharmacology study (Pagan, Coudron, and Kaneria 2009). The pupils found some sections of the scientific publication and some of the scientific language challenging. However, they found the figures very helpful and after asking questions about the parts they found challenging, they understood the study. The pupils and scientist discussed how this method could be altered to incorporate their ideas.

Experimental materials and methods (Session 4 and 5)

The pupils spent two school days at the Medical Toxicology Centre to conduct their practical experiments under the guidance of the scientist and the science teacher. Before starting, pupils were given a safety talk and provided with disposable laboratory coats, safety spectacles and gloves.

Twenty flatworms (*Dugesia*, large brown) were purchased a week before the experiment (Blades Biological Ltd, UK). They were kept at room temperature in shallow water (tap water allowed to stand for at least one day) in an aerated plastic container in the dark. They were fed once a week with raw chicken and the water was changed regularly (2–7 days).

A transparent propylene dish (85 × 15 mm) containing 20 ml of water was placed on a piece of paper with 1 cm² gridlines. One worm was transferred from the home container to the dish using a paintbrush (Figure 1). The lid was placed on and a timer started. Locomotor activity was quantified by counting the number of times the worm moved across a line in a 1-min period (if it turned and recrossed a line immediately the second crossing was discounted, if it crossed the intersection of two lines this was counted as 1). Descriptions of locomotor activity were also recorded (e.g. position in Petri dish, consistent gliding or random movements and twisting in one space).
All chemicals were purchased from Sigma–Aldrich except the aspartame which was purchased from the supermarket as an artificial sweetener containing 3% aspartame. All the chemicals were assessed for risk according to Control of Substances Hazardous to Health regulations. Methanol is regarded as toxic, caffeine as harmful and aspartic acid and phenylalanine as not harmful. Therefore, the pupils were provided with diluted stock solutions which contained the chemicals at levels below those known to have acute and long-term systemic effects. Stock solutions of caffeine (10 mM; 0.097 g/l), DL phenylalanine (10 mM; 0.08 g/l), DL aspartic acid (10 mM; 0.067 g/l) aspartame (10 mM; 1 g of artificial sweetener/l) and methanol (100 mM; 3 g/l) were made in water. The pupils diluted the stock solutions of caffeine, DL phenylalanine and DL aspartic acid 1:200 to make 50 μM working solutions, the aspartame stock solution 1:10 to make a 1 mM working solution and the methanol stock solution 1:10 to make a 10 mM working solution. Although fine balances were used to measure mg quantities of the chemicals and Gilson pipettes were used to measure small water volumes, the dilutions could be achieved in a classroom using serial dilutions.

**Behavioural observations**

In pairs pupils observed and recorded the behaviour of a flatworm in a dish for 10 min and then repeated this with another worm. This provided an opportunity for the pupils to become familiar with the flatworm behaviour before the experiment but also to gather data on variability. The scientist explained that behaviour of one individual could change over time (intra-individual variability) and that behaviour could be different between individuals (inter-individual variability). In addition, behavioural assessments can be subjective and vary depending on the tester (inter-rater variability) and if the data are qualitative vs. quantitative. She then explained that in an experiment we normally try to test one variable (the chemical) and so variability as a consequence of assessing behaviour needs to be understood and minimised if possible. The pupils were asked to compare the data they had collected with their partner’s data and determine if their qualitative and /or quantitative assessments varied (inter-individual variability).

**Chemical testing**

Flatworms were placed for 5 min in a dish of water and then transferred to a dish containing the working solution of the test chemical (20 ml) for 10 min and then transferred back to the dish of normal water for 5 min. Each chemical was tested on 6 different worms except for caffeine which was tested
on 12. Worms were not reused for a different chemical on the same day. Worms tested with methanol were not reused at all. At the end of testing, all worms were replaced in their home container and maintained until the end of their natural life cycle.

**Analysis (Session 6)**

Pupils entered their data into an Excel spreadsheet. The scientist visited the school to discuss the different ways the data could be presented (individual data vs. averaged, continuous data vs. time intervals). Statistical tests were conducted by the research scientist but the tests were explained to the pupils. Quantitative data collected each minute from individual worms during the behavioural observations were analysed using repeated measures ANOVA and paired samples correlations. For the chemical testing experiments average activity in 5-min intervals was calculated for each worm. The effect of the chemicals was analysed using one-way ANOVAs and paired samples *t*-tests.

**Evaluation**

Following the project, pupils were emailed a questionnaire via their science teacher to evaluate their experience of the partnership and project. They were given a few days to complete it in their own time and then emailed it to their teacher. A set of open and closed questions were used in the evaluation questionnaire, focusing on overall experiences, barriers and facilitators related to students involvement in this project. This flexible approach allowed the overall structure of the questionnaire to be set whilst capturing individual thoughts, beliefs and realities. There were 17 questions in total with a focus on knowledge, interest, relevance benefits and barriers to learning. The questions were developed by the team and based on past evaluations of children engaging in scientific research (Chen and Cowie 2013; Palmer 2009). All six students completed the evaluations which were analysed using thematic content analysis (Braun and Clarke 2006; Cohen, Manion, and Morrison 2009; Graneheim and Lundman 2004). Thematic content analysis goes beyond statistics towards the examination of language and classification of text (Graneheim and Lundman 2004) and is an analysis method for the subjective interpretation of the content of text data through the systematic classification process of coding, categorising then identifying themes or patterns (Hsieh and Shannon 2005). As supported by Graneheim and Lundman (2004), the data were coded (78 codes), searching not for words, but meanings. The coded data were read through and broad overarching labels or themes into which the coded data seemed to fit emerged. Broadly following Braun and Clarke’s (2006) model, overarching descriptive labels or themes (four themes) were developed from the codes, while ensuring that all coded material was encompassed into one of these themes. The scientist’s evaluation of the partnership with the pupils was written at the suggestion of the editor.

**Results**

**Independent information gathering**

Pupils identified some supermarket products contain caffeine (coffee and soft drinks), nicotine (aubergine), alcohol (wine and spirits) and artificial colourings and sweeteners such as aspartame. They reported that different products contain different chemicals in differing quantities and that some had depressant effects on behaviour and some were stimulants and caused increased activity. The artificial sweetener aspartame was of particular interest to the pupils as they had found many webpages, and an alarming video, dedicated to this chemical.
**Development of the experimental design**

On the basis of their research, the pupils decided to test caffeine and aspartame, both ingredients commonly found in soft drinks. The research scientist advised them that aspartame is rapidly broken down in the body. Therefore, the aspartame metabolites phenylalanine, methanol and aspartic acid were tested as well. During the basic behavioural observations (see below) it was evident that inter-individual variability was high and so the scientist suggested that the experimental design should be within subject (data collected from each worm in absence and presence of the chemical) rather than between subjects. One pupil asked if the effects of the chemicals would reverse and so a reversal period was incorporated into our experimental design (5 min back in water). Another pupil asked what would happen to the behaviour if two different chemicals were mixed. Due to time limits this was not incorporated into these experiments but could be explored in the future.

**Behavioural observations**

Pupils reported that the behaviour of individual worms changed during the 10-min observation period (intra-individual variability). Five out of six worms were reported to ‘slow down’ towards the end of observation period but the quantitative data indicated that activity did not change over time (Figure 2(A), repeated measures ANOVA). There was a difference in activity between individual worms (inter-individual variability, $F_{1,2} = 32; p < 0.01$). Quantitative data collected by two pupils observing the same worm were highly correlated ($0.98$ paired samples correlations, $p < 0.001$). Likewise, qualitative data collected by two pupils observing the same worm were similar. For example:

Pupil 1 ‘Moved steadily around the perimeter of the dish anticlockwise. It did a flip 3 times, varying from in the water to on top on the water. It slowed towards the end of the time, after 4 minutes it started to move more to the centre of the dish, not around the dish.’

Pupil 2 ‘Moved at a steady pace quick pace around the perimeter of the petri dish for around 4 minutes. It then flipped over and moved more into the central area. As it moved round the central area it got slower and moved less. Worm got gradually calmer’.

The qualitative data provided information additional to the quantified activity data. It provided information on the types of movement, for example ‘moved steadily’ or ‘steady pace’ were used in four accounts, ‘random movements’ or ‘wriggling’ was used in five accounts and ‘flips’ and ‘twists back around’ was used in five accounts. It provided information on the position of the worm in the dish, for example ‘Moved around the edge/rim/perimeter of the dish’ was used in nine accounts. Emotions were also used to describe behaviour, for example, ‘looked cautious,’ ‘got gradually calmer’ and ‘more adventurous’ were used.

**Chemical testing**

The average locomotor activity of flatworms before chemical exposure was $7.5 \pm 0.6$ crossings/minute ($n = 36$). During the first minute of exposure to caffeine average locomotor activity increased to $9.3 \pm 2.6$ crossings/minute ($n = 12$) and then decreased to $6.6 \pm 2.3$ crossings/minute ($n = 12$) after 5 min. However, when analysed there was no significant effect of caffeine on average locomotor activity (Figure 2(B); $n = 12$). Ten minute exposure to aspartame (artificial sweetener; Figure 2(C); $n = 6$) and the aspartame metabolite aspartic acid (Figure 2(D); $n = 6$) had no effect on flatworm activity. In contrast, exposure to the aspartame metabolite phenylalanine significantly affected activity (Figure 2(E); $F_{3,15} = 8; p < 0.05; n = 6$). Locomotor activity decreased by 58% at 6–10 min. Returning to water reversed the phenylalanine-induced decrease in activity. Exposure to the aspartame metabolite methanol significantly affected activity (Figure 2(F); $F_{3,15} = 9; p < 0.05; n = 6$). Locomotor activity was rapidly inhibited by 55% in the presence of methanol and was inhibited further at 6–10 min.
Figure 2. Soft drinks ingredients altered locomotor behaviour in the flatworm Planaria. (A) Locomotor behaviour between individual flatworms (n = 6) was significantly different. Exposure to caffeine (B, 50 μM, n = 12), aspartame (C, 1000 μM, n = 6) and the aspartame metabolite aspartic acid (D, 50 μM, n = 6) did not affect mean locomotor activity. Exposure to the aspartame metabolites phenylalanine (E, 50 μM, n = 6) and methanol (F, 50 μM, n = 6) decreased mean locomotor activity. Returning the worms to normal water reversed the inhibition in activity. Mean ± SD activity in 5 min interval.

*p < 0.05 in comparison to ‘before’ interval (paired t-test).

Pupil’s evaluation of the partnership

The four themes emerging from analysis of the pupil evaluation (Figure 3) include:

- Approach to learning, others, action and meaningfulness.
What new facts did you learn?

Pupil 1: Lots of fizzy drinks and other products contain high levels of behaviour changing chemicals, such as caffeine. Aspartame contains metabolites; phenylalanine, aspartic acid and methanol. Behaviour differs according to the worms they act unpredictably. Aspartame is an artificial sweetener in several products.

Pupil 2: Aspartame is an artificial sweetener used in soft drinks. When the body breaks down Aspartame, it produces methanol, which is poisonous. Flatworms are used for chemical testing. Artificial chemicals are used in things you wouldn’t expect them to be in, i.e. Milk. Rabbits are banned for chemical testing. Protests against animal testing and GMO’s are a lot more popular, with companies like ‘Lush cosmetics’ leading the way. We have been chemically testing and modifying organisms hundreds of years. Chemicals in drinks can make children more aggressive, overactive, and less aware. Companies can use GMO’s and chemicals in their products without putting it on the label.

Pupil 3: Fizzy drinks have lots of caffeine in. Aspartame is an artificial sweetener that is found in everyday products. Flat Worms are used as a substitute of mammal/rodent testing models. Animals have different reactions to different chemicals just like humans.

Pupil 4: Chemicals that in our food and drink can affect our behaviour. Rabbits are banned from chemical testing. We can use invertebrates for chemical testing. There are moral and ethical issues with animal testing. Artificial chemicals are in nearly everything we take in in our daily lives i.e. ice cream. Companies can put chemicals into their products and not put it on the label.

Pupil 5: Aspartame is an artificial sweetener that is present in many fizzy drinks. Rabbits are banned from animal testing.

Pupil 6: Aspartame is an artificial sweetener and has links with cancerous tumours. In lots of products the aspartame label has been taken off so that the person doesn’t know that they are consuming it. Aspartame is broken down into phenylalanine, aspartic acid and methanol in metabolism in the body. Lots of fizzy drinks and other products such as coffee have a lot of caffeine in them. Behaviour is always different in different animals. You cannot control the ways that animals react to different things.

What new scientific words did you learn?

Pupil 1: Toxicology, Aspartic Acid, Gilstein Pipettes, Phenylalanine
Pupil 2: Phenylalanine, Gilson pipette, Flatworm, Aspartame, Aspartic acid, Incubate, Bio-reactor, Centrifuge
Pupil 3: Neurotoxicology, Aspartame, Gilstien pipettes, Quantitative data, Qualitative data
Pupil 4: Phenylalanine, Gilson pipette, flatworms, aspartic acid
Pupil 5: Aspartic acid, Gilstien Pipettes, Neurotoxicology
Pupil 6: Neurotoxicology, Gilstein pipettes, Aspartame, Aspartic Acid

What new skills did you learn?

Pupil 1 Using Gilstein pipettes, Using grids to measure behaviour, Recording quantitative and qualitative behaviour, How to work in a research environment.

Figure 3. Partnership evaluation questions and each pupil’s response.
Pupil 2: How to use a Gilson Pipette, How to measure out the right concentration of the chemical, How to extract DNA from plants like strawberries, How to adjust a microscope’s focus.

Pupil 3: How to use a Gilstien pipette, How to measure tiny amounts of chemicals using a two decimal place balance, How to work effectively in a small science lab.

Pupil 4: How to use a Gilson pipette, How to test behaviour changing chemicals, How to make a chemical mixture, How to extract pure strawberry DNA, How to use a microscope.

Pupil 5: How to measure out small amounts of chemicals to be able to dilute them. How to use Gilstien pipettes.

Pupil 6: How to use Gilstein pipettes, Using grids to measure the behaviour of the flatworms, How to measure out tiny amounts of chemicals using scientific scales, How to record quantitative and qualitative.

Did you learn more new facts, skills and words from the scientist’s presentation, your on-line research task, the experiments or from talking to the scientist?

Pupil 1: From talking and experimenting with my scientist because I was physically doing what I was learning.

Pupil 2: From talking to our scientist, and doing research, as I find both of these methods best for learning.

Pupil 3: In the on-line research task I found many new facts.

Pupil 4: From doing the experiments and talking to the scientist because we could talk about something and then go and test it out.

Pupil 5: Yes, I learnt more facts, skills and words from the project as we got to use new equipment and conducted new experiments.

Pupil 6: I learned more new facts from doing the experiment and talking with Dr Judge about the experiments because I am a kinaesthetic learner.

Which parts of the project interested you the most? Think about all elements of the project, the sessions, what you learnt, what you did etc

Pupil 1: I found the actual experimenting the most interesting because I was putting all my skills into practice and it was interesting to see the links and differences in the behaviour of the worms. However I found that the skills workshop was very useful as it taught me the fundamental skills I needed in the actual process.

Pupil 2: I was most interested in talking to professionals, and the workshops like the confidence workshop and the introduction workshop. I enjoyed these both, as they are engaging, and I enjoy that sort of thing.

Pupil 3: Learning how to do experiments that contributed to research that would affect what was in our drinks really excited me as well as working in the lab.

Pupil 4: I was most interested in talking to the scientist and then being able to take what we were talking about and get on and test it straight away which to me was very interesting.

Pupil 5: I was most interested in the experiment parts of the project as we learnt how to mix chemicals to precise concentrations. This also interested me as it was a new experience to work in a lab and we learnt how to find data by testing using certain methods.

Figure 3. (Continued)
Pupil 6: I was most interested in the experiments we did in the labs at Newcastle University medical School because it was interesting to see and use equipment that we would never be able to use in a lab at school.

Which parts interested you the least? Why?

Pupil 1: I found the entire project interesting as everything I learnt was new to me so I was always learning.

Pupil 2: I didn’t enjoy the experiments themselves; for all tests, we had to sit and look at a worm for 20 minutes, and I found it boring. We also had to do this multiple times, resulting in a lot of boredom.

Pupil 3: I found 20 mins quite a long time to sit and watch a flat worm turn in circles and tally, and then repeat 3 or 4 times.

Pupil 4: I didn’t really enjoy the time we had to spend collecting the results. I enjoyed doing the experiment and planning it but I was surprised at how long it took to get our results. This was probably because I had never done a scientific experiment like that before and that is how long it takes normal scientists to get results.

Pupil 5: The area that interested me the least was the poster session as it was more to do with applying skills in a slightly different way. However this was still enjoyable and it is a valuable skill.

Pupil 6: Everything about the project interested me, especially the experiments, but the part I was least interested in was the Speaking skills workshop (part of the leading edge programme).

Do you think other pupils would be interested in conducting the experiments you conducted in school? Why?

Pupil 1: Yes, because it is not something that is usually done in school and would help improve their understanding of animal behaviour and professional research.

Pupil 2: I do think other students would find these things interesting, as it’s a break from the regular look-at-the-board-and-learn lessons, and the students would be able to understand the effects of chemicals without witnessing them.

Pupil 3: I think other pupils would be interested in this experiment as they would learn exciting new skills and it is something that is relevant to their lives.

Pupil 4: I believe there would be a lot of interest in our school. 20 of us wanted to take part in this project but sadly only 6 can go. I think this shows the level of interest our school has in science as a whole.

Pupil 5: I believe that students would be interested in conducting the experiments in school as it would be a new experience for the students. One of the things they would learn would be that animals will not behave in the same manner, even if being put in the same chemical as they are all different.

Pupil 6: I think that they would be interested in doing the same experiments that we did in our project but they would have to understand that they aren’t the most interesting experiments in the world.

How would you make the experiments more interesting for other pupils?

Pupil 1: I would maybe use a smaller amount of chemicals as the observation period could sometimes be quite tedious especially for a class of students.
Pupil 2: The time spent looking at the worm can be a bit boring, so if something could change that, or you could shorten it, as that would decrease the boredom of the students, resulting in less noise, and better behaviour of the students.

Pupil 3: To make it more interesting I would use less chemicals so it would not take as long and work in pairs so that one person can tally the amount of squares the worms crossed and the other writing down a detailed description of the movement of the worm.

Pupil 4: The time it took to monitor the worms could be a bit too long if a whole class was doing it all at once so if that time could be shortened it would improve the behaviour and keep their interest in the experiment if they aren’t so interested in science as us.

Pupil 5: This experiment would help to promote interest as you would always have different results; this means it is not a fixed result unlike in some experiments, e.g. burning certain powders.

Pupil 6: I would make the time that they’d watch each worm for shorter so they wouldn’t lose interest or become bored.

Before being part of this project did you think research in toxicology was relevant to your life?

Pupil 1: No, it was not really something I had ever thought about as it is not an everyday topic.

Pupil 2: No, not really—it didn’t interest me much.

Pupil 3: Before the project I did not even know what toxicology or that it was relevant to my life.

Pupil 4: No, not at all. I didn’t even know that it was a field of science.

Pupil 5: Before this project, toxicology was not an area of science that I was particularly aware of.

Pupil 6: I knew that there were some chemicals that are dangerous for your body but I never realized how relevant it was to my life.

Did being part of this project change your mind? Why?

Pupil 1: Yes, after seeing how the worms react it made me think about how these chemicals are affecting my body and behaviour.

Pupil 2: Yes, it definitely changed my mind. To see the effects of artificial chemicals right in front of my eyes were shocking, and to see them in graphs put it into perspective that millions of people consume this substance daily, temporarily altering the brain.

Pupil 3: Now I know that toxicology is the study of toxins in the brain and that it affects my life a lot.

Pupil 4: Yes, because toxico logical affects our lives every day and is very important to keep us safe from dangerous chemicals.

Pupil 5: This project has shown that toxicology is an area of science that I find interesting. This is due to the way that by developing the understanding of the effects of chemicals on animal models e.g. the flatworms helps us to understand how the chemicals will affect humans.

Pupil 6: Yes, because I now know more about what toxicology is and how knowing some things about it can help you.

Figure 3. (Continued)
You chose the chemicals to test. Do you think this made the experiments more relevant to you and other pupils?

Pupil 1: Yes, because they were chemicals we had either researched or use on a day to day basis so we knew about them before the experiment and allowed us to be able to relate to the experiment.

Pupil 2: Yes, as -like I said- people consume Aspartame and Caffeine on a daily basis, i.e. Coca-Cola.

Pupil 3: I liked the fact we could choose what chemicals we could test as it definitely made it more relevant as we could find out what we wanted to find out.

Pupil 4: Yes, it made us feel like we had more of an input on the experiment and get results on what affects us.

Pupil 5: I believe that choosing the chemicals made the test more relevant to us as it would make the experiment different to if another group conducted the same test but with different chemicals.

Pupil 6: I think it made it more interesting because we knew about the chemicals and what their usual effects are on people where as if we didn’t know what the chemical were then we would probably have found it quite boring.

What other chemicals are you concerned about and think should be tested? Why?

Pupil 1: I think alcohol and glucose would be interesting to test as they are so commonly used yet have such large side effects.

Pupil 2: I think chemicals that are a lot more common and life changing, like Nicotine, Ethanol, and some drugs, should be tested.

Pupil 3: Alcohol, Nicotine

Pupil 4: We should test every chemical that we ingest to test if they are safe but that would cost too much and is impossible.

Pupil 5: I think that new chemicals/drugs should be tested upon flatworms as they have similarities to vertebrate models.

Pupil 6: Before we did the experiments we had a list of chemicals that we were considering testing but we had to narrow it down and cut out a few. We were considering testing alcohol because it has very well-known side effects and would be interesting to see if humans reacted in the same way as flatworms do, however we didn’t test alcohol on the worms.

You were partnered with a real scientist. How did this benefit you and the project? Were there any down-sides to working with a real scientist?

Pupil 1: I think it helped a lot because this was something these scientists do every day so to be taught by someone who is in the industry it helped us learn a lot of new information.

Pupil 2: Professional scientists know a lot more than we do, and there were no downsides to having a professional scientist.

Pupil 3: I loved working with real scientists as it helped me learn a lot more as they specialise in the subject.

Pupil 4: She had a wealth of knowledge at hand for us. This was very helpful for any questions we could have had. I don’t think there was any down sides to working with our scientist.

Pupil 5: Being partnered with a scientist was a good experience, this is because she was glad to show us around and answer our questions about the experiments.

Figure 3. (Continued)
Pupil 6: Working with a scientist made the project a lot more interesting and we learned so much more than we would have if we didn’t work with a scientist.

Did you share your experience of the project with other people? Which parts, with whom?

Pupil 1: Yes, with my friends and family I talked more about the skills workshop and experiment because they were the newest experiences to me. I will also be sharing my experience with and audience when my team and I present our data, project and experiences.

Pupil 2: I shared everything with my parents and friends.

Pupil 3: I shared new facts that I had learnt and what I had done with my family who were very impressed.

Pupil 4: Yes, all of it to my friends.

Pupil 5: Yes, I believe that we have all had a brilliant experience with the project, working in labs and working with Sarah.

Pupil 6: We will be sharing our experience with an audience of parents, scientists and the other teams when we present our final presentation about the project and what we discovered. I also talked to my friends and family about the experiences I have had.

Did being part of the project make you more interested in toxicology? It would be great if you could provide examples that demonstrate your interest.

Pupil 1: Yes, I found the fact that no two worms were the same extremely interesting—when we were using the same chemical all the worms had slightly different behaviours and I think this is interesting because it is similar to how humans react.

Pupil 2: It did, as knowing some chemicals that are used in daily products can change your behaviour can be extremely helpful.

Pupil 3: I now know what toxicology is and its relevance to my life and I would consider it as an option to develop.

Pupil 4: Yes I now realise just how important this field is and I’m now considering becoming a toxicologist in the future it seems really interesting and important.

Pupil 5: The project has made me more interested in toxicology as it was an area of science that we had not heard a lot about.

Pupil 6: I now definitely consider toxicology a very important part of science whereas before I probably wouldn’t have even know what it was and what people did in this study.

Some people think scientists should listen more to what ordinary people think. What do you think might be the best way of doing that?

Pupil 1: Maybe by listening to what the members of public think should be looked into more, perhaps by sending out questionnaires to members of public who are interested in scientific research.

Pupil 2: The best way would be to hear out what the public are saying. Listen to banning of animal testing, the use of GMO’s, and all the rest of it.

Pupil 3: On-line questionnaires would be easy to access and use.

Pupil 4: I think a survey would be the best way but not posted through peoples door because those more interested in science will fill it out and those who are not
Approach to learning: Students appeared very aware of their preference and ideal environment for learning. They suggested an experiential approach to learning was ideal and the experience of 'doing' the experiment was valuable. The planning and talking to the scientist was also an important aspect to them.

Others: The students interestingly did not discuss any collaboration with peers as important or valuable. One student mentioned another student could 'scribe' but this was not a cooperative or collaborative suggestion. The discussions and guidance with and from the scientist was imperative. The scientist was seen as a context expert, not facilitator.

Action: The third theme was one of action: what students learnt from the active experience, how to improve this learning process and what they did with the experience. Students of course suggested they learned typical academic content. However, there were several comments concerning the value of just being in a research lab, suggesting that they learned how to work in this environment. They suggested that the experiment itself was too long. Suggestions for improvement included using chemicals that were faster or decreasing the time. Finally, they suggested they would take from this experience and share it with their family and friends. The action in the lab was important, too. Insufficient action caused them to lose interest. After finishing the experiment, they actioned their experiences by sharing with family and friends.

Meaningfulness: was the final theme identified. Students found three aspects important within this theme: academic content, animal rights and health considerations. These three areas were shared by students when asked about 'what' they had learned and what was relevant. Specific academic content, as expected, was highlighted concerning toxicology, etc. However, students consistently flagged

Figure 3. (Continued)
up areas of societal responsiveness. Concern over animal rights and an awareness of personal health issues were emphasised.

**Scientist’s evaluation of the partnership**

The experience was very rewarding. The pupils were fully engaged with the activities asking highly relevant questions and making very astute observations. Their enthusiasm for the project refreshed my own scientific curiosity. I had predicted they would select alcohol or nicotine to test and was surprised they chose aspartame, a chemical I had not previously considered investigating. The data they produced clearly demonstrate that aspartame metabolites can affect behaviour. Their choice illustrated that a scientist’s perception of the public’s interests and concerns may not be entirely accurate and reinforces the need for public involvement in science. I was also surprised, perhaps naively as I do this routinely, that they grew bored of the repeated testing. For future public engagement activities, the period of any practical work will be minimised to prevent boredom but not to the extent that it gives a false impression of the reality of scientific research, and the need for repeated testing. The results also support the growing evidence that invertebrates can be used instead of rodents as animal models in neuropharmacological and neurotoxicological studies.

**Discussion**

The task of conducting independent research on chemicals found in supermarket products increased the pupil’s knowledge about different chemicals and their effects on the nervous system. It also stimulated their interest in pharmacology and toxicology. They were particularly interested in aspartame having found many web pages dedicated to the effects of this chemical. Allowing the pupils to choose the test chemicals gave them the opportunity to pursue their own line of inquiry and motivated them to conduct the experiments. This was also evident through the questions they asked, for example do the effects of the chemicals reverse. The behavioural observation experiments yielded useful data on behavioural variability and formed the basis of discussions on variability in experiments and the need to control variability. The chemical testing experiment clearly demonstrated and reinforced the concept that exposure to some chemicals can significantly alter behaviour.

**Animal use**

Flatworms have been previously used in research laboratories as a tool for pharmacological and toxicological research (Raffa, Cavallo, and Capasso 2007; Rawls et al. 2009; Sacavage et al. 2008) and as a model in undergraduate teaching (Pagan, Coudron, and Kaneria 2009). However, as far as we are aware, this is the first time they have been used by school pupils to assess the effects of chemicals. They are easily obtained and maintained and relatively inexpensive. They do not come under the Animals Scientific Procedures Act 1986 but understandably, some people may have ethical issues using any type of living organism for an educational purpose. The pupils understood they were using living organisms and treated them with respect. They were very careful transferring them and the chemicals were used at very low concentrations. The changes in behaviour appeared to reverse indicating they were pharmacological, like the effects of coffee. There did not appear to permanent adverse effects on the flatworms. After testing, worms were replaced in their home container and maintained until the end of their natural life cycle.

**Pharmacology**

Caffeine and aspartame are ingredients commonly found in soft drinks. It is well-established that caffeine is a stimulant in mammals and causes dose-dependent increases in locomotor behaviour by inhibiting adenosine receptors (Yacoubi et al. 2000). However, its effects are biphasic and higher caffeine doses depress activity (Svenningsson, Nomikos, and Fredholm 1995). Previous reports on the effects of caffeine on flatworm locomotor activity are inconsistent. Pagan, Coudron, and Kaneria (2009)
reported that caffeine increased activity, whereas Moustakas et al. (2015) reported no overall effect. Indeed, Moustakas et al. observed a slight short-term stimulatory trend followed by an inhibitory trend in locomotor activity, consistent with our observations. This biphasic response may be dependent on caffeine concentration (the concentration in the flatworm increasing with time) and thus similar, to the concentration-dependent effects observed in mammals. Interestingly, guarana, an ingredient often found in energy drinks which contains caffeine and other stimulant alkaloids, moderately increases flatworm locomotor behaviour (Moustakas et al. 2015). Therefore, we would recommend that guarana is used in the future for flatworm classroom experiments as a stimulant instead of caffeine alone.

Aspartame did not affect flatworm behaviour. This was predicted as there is little evidence that aspartame has pharmacological or toxicological effects in mammals. Once consumed, aspartame is rapidly broken down into aspartame metabolites. Methanol is an aspartame metabolite and has well-known toxic effects, hence the public concern over aspartame consumption. Like other alcohols such as ethanol, it has depressant effects on the nervous system. The depressant effect is mediated via inhibitory GABA receptors and causes a decrease in locomotor behaviour (Palmer et al. 2002). The effect of methanol on planarian activity has not been reported previously. Planaria respond to GABA receptor modulators (Raffa, Cavallo, and Capasso 2007). The rapid and reversible decrease in locomotor activity we observed is likely to be due to the modulation of inhibitory GABA receptors producing a transient sedative effect on the nervous system rather than toxic effects. Phenylalanine is another aspartame metabolite and products containing aspartame state that it is a source of phenylalanine. Phenylalanine is an amino acid and a precursor for a number of mammalian neurochemicals. It is also an antagonist at the excitatory N-methyl-D-aspartic acid (NMDA) glutamate receptor (Glushakov et al. 2002). NMDA receptors are involved in learning and memory and it has been suggested that NMDA receptor blockade may underlie the developmental disability observed in phenylketonuria, a condition characterised by high phenylalanine levels. Given that planaria possess functional NMDA receptors (Rawls et al. 2009) it is likely the relatively rapid decrease in activity we observed with phenylalanine is due to NMDA receptor blockade.

These experiments clearly demonstrate that aspartame metabolites can reversibly affect behaviour. However, this does not mean that drinking one can of a soft drink containing aspartame will affect a child’s behaviour. As with many chemicals, the effects will be dose-dependent.

**Recommendations for classroom implementation**

The experiment can be used to illustrate that chemicals, such as recreational drugs, can stimulate or depress behaviour (Department of Education 2013). Although conducted in a university research laboratory it does not require sophisticated equipment and can provide results quickly (<30 min) and so can be used in a classroom or public engagement workshop. There are a number of aspects to this experiment that we think will engage pupils and children. Firstly, the chemicals are soft drink ingredients making the experiment relevant to their present lives and therefore ‘meaningful’, secondly the experiment is hands-on and so the pupils are active participants in the learning process and thirdly, observing the behaviour of an organism provides a source of novelty (Palmer 2009). However, the pupils identified that repeated testing of different chemicals and 20-min observation periods reduced the novelty of the experiment. Thus, we would suggest limiting the chemicals tested to two, perhaps one depressant such as methanol, ethanol or phenylalanine and a stimulant such as guarana and limiting the observation period to 3 min, before the chemical and 10 min in the chemical. In addition, we would recommend working in pairs, with one person observing and the other scribing. Incorporating these recommendations suggested by the pupils will make this novel, low-cost experiment more interesting for their peers.
Educational implications

These pupils found the opportunity for experiential learning valuable. The concrete experience of the laboratory work and reflective observation (Kolb 1984) with the scientist was essential. Pupils saw the cyclical nature including: talking and discussing things through with an expert and the abstract conceptualisation of learning from experience worthwhile. For educators, creating these, or similar opportunities for students may be a significant platform for learning.

No peer collaborative activities were suggested by pupils. Indeed, the scientist was the primary active agent in the learning and was seen as a knowledge expert and authority. The role of the scientist (as teacher) was not to facilitate or supervise, but to transmit knowledge and instruct. This is consistent with a scholarly academic approach to teaching. In this ideology, teachers are transmitters of knowledge, learning occurs from the perspective of the teacher and children are relatively passive (Schiro 2008). As educators, this is not necessarily a concern, but an awareness of students’ perceptions surrounding teachers is important. Furthermore, although the promotion of cooperative and peer learning is trendy in UK schools (Gielen and De Weyer 2015), students did not suggest this was lacking in the activities in which they participated.

It was also evident these students needed action. The wanted activity in their learning and this dynamic clearly continued after the programme was finished as pupils shared information with their family and friends. It is well documented that students appreciate activity in science curricula and a lack of it can cause boredom, making science appear tedious and boring (Delpech 2002). Given the difficulties for teachers in trying to balance the demands of prescriptive curricula and the inclusion of practical work, this is an important factor to note.

Finally, for educators, the value of a curriculum as being an agent of social change is unequivocal. Although pupils flagged up the academic basis of what they had learned (toxicology, etc.), it was clear there were other aspects as well. For example, awareness and concern surrounding animal testing and the adverse effects everyday chemicals may have on their individual health. This demonstrated the power of this programme. Aspects of a social reconstructionist approach (Schiro 2008) were seen as students demonstrated their desire and convictions regarding a future ‘good’ society. This suggests these pupils were able to consider their ability to contribute to an improved society. Shaping children to be socially and personally aware was an invaluable outcome of this partnership, and arguably one of the most important roles of today’s teacher.

Acknowledgements

The authors would like to thank Drs A. Fitchett and C. Morris for comments on the manuscript.

Funding

This work was supported by the Faculty of Medical Sciences at Newcastle University under the Leading Edge Grant.

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