



Professor Paul Fowler tells Josh Howgego how the REEF project is beginning to make sense of how the myriad chemicals we are exposed to in daily life impact human female fertility.



Fertile ground for investigation

If you're anything like me, whenever you pick up an apple to eat, you'll give it a quick wash. It's probably not going to do you too much harm if you don't, but – well – it's best to be on the safe side. You never know what chemicals it has had on it.

This everyday uncertainty is a microcosm of a wider problem. In general, scientists have little idea of how the diverse cocktail of low level environmental

chemicals (ECs) we are continuously exposed to impacts our bodies. The Reproductive Effects of Environmental chemicals on Females (REEF) project, led by Professor Paul Fowler of the University of Aberdeen, UK, is one group trying to understand how these chemicals might be affecting human health.

There is a growing body of evidence that infertility in males is linked to ECs.

Specifically, Testis Dysgenesis Syndrome (TDS) seems to have links with specific ECs, and can cause abnormally formed genitalia, low sperm counts and consequential infertility in males. And the occurrence of male reproductive disorders in general is on the rise.

'In Denmark, for example,' says Fowler, 'the latest figures suggest that about half of young men have sperm counts that are

approaching the sub-fertile level. It is pretty shocking.'

Female fertility is different

It's relatively easy to measure fertility in males – you just count the sperm in a sample – and so progress in research which relates ECs to TDS has moved steadily forward. But for women, assessing fertility is much more difficult. In females, hormone levels fluctuate during the menstrual cycle, and there is still no single simple method for counting eggs in living females. REEF is hoping to address the imbalance of knowledge by improving our knowledge of how ECs affect female reproductive health.

The key questions

A startling and key observation occurred when scientists noted that particular types of long-lived ECs accumulate at higher levels in foetal than in maternal tissues. Di-2-ethylhexylphthalate (DEHP) and certain polychlorinated biphenyls (PCBs) are known to act in this way, but there may be others. These compounds are widely used in consumer goods and include chemicals such as plasticisers and coolants, and so we come into contact with them all the time.

'Part of the issue is that we are exposed to very complex mixtures of chemicals' explains Fowler. 'There's considerable evidence that these mixtures have a greater biological effect than you would expect from just adding up their individual potencies. But we – and others – are convinced that phthalates are of particular concern.'

With this in mind REEF has set out to shed light on these hugely complex biological questions. The project's consortium of research groups from four countries (UK, France, Germany & Italy) wants to know, once accumulated, do DEHP and PCBs affect the development of foetal ovaries?

The project is also looking to find out if the effects of ECs get passed down through generations. Worryingly, since all the egg cells a female will ever produce are formed after just six months in the womb, the next generation may be affected before the mother is even born. Another, still controversial, issue is epigenetic changes – changes in methylation of the DNA – may mean that exposure of mothers to ECs could affect their offspring for the next two generations – and if there are trans-generational effects, maybe even the third and fourth.



REEF also has a broader goal – to learn more about if and how ECs in general pose a threat to the developing human foetus. There is a reason why we don't know more about how ECs affect health though. It's a hugely complex issue. For a start, we don't know how many individual ECs might interact with the body. Some ECs are endocrine disruptors, which means they have an effect on one or more of the hormone systems of the body. But a chemical can produce this effect in a multitude of different ways. It could bind to a hormone and act like the hormone; both are potentially damaging. Equally, an EC can interact with the receptors, which recognise hormones, by blocking them or prodding them to behave in a different way upon sensing the chemical messages.

Simply making measurements of how hormone levels are affected upon exposure to ECs is not enough either. 'One issue is the genotype of the mother,' says Fowler, 'some people are more susceptible to chemicals than others. That might be due to small differences in the genes coding their liver enzymes, resulting in less efficient breakdown of ECs, for example. Some ECs are perhaps not endocrine disruptors at all, but can have effects on reproductive health through other pathways. All this complexity means that incredibly detailed studies need to be completed before conclusions can be drawn.'

Eventually, a combination of studies in this field could lead to the identification of specific chemicals which are doing the human population serious harm. But from another perspective, they're actually helpful additives, widely used in consumer goods. When scientists recommend that

the use of a particular compound should be scaled down it could present economic challenges for industry. 'It would be very important for any ban to be based on solid, good quality data,' says Fowler, 'some of these chemicals would be very expensive to ban and it could well be that a chemical that has an effect on a mouse has no effect on a human. That's why we're referring our sheep and mouse models back to humans.'

These are big questions with potentially huge impacts on public life. If ECs are having serious impacts on reproductive health, those effects could be long lasting and extremely serious. As Fowler says 'this issue is something that needs to be looked at really closely – and that is expensive and difficult.'

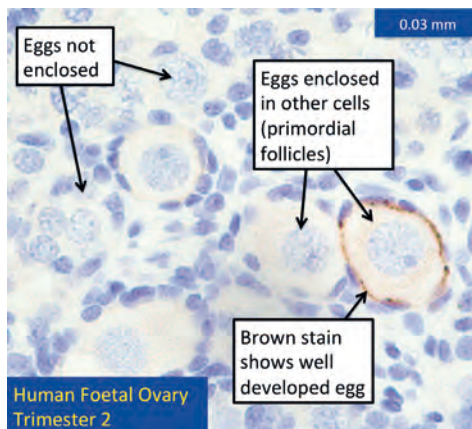
A real-life simulation

A lot of previous work on EC toxicity has looked at injecting or administering single (or just a few), specific, environmentally persistent chemicals into organisms and measuring the short-term effects. Although useful, this approach fails to replicate the way in which we are exposed to chemicals in real life. The approach of the REEF project is longer term, more comprehensive and far more representative of how humans really experience ECs.

REEF is using sheep fed on pastures which have been fertilised with solid pellets made from processed sewage sludge to model real-life human exposure to ECs. The sewage sludge contains all the nitrogen the grass needs to grow healthily, but also throws in a dose of pollutant chemicals. Since sewage contains, of course, human waste as well as agricultural and industrial run-off, this material provides an

Left: A ewe with her lambs on a sewage-sludge fertilised pasture.

Right: Micrograph of a human foetal ovary at 19 weeks of pregnancy.



accurately constituted cross-section of the ECs to which the contemporary human population are routinely exposed. Sheep fed on nearby pastures fertilised with inorganic fertilisers form the control group.

Mice are also used as models, and in this case the research also aims to determine how the effects of ECs are expressed across generations. Since mice reproduce more quickly than sheep, potential transgenerational effects are easier to study in this model. In the mouse model EC-exposure caused metabolic changes closely related with normal fertility, as well as affecting reproduction directly. These findings show that DEHP may also act as an obesogen (something that makes an individual fatter).

Working with these two animal models, REEF has already uncovered some key genes that are altered in the EC-exposed animals, but not in the control groups. 'Some developmentally important genes are affected' says Fowler, emphasising that the details are soon to be published in full. Once the team have leads as to which genes and pathways are being affected in animals they can look at the analogous cascades in humans.

They do this by looking at human foetuses that have been electively terminated between three and six months of pregnancy. This is the stage where the future reproductive capacity of the female foetus is determined.

All of the primordial follicles – the earliest stage of cell clusters enclosing the eggs – a woman will ever possess are formed during this second trimester of pregnancy. Fowler's team have gained new insights into the genes that help control the production of primordial follicles and can now study how these currently poorly understood processes are affected by exposure to ECs.

Although all humans are exposed to ECs regularly, the REEF team have also

investigated the effects of how smoking can affect foetal reproductive health. Maternal cigarette smoking is another useful model of the EC landscape since it is one of the few defined adverse chemical exposures in pregnant women. The researchers investigate genes that have been identified as sensitive to chemicals in the animal studies in order to see whether they are also affected by cigarette smoke exposure in the human foetus.

The beginning of the end?

The REEF project draws to a close at the end of October 2011, having reached the end of its 42 month, €2.9 million European Commission funding period. Importantly, the project has shown that the timing of exposure to ECs during development affects how severely the foetus is affected. PCBs and DEHP have also been shown to adversely affect ovaries in mice, and other ECs have been proven to have negative effects on foetal ovaries, especially during the crucial stage when primordial follicles are being formed.

The broad conclusions from REEF can be summarised as follows. The effects of mixtures are not the sum of isolated chemicals, and that these effects depend on:

- * Stage of gestation
- * The organ in question (ovary, pituitary, mammary gland)
- * The sex of developing fetus
- * The class of chemical(s) under investigation.

As the consortium collates the data from the project and publishes its conclusions, a picture emerges which depicts the female reproductive health as extremely sensitive to the chemicals that we eat, breathe or rub in to ourselves on a daily basis. More data are needed, of course, but REEF has succeeded in pioneering a path to understanding this complex yet vital, area of science. ★

At a glance

Project Information

Project Title:
REEF: Reproductive Effects of Environmental chemicals in Females

Project Objective:
To determine effects of ECs, including those in sewage sludge, on the foetal ovary during critical developmental windows, also establishing effects of environmental concentrations of DEHP and PCBs on female development. We also aimed to establish any transgenerational effects of ECs on female development and to integrate human and animal models.

Project Duration and Timing:
42 months, 01/05/2008-31/10/2011.

Project Funding:
REEF is funded by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no 212885, ending October 31st 2011. €2,923,363.

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Prof Fowler graduated in 1982 (1st Honours Zoology) at the University of Aberdeen, completing his PhD in 1986. After post-doctoral and academic posts, in 2006 he moved to the Institute of Medical Sciences (remaining in Aberdeen), as chair in Translational Medical Sciences. Over the last decade his research has focused on endocrine disruption of fetal development in humans and animals.



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