Why do methods get stuck?

The process of implementing alternatives for regulatory use is complicated by a number of factors; regulatory reassurance usually needs to be given for each specific alternative method for each specific sector (e.g. cosmetics, pharmaceuticals or chemicals), several regulatory bodies may need to be involved and legislative and other documents may need to be updated. Failure by regulatory bodies to recognise these steps and take responsibility for them has, in our opinion, been a major reason for some of the delay in the implementation of these methods.

Companies may also need to perform in-house validation studies - particularly for alternatives to batch tests and will need to update their market authorisations. Failure to keep up with updates to the pharmacopoeias and other medical legislation has, in our experience, been one reason why some of these tests are still commissioned.

Finally, there needs to be improvements in the harmonisation of testing requirements between EU and non-EU countries. We believe that companies and regulators should not permit animal testing for regions that do not accept the alternative if the alternative has been recognised in at least one region and there is no scientific reason why the other region should not accept it.

What regulators can do

Communicate	Ensure there is a mechanism for continual dialogue on the acceptability of these alternatives between the regulatory agencies in your region that are responsible for chemicals, medicines, pesticide, biocides, cosmetics and food as well as those responsible for authorising animal experiments.
Analyse	Identify and map the reasons for the continued use of these tests and take action where possible.
Promote	Make sure those that use animals are aware of these alternatives or waiving options.

What companies can do

Validate	Perform in–house validation of the alternative for your product, where necessary and update your licences.
Analyse	Proactively evaluate the need for animal testing and take your results to the regulators.
Use	Make sure those that throughout your business there is awareness of - and commitment to use - these alternatives or waiving options.

For change to happen it is important that a desire to minimise animal testing is matched with actual policy and resource. Both regulators and companies need to invest in people who will look out for and assess alternatives as they come on board, as well as the science to ensure that new alternatives continue to be developed.

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Implementing alternatives to animal testing

The RAT (Replace Animal Tests) list

Over the last 30 years there have been great developments in the replacement of tests on animals for regulatory purposes. Alternatives have been developed that can now replace wholly, or in part, a number of animal tests for several product sectors.

However, our experience has been that these methods can become 'stuck' in the process and can take much longer to actually replace animals than most people think.

In recent years alternatives to animal tests such as skin irritation, acute toxicity and various batch safety tests have taken years to be completely accepted and in many cases the animal test is still being conducted for regulatory purposes. Everyone will agree that this is something that should be avoided, both in the interests of animal welfare and good regulatory science, as alternative methods are usually cheaper, faster and more accurate than the animal tests they replace.

Our experience is that there are a number of reasons why methods may become 'stuck'. For example, companies may need reassurance from their relevant regulatory authority that these methods will be acceptable for regulatory purposes. Failure to provide this reassurance, or delay in doing so, can mean that animal tests are still conducted when an alternative method is actually scientifically acceptable and available. Additional validation on a product by product basis may be required for alternatives for quality control testing and there may be insufficient motivation for companies to do this.

Furthermore, lack of communication between regulatory sectors can mean that information about the availability and acceptability of new alternative methods simply falls through the gaps.

We have created the RAT list to draw regulatory and industrial attention to this issue. We have selected just 10 animal tests that are still being conducted in Europe despite evidence that they are either redundant or have valid replacements. We have estimated that half a million animals are being used in these tests in Europe alone despite the fact that EU law (Directive 2010/63) states that "Member States shall ensure that, wherever possible, a scientifically satisfactory method or testing strategy, not entailing the use of live animals, shall be used instead of a procedure".

We highlight where these 10 tests are 'stuck' in the process and provide some practical solutions that regulators and companies can employ to get them moving again.

Cruelty Free International

Cruelty Free International is the leading organisation working to create a world where nobody wants or believes we need to experiment on animals.

We are widely respected as an authority on animal testing issues and are frequently called on by governments, the media, corporations and

Cruelty Free International

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The RAT list

Test and number of animals used annually	Description of test	Options for replacement	What needs to happen
Pyrogen test – Rabbits 3,167 pyrogen tests were done in the UK alone in 2014. Across the EU the number is thought to be around 200,000. Sector: HP, VP	Rabbits are restrained in boxes for up to eight hours per test, with food and water restriction prior to this. Rabbits can suffer a fever reaction (in the rare occasion the batch is contaminated), and damage to ears from repeated injections. Temperature probes are inserted deep into their rectums during the test. Rabbits may be housed singly during their lives and are typically re-used several times.	This has been replaced by a test which uses blood from horseshoe crabs, and more recently with a more sensitive test based on cryo-preserved human blood.	**Regulators** need to ensure that the rabbit test is no being conducted when the relevant pharmacopeia specifies otherwise. **Companies** need to be encouraged to validate the human blood test for their product and update their licence.
Botulinum toxin test – Mice 220,544 mice were used in LD50 tests in Europe in 2011 alone. A large proportion of these tests were botulinum toxin (botox). Sector: HP	This is an LD50 (Lethal Dose 50%) test aimed at determining the dose that kills exactly half of the animals used. The mice are injected into their abdomens with the botulinum toxin and over the next three days become increasingly paralysed. If left, mice in the higher dose groups will suffocate to death within approximately three days.	Some toxin manufacturers have now developed a cell-based test to replace the batch test, but they continue to use the mouse test for other purposes.	**Regulators** need to ask all companies to validate the cell-based test. **Companies** need to be encouraged to validate the cell-based test for their product.
Acute toxicity test – Rats 4,431 rats were used in acute tests across Europe in 2011. Sector: HP, VP, C	Animals are exposed to very high doses, which can cause irritation, difficulty breathing, weight loss, convulsions, bleeding and death. Death is still used as the 'endpoint' in tests via the dermal or inhalation route. In tests where the animals are force-fed, the researchers may kill the animal before they die but only if they are extremely ill and they are found before they die.	This test is redundant in many cases as companies use the repeated dose test for their safety purposes. This was demonstrated for pharmaceuticals in 2008. A humane simple cell-based test was validated in 2013 for chemicals and now can be used to demonstrate absence of toxicity but is not yet in common use.	**Regulators** need to assess the need for the oral test across all sectors. **Regulators** need to promote the use of the cell-based test to waive testing. **Companies** need to be aware of changes to the requirements for this test in all sectors.
Ecotoxicity – Fish 71,406 fish were used in acute and chronic toxicity tests in Europe in 2011. Sector: HP, VP, C	Young fish are exposed to the test substance dissolved in their tanks for 96 hours. The acute test is a lethal test - 50% of the fish are expected to die. Fish tend to be found dead and are not humanely killed. Chronic tests can also cause death.	The Zebrafish Embryo Acute Toxicity Test Method (ZFET) and the Short-term Toxicity Test on Embryo and Sac-Fry Stages are replacements of the acute and chronic fish toxicity tests respectively, and use fish embryos rather than young fish. The ZFET has been shown to agree with adult acute fish test results 90% of the time.	**Regulators** need to promote the use of the embryo-based test. **Companies** need to be aware of these alternatives and use them.
Carcinogenicity – Rats & mice 11,826 rats and mice were used in carcinogenicity tests across Europe in 2011. Sector: HP, VP, C	Mice or rats are given a substance either in their diet, drinking water or are force-fed every day for two years. All of the animals are then killed and dissected to see if the substance leads to signs of cancer. Animals often suffer from spontaneous cancers during the experiment that may not be due to the substance.	Because of its unreliability and expense, this test is losing popularity and is rarely required in practice for chemicals and cosmetics and is being examined for replacement for pharmaceuticals. However, it is still a requirement in the legislation and guidance.	**Regulators** need to speed up their analysis of the redundancy of this test and make sure it is removed from all requirements. **Companies** need to continue to encourage regulators to end the requirement for this test.
Eye irritation – Rabbits 2,080 rabbits were used in eye irritation tests across Europe in 2011. Sector: C	The substance is left in one rabbit's eye for at least one hour before it may be washed out. The eyes are then examined for signs of irritation and damage over 3 days. If there are no signs of severe irritation in the initial test, two more rabbits are used. Rabbits are forced to suffer restraint whilst being dosed and examined and can experience painful damage in their eyes that can cause blindness.	Eyes from dead hens and cows can be used in validated tests to identify severe irritants and non-irritants. Reconstituted human eye models have also now been accepted.	**Regulators** need to promote the alternatives and ensure licences are not issued for testing unless absolutely necessary. **Companies** need to ensure they use the alternatives and continue to develop the methods for mild irritation.
Skin irritation – Rabbits 3,151 rabbits were used in skin irritation tests across Europe in 2011. Sector: C	The product is rubbed onto a 6cm area of a rabbit's shaved skin on their backs and held in place with a bandage for four hours. The rabbit is then examined for signs of skin damage for 14 days. If there are no signs of irritation in the initial test, two more rabbits are used in a 'confirmatory test'. Rabbits are singly housed and can suffer from painful skin reactions and rashes.	The test can now be completely replaced with reconstituted human skin models, which are validated and widely accepted. They are more predictive than the rabbit test.	**Regulators** need to promote the alternatives and ensure licences are not issued for testing unless absolutely necessary. **Companies** need to ensure they use the alternatives.
Skin sensitisation – Guinea pigs & mice 15,214 guinea pigs were used in guinea pig maximisation test (GPMT) and 16,846 mice were used in LLNA (Local Lymph Node Assay) skin sensitization tests in Europe in 2011. Sector: C	In the GPMT guinea pigs are injected six times in their backs with a substance that increases their body's immune response to the test chemical. Six and then 20 days later the test chemical is rubbed onto their shaved skin. The animal is observed daily for allergic reactions for 23 days. The guinea pigs may be killed and dissected to confirm any unusual reactions. They may be singly housed and suffer from painful skin reactions and rashes. In the LLNA the test substance is painted onto the ears of mice every day for three days. The mice are then killed three days later and their ears are dissected.	The GPMT was replaced by the LLNA in 1999; but the LLNA itself is now replaced. Chemical based (DPRA) and cell based tests (ARE-Nrf2 -KeratinoSens) have been formally accepted by the OECD in 2015. Currently, at least two alternative tests need to replace the LLNA, however this strategy has been shown to consistently predict 90% of human skin reactions.	**Regulators** need to promote the alternatives and ensure licences are not issued for testing unless absolutely necessary. **Companies** need to ensure they use the alternatives.
2nd species repeated dose toxicity test – Dogs & monkeys 2,785 dogs were used in repeated dose tests along with 1,306 monkeys in Europe 2011. Sector: HP	Dogs or monkeys are used as a second species after mice or rats to test the safety of human medicines. Animals are dosed every day for between two weeks to nine months with drugs that might lead to harmful side effects that can include vomiting, diarrhoea, internal bleeding and organ damage, seizures, paralysis and even death. They are also subjected to other stressful tests such as repeat blood sampling and daily gavage. Monkeys are usually imported from Africa or SE Asia for these tests and may have been born to parents or grandparents that were taken from the wild.	Research conducted by Cruelty Free International has recently provided more evidence that this test does not help show whether a drug is likely to be toxic to humans. Cell based tests and computer models are in use but are not currently considered adequate by regulators or companies.	**Regulators** need to assess the justification for this test in collaboration with industry. **Companies** need to work with regulators to assess the need for this test.
2nd species prenatal toxicity test – Rabbits & monkeys 2,560 rabbits and 281 monkeys were used in developmental toxicity tests in Europe in 2011. Sector: HP, VP, C	Rabbits or monkeys may be used in an additional 'second species' test after similar tests in rats. The animals are force-fed the test chemical during most of their pregnancy and are killed the day before they are due to give birth. Their pups are extracted by caesarean section and examined before being killed. Due to the high doses used, the chemicals may cause the mother to become ill and some chemicals could lead to deformities, stillbirths or miscarriages.	There is little evidence that testing on a second species adds to the safety of chemicals. Several studies have indicated that the test in rabbits may be unnecessary and should not be conducted, but regulators are inconsistent in their rules.	**Regulators** need to assess the justification for this test in collaboration with industry. **Regulators** need to ensure there is consistency between sectors in the waiving options. **Companies** need to work with regulators to assess the need for this test.

HP - Human pharmaceuticals, VP - Veterinary pharmaceuticals, C - Chemicals including biocides, pesticides, cosmetics, industrial chemicals