G: NON-TECHNICAL SUMMARY (NTS)

NOTE: The Secretary of State considers the provision of a non-technical summary (NTS) is an essential step towards greater openness and requires one to be provided as part of the licence application in every case. You should explain your proposed project clearly using non-technical terms which will be understandable to a lay reader. You should avoid confidential material or anything that would identify you, or others, or your place of work. Failure to address all aspects of the non-technical summary may render your application incomplete and lead to it being returned.

This summary will be published (examples of other summaries can be viewed on the Home Office website at http://scienceandresearch.homeoffice.gov.uk/animal-research/).

(WORD LIMIT: 1000 WORDS)

Please complete the following:

<table>
<tr><td>Project Title (max. 50 characters)</td><td colspan="3">Understanding cerebral folate metabolism</td></tr>
<tr><td>Key Words (max. 5 words)</td><td colspan="3">Hydrocephalus, brain development, folate</td></tr>
<tr><td>Expected duration of the project (yrs)</td><td colspan="3">5</td></tr>
<tr><td>Purpose of the project (as in Article 5)[1]</td><td colspan="3"><table>
<tr><td>Basic research</td><td>Yes</td><td></td></tr>
<tr><td>Translational and applied research</td><td>Yes</td><td></td></tr>
<tr><td>Regulatory use and routine production</td><td></td><td>No</td></tr>
<tr><td>Protection of the natural environment in the interests of the health or welfare of humans or animals</td><td></td><td>No</td></tr>
<tr><td>Preservation of species</td><td></td><td>No</td></tr>
<tr><td>Higher education or training</td><td></td><td>No</td></tr>
<tr><td>Forensic enquiries</td><td></td><td>No</td></tr>
<tr><td>Maintenance of colonies of genetically altered animals[2]</td><td>Yes</td><td></td></tr>
</table></td></tr>
<tr><td>Describe the objectives of the project (e.g. the scientific unknowns or scientific/clinical needs being addressed)</td><td colspan="3">Periconceptional maternal supplementation with folic acid has demonstrated benefits in reducing the incidence of neural defects (spina bifida) but has no clear reported benefits on other neurological condition. Many neurological conditions have now been identified with a folate problem affecting the brain but not the rest of the body. Furthermore, one of these, hydrocephalus, responds to natural folates but not the unnatural folic acid with a decrease in incidence. Understanding the precise nature of how the brain is supplied with and handles folates will allow the development of a maximised effective treatment of such conditions with a cerebral folate issue.</td></tr>
<tr><td>What are the potential benefits likely to derive from this</td><td colspan="3">With knowledge of how the brain is supplied with, and handles folates, a combination folate therapy can be defined that compensates for cerebral folate</td></tr>
</table>

[1] Delete Yes or No as appropriate.
[2] At least one additional purpose must be selected with this option.

project (how science could be advanced or humans or animals could benefit from the project)?	insufficiency or imbalance and which can be used to maximise normal brain development in humans and animals. Too much of any folate can push the balance too far in specific directions leading to different problems so the proper dose and combination to provide a balance is essential.
What species and approximate numbers of animals do you expect to use over what period of time?	The best characterised model for human foetal-onset hydrocephalus is the hydrocephalic Texas (H-Tx) rat as well as the less well characterised hydrocephalic (hyh) mouse. These will be used to investigate the natural history of the brain folate problem underlying the development of the condition. The mouse is important as it is driven by a single gene mutation that gives hydrocephalus as opposed to the multi-gene susceptibility in the H-Tx rat. The consequence of fluid drainage problems should be the same so the mouse will give the important opportunity to test this. This will require maintaining a colony of both animals as well as using normal rats and mice as controls to see how the system develops in them. A maximum of 4000 animals from the two colonies (3000 from protocol 1 used in protocol 2) will be used as well as significantly fewer normal animals (max 2000 wild type bought in) over the course of the study but hopefully far less since we will analyse data as we go and stop experiments as we reach significance or we see them not producing significant data.
In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?	Minimal adverse effects are expected from the experimental program and we therefore expect a mild or moderate severity limits. All animals will be sacrificed for brain analysis at the termination of experiments. Any unused animals produced from the hydrocephalic colonies will be processed into a brain bank for future study or supply to other researchers. Any animal showing any adverse effects likely to exceed mild-moderate levels, will be humanely killed. The H-Tx rats have a susceptibility to hydrocephalus which ranges from 20 to 100% of any particular litter dependent on the mother and her conditions. Stress leads to a greater incidence. Each individual neonatal rat pup is either born unaffected or affected with the degree of hydrocephalus measured by the obvious doming of the head that occurs as fluid accumulates within the brain. Hydrocephalus itself is not associated with any pain or distress but as fluid accumulates and pressure rises within the head neurological signs and symptoms indicate possible distress. These signs occur following skull plate fusion at postnatal day 10 and subsequent fluid accumulation and rising intracranial pressure. All affected animals will be culled on or before postnatal day 20 at which point they will have no more than mild clinical signs including doming of the head and some weeping and sunsetting (partial closure) of the eyes due to pressure on the occulomotor nerves. Similarly all affected hydrocephalic mice would also be

	culled by postnatal day 20. It is important to sample animals after day 10 as this is the point when the skull plates fuse and raised intracranial pressure is first detected.
Application of the 3Rs	
1. Replacement State why you need to use animals and why you cannot use non-animal alternatives	We need to investigate a complex condition and functional outcomes and this can only be achieved with the special colonies and by comparison to normal rats and mice. The H-Tx rat is the most well characterised model equivalent to human foetal-onset hydrocephalus and has proven to be an ideal model.
2. Reduction Explain how you will assure the use of minimum numbers of animals	We will analyse data as we collect them and stop experiments as we reach significance or it is clear the experiment is showing no differences to controls or untreated animals.
3. Refinement Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.	The objectives require a close model to the human condition and the H-Tx is widely regarded as the best model. The experimental plan involves minimal harm to animals and any possibility will be minimised by proper training of personnel.
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Will the project be subject to Retrospective Assessment?[1]	☐ No ☐ Date due[3]:

[3] The retrospective assessment should be completed, agreed with the establishment AWERB, and submitted to the Home Office within 3 months of this date (or when the project terminates if earlier).