The ASC
Animals in Science Committee

ANIMALS IN SCIENCE COMMITTEE

FORCED SWIM TEST REPORT

JUNE 2023

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1. Introduction

1.1. In 2021, the Animals in Science Committee (ASC) established a Task and Finish Group for the Strategic Review of Project Licences. This was in response to a commissioning letter from the Home Office. The purpose of this group is to carry out reviews of selected project licences falling under a theme, for discussion and ratification by the ASC, in order to provide strategic advice to the Minister on specific topics.

1.2. On 22 August 2022, the Home Office commissioned the ASC stating, "the UK Government wishes to receive advice from the Committee on the evidence of alternative methods and appropriate justification for the use of the forced swim test, under the Animals (Scientific Procedures) Act (ASPA), with due consideration to the legitimate requirements of science and industry and to the protection of animals". In addition, the ASC was asked to answer the question: "How should the 3Rs be applied regarding the forced swim test, drawing on the available evidence and licence review?" According to this commission, ASC advice on the availability of alternative methods and appropriate justification for the use of the forced swim test will be used to inform good practice in the science sector and better regulation by the government.[1]

1.3. In recent years, both scientific and welfare concerns have been raised by a number of stakeholders about the use of the forced swim, or Porsolt, test (FST) (e.g., Reardon, 2019; FRAME, 2022; Hutchinson, 2023; PETA, 2023). Organisations that use or support the use of the FST have also presented their case for when and how its use is justified (UAR, 2022).

2. Executive summary

2.1. This report considers evidence of alternative methods and justification for the use of the FST, its severity, its validity in the context of experiments with different research purposes, and opportunities for more fully implementing each of the 3Rs. On the basis of the findings from the review, we make several recommendations for consideration by the Minister.

2.2. Our review of project licences authorising the use of the FST was complemented by questionnaire responses received from project licence holders and stakeholder groups that covered a breadth of perspectives on the FST.

2.3. In reviewing the licences, the group identified that the FST is potentially being used for a number of different purposes, and that in many cases it was not clear from the licence whether or not the test would be used. We also found differences in views relating to the severity of the procedure and in the descriptions of what the procedure entailed.

2.4. We did not find any evidence of reliable, reproducible and accepted non-animal alternatives to address the purposes for which the use of the FST may be justifiable. However, it was encouraging to read about promising developments that may provide replacement technologies in the future.

2.5. Until such replacements are available, we consider that our recommendations will help to ensure that any future use of the FST will be robustly justified and scrutinised.

[1] https://www.gov.uk/government/publications/use-of-the-forced-swim-test-under-the-animals-scientific-procedures-act-1986/commission-of-policy-advice-from-the-animals-in-science-committee-the-use-of-the-forced-swim-test-under-aspa#fn:1

2.6. The review of licences and stakeholder evidence has highlighted that the forced swim test may be justifiable for the screening of potential antidepressants and when studying the neurobiology of stress.

2.7. However, while the FST has enabled the development of established antidepressants, there is a need to confirm its predictive validity for compounds with novel mechanisms of action.

2.8. When the FST is used to induce a stress response we recommend considering why the FST is the most appropriate method for the types of stress being investigated.

2.9. We recommend that the FST should not be used as a model of depression or to study depression-like behaviour (including in the phenotyping of genetically altered mice) or for studies of anxiety disorders and their treatment.

2.10. When applying for a licence that includes the FST, the applicant should ensure that the parameters of the test are optimised so that the most refined protocol is being used, commensurate with the power of the test being adequate to meet the objectives of the study. Details of how the FST is conducted should be recorded and reported in publications.

2.11. There were inconsistencies in descriptions of the severity of the test and we recommend that the Animals in Science Regulation Unit (ASRU) guidance in this area should be followed.

3. The forced swim test

3.1. For the purposes of this review, we have defined the FST, or forced swimming, as any procedure in which an animal is placed into a container of water, out of its depth, with no means of escape. The swimming is classed as a forced procedure because it is not a voluntary behaviour of the animal. In the licences we reviewed, this involved rats or mice exclusively. The procedure is regarded as aversive (unpleasant) because they have no means of escape; the inescapability differentiates the FST from other tests, such as water mazes, where animals swim but have a potential means to get out of the water.

3.2. When placed in the water, the animals swim until either a predefined time has elapsed, or until they are observed to 'float'. Throughout this report we use the term 'immobility' to describe the animals floating in the test. The latency (time taken) to develop immobility (and total time spent immobile) varies from animal to animal and is the measure of interest. The animals are observed for the full duration of the test, which can vary.

3.3. The FST was first developed by Porsolt in the 1970s (e.g., Porsolt et al. 1977). The behavioural test was found to be responsive to antidepressant drugs. For all classes of compounds that are licensed for use as antidepressants in humans, there are peer-reviewed reports stating that they reduce animals' immobility during the FST (See Appendix 1). That feature of animals' response to drug treatment has been used as a predictive screen for candidate antidepressant treatments ever since (Kara et al., 2017 (mice); Bogdanova et al., 2013 (rats)). As noted in paragraphs 3.5, 5.15-19, there are differing views on the value of the FST in this context (Trunnel and Carvalho 2021; Stanford 2020).

3.4. Because the immobile (floating) posture has been described, albeit arbitrarily, as 'behavioural despair', it is evident from the literature that it has been inferred by some researchers that animals' immobility in the FST is analogous to a state of depression (or depression-like behaviour) in humans (Porsolt et al., 1978). That inference is apparently based on the reduction in immobility when animals are treated with an antidepressant drug before experiencing the FST. However,

there has been scientific challenge in relation to the inference that animals subjected to the FST are depressed or expressing depression-like behaviour. Alternative explanations for the immobility have been discussed in a number of reviews (e.g., Armario, 2021; Molendijk and De Kloet, 2022; Reardon, 2019).

3.5. An important possibility is that the use of the FST to screen antidepressants and its use as 'a model of depression', analogous to the human disorder, should be appraised separately. Some researchers have the view that depression and relief of depression recruit different mechanistic pathways in the brain: i.e., that antidepressant treatments might inactivate neuronal mechanisms that cause depression and/or promote mechanisms that compensate for (or mask) depression (e.g., Reid and Stewart, 2001; Han and Nestler, 2017). In the latter case, the use of the FST as a predictive screen for promising antidepressants does not require the FST to induce a state of depression in animals (Stanford, 2020). Some dispute the reliability of the screen because some specific compounds that produced a positive response in the FST are not in clinical use as antidepressants (Trunnell and Carvalho, 2021). However, there are many reasons why potential candidates are not used as originally intended (Stanford, 2020; e.g., Luque and Rey, 2002, Hay et al, 2014, Waring et al, 2015).

4. Our methodology

4.1. The ASC has been asked to provide independent, balanced and objective advice on evidence around the justification for the use of the forced swim test, alternative methods, and how the 3Rs should be applied regarding the test, drawing on the available evidence and licence review. Our findings are also informed by stakeholder responses and peer-reviewed publications, including reviews undertaken by others.

4.2. We undertook a qualitative review of eighteen project licences and identified important themes related to the advice we were asked to provide. These 18 licences were provided by the Home Office (identified using the search criteria 'swim test', 'FST' and 'Porsolt'). The licences authorised the use of the FST at the time of the review. Although we were provided with 18 project licences, the feedback from Project Licence Holders (PPL holders) indicates that the FST was not currently being used in all these cases, since the FST is often included in a suite of potential tests that may or may not be used.

4.3. In addition, we sought feedback from PPL holders asking how they use, or plan to use, the FST in the course of their research project as well as asking about potential alternatives and the severity of the test. A call for evidence from stakeholders also enabled us to gather and understand a full range of perspectives on the FST. We circulated two questionnaires: one was sent to Project Licence Holders and the other to stakeholder organisations. The responses to these questionnaires have further informed our review of licences involving the FST. An open call for evidence was also included in the ASC letter responding to the FST Commission, published on the ASC website.[2] We would like to thank everyone who contributed evidence for the review and acknowledge the breadth of opinions we received. The questionnaires used can be found in the appendices to this report. In addition, a full list of all references provided by stakeholders is included in the appendices to this report.

[2] Ltr_ASC_Chair_response_to_FST_Commission_14_10_2022_-_Final.pdf (publishing.service.gov.uk)

5. Findings

5.1. In reviewing the licences, the group identified that the FST is potentially being used for a number of different purposes, as set out below, and that in many cases it was not clear from the licence whether or not the test would be used. In response to our questionnaires, we also found differences in views relating to the expected severity (the expected level of harm to the animal), and severity limits (how the procedure should be classified in the licence), for the FST and in the descriptions of what the procedure entailed.

5.2. The ASC's review of the eighteen project licences confirmed that the FST is reported as potentially being used for several different purposes:

- To try to induce a change in the behaviour of animals in studies of neurobiological processes that could explain depression in humans, often as one of a suite of behavioural tests. Such studies include investigations of behavioural abnormalities in the phenotype of animals following an experimental procedure (e.g. genetic alteration, drug challenge or neurotoxic lesion).
- As a predictive screen for novel antidepressant treatments.
- To explore the neurobiological processes that are recruited during exposure to an inescapable stress in order to explore mechanisms underlying resilience or adaptation to stress.
- To study the mechanisms underlying anxiety.

5.3. Arising from our wider consultations, we have identified several concerns, raised by different stakeholders. In some cases, these relate to the use of animals in experimental research more generally, but others question the scientific value and validity of the use of the FST, specifically.

5.4. Regarding the former category, the view of at least one stakeholder was that no animal research is ever acceptable. However, the latter group commented that reward-based behavioural testing is preferable to aversive categories of behavioural testing. Several PPL holders we consulted stated that they would not use the FST were they to consider that a suitable animal or non-animal alternative was available.

Severity

5.5. In the licences we reviewed, the severity of the protocols that included the FST was usually described as moderate. However, the protocols typically contained several different optional procedures and so it was impossible to establish the expected severity of the FST in isolation.

5.6. There were differences in opinion among PPL holders and the stakeholders we consulted regarding the severity of the FST. While the majority regarded the FST to be of moderate severity, some considered it as mild severity, and others considered it as severe. This may relate, in part, to different parameters for the test, as applied by different users: e.g., its duration; or the temperature of the water; or the use of different rodent species or strains that respond in different ways in the FST.

5.7. The disparity might also be explained by a commonly held view that the immobility is explained by a state of exhaustion, in which case the procedure should be classified as severe. However, there is no information on any of the range of physiological parameters that would be required to confirm that assumption. None of the licences we reviewed included exhaustion as the endpoint, and all included a maximum time that the animal would be in the water. The differing

perspectives on severity may also relate to the position the respondent takes to the questionnaire on whether to permit or ban the test: those wishing to use it may under-estimate the severity, while those opposed to its use may over-estimate the severity. This point was noted specifically by one stakeholder organisation.

5.8. We understand that in Australia there have been reports of animals dying following the FST, from aspiration of water during the test that was not detected during the procedure. (New South Wales Parliament, 2022). As no mention of this as a potential adverse effect of the FST was made in any of the licences we reviewed, and it was not referred to by any of the PPL holders who responded to our questionnaire, we have not been presented with any evidence that this has happened in the UK. Were this to have happened, it would have been reported under Standard Condition 18 (SC18) of project licences under A(SP)A. The ASRU has reviewed its SC18 database and confirmed that no SC18 reports have been made, involving rodent death following the FST, since the development of the database on 1 July 2021. ASRU cannot verify SC18 records before the development of the database.

5.9. Regardless of these disparate views, we note that the Guidance on the Operation of A(SP)A classifies this type of procedure as moderate because the animal is unable to escape and therefore the test is aversive. If the animal were expected to swim until it is exhausted, which was not the case in any of the licences we reviewed, then the test would be classified as severe, as set out in the Guidance (Home Office, 2014).

Validity as a model of depression

5.10. It is evident from the literature that some scientists believe that immobility in the forced swim test is analogous to a state of depression, or 'depression-like behaviour' in humans (e.g., Jalewa et al., 2014). However, none of the PPLs we reviewed that referred to depression specified how this behaviour is interpreted in terms of the many and diverse diagnostic features of depression in humans.

5.11. Also, none of those licences explained how immobility in the FST can be assumed to be confined exclusively to a depressed phenotype, rather than being analogous to one of the many features of depression that are shared with other human disorders that affect mood, motor behaviour and cognitive performance. This is important because interpretation of immobility in the FST has been proposed by some researchers as being more likely to reflect a passive coping behaviour, at least in some animals, rather than a depressive state (Molendijk and de Kloet, 2015; Amario, 2021).

5.12. Given this controversy, and the absence of scientific evidence to link any aspect of behaviour of rodents in the FST with any aspect of depression in humans, many argue that it is hard to justify the use of this procedure as a 'model' of depression or even 'depression-like' behaviour (Commons et al. 2017; Stanford, 2017).

5.13. This limitation is especially relevant to licences that include the use of the FST as a tool for phenotyping of the animals after an experimental intervention (such as genetic alteration or drug challenge). Licences that authorise the use of the FST for such phenotyping studies did not make clear how any behavioural change in the test would be interpreted: in particular, how, and the extent to which, any change would be interpreted as relevant to a depressive phenotype. This limitation reflects the need to avoid anthropomorphic interpretation of the animals' subjective state and to limit conclusions to objective observations of the animals' abnormal behaviour (Molendijk, 2022).

5.14. Several stakeholders pointed out that this 'modelling' was also somewhat undermined by the marked variation in the behavioural response according to the strain of animal used in the test.

However, some licence holders argued that strain differences in the behavioural response to a specific experimental challenge, such as the FST, can be useful when trying to pinpoint the underlying biological factor(s) that influence, or even determine, an abnormal phenotype for new genetic strains of rodents.

Validity as a predictive screen for antidepressant treatments

5.15. The group noted that, although there is no regulatory requirement to include data from the FST in applications for clinical trials, the regulator does require convincing evidence that a compound is likely to be efficacious before authorising tests in humans. The evidence put forward can include data from the FST. However, variability of findings across different strains and experimental parameters (see Bogdanova et al. 2013) was cited as undermining its validity when used for this purpose (but see paragraph 5.14).

5.16. Another criticism of the FST as a screen for antidepressants is that it gives no indication of the latency of the therapeutic response. For most, but not all antidepressants, a response to treatment of depression in humans has a latency of several weeks. Yet, a positive response in the FST is evoked after treatment within 24h preceding the test (e.g., Porsolt, 1978). This anomaly is considered by some as further evidence that antidepressants are not preventing a state of depression in the FST and supports the view that the immobility is not analogous to depression in humans (Willner and Mitchell, 2002). For that reason, it cannot be assumed that an experimental intervention that increases animals' immobility in the FST has induced depression or depression-like behaviour (See *'Validity as a model for depression'*, above).

5.17. However, it could be argued that these disparities do not undermine the validity of the test as a predictive (qualitative) screen for putative antidepressant treatments, i.e., whether or not a novel compound is likely to act as an antidepressant in humans (Kara et al., 2018). This is not least because a predictive screen does not require the animals to be expressing any aspect of depression. The validity of the FST, as in many other predictive screens, rests merely on all treatments with established therapeutic efficacy in humans also having a consistent effect on the behaviour of animals: in this case, a reduction in their immobility in the FST (Kitada et al. 1981).

5.18. A key limitation is that because the use of the FST to screen for antidepressants is not based on a mechanistic understanding (rather it is a black-box test), there is a risk that some novel compounds, which would have turned out to be effective antidepressants, might not be responsive in the FST screening process and so are excluded from further development ('false negatives'). However, this uncertainty afflicts all predictive screens that lack an understanding of the underlying mechanism of action. As a consequence, the FST, like other such predictive screens, needs continual empirical revalidation as new candidate treatments emerge.

5.19. The working group acknowledges that on the basis of available evidence it cannot be certain whether or not the FST will be a useful screen for new classes of antidepressant treatments that have yet to be developed (Trunnell and Carvalho, 2021). This is a notable limitation given that more effective treatments for depression are required (Marwaha et al. 2023).

Forced swimming as a stressor

5.20. In some cases, the FST, as we define it above, is being used to subject the animals to a stressor (de Kloet et al, 2016; Molendijk and de Kloet, 2015; Molendijk and de Kloet, 2019; Commons et al, 2017, Sze et al, 2018). According to some of the PPLs we reviewed, and some of the stakeholder comments we received, the rationale for its use in this context is based on evidence that uncontrollable stress is a factor that provokes or aggravates several psychiatric disorders (e.g.

schizophrenia, post-traumatic stress disorder, depression, and autism spectrum disorder: See refs cited in Lenart-Bugla et al., 2022).

5.21. Nevertheless, our review confirmed that PPLs authorised to use forced swimming for this purpose do not always explain the ways in which the features of this test are qualitatively analogous to the types of stress that are typically experienced by humans. This is also true of other aversive tests that may be used to study the neurobiology of stress. We understand that this is important because stressors of different types, duration and intensity provoke different profiles of biological responses (Henry, 1992; Stanford, 1995; Jaggi et al., 2011).

5.22. For this reason, it is important that PPL applications, which seek authorisation for the use of the FST as a stressor, should explain how the response to forced swimming in rodents is naturalistically relevant, why the use of the FST is necessary for achieving the experimental objectives and why it is the most refined among in vivo tests that could be used to achieve the experimental objectives.

Studies of anxiety-like behaviour

5.23. Our review found that the FST is also being used to study the neurobiology of anxiety. However, depression and anxiety comprise different families of disorders with different clinical features and treatment strategies (Baldwin et al, 2014; Cleare et al, 2008). From a recent appraisal, there appears to be no scientific justification for inferring that a change in behaviour in the FST reflects animals' anxiety status (Armario, 2021). Moreover, anti-anxiety drugs that have no efficacy as antidepressants do not reduce immobility in this test (Porsolt, 1977). On that basis, the use of the FST, either to study anxiety or as a screen for anti-anxiety drugs, cannot be justified.

6. Appropriate Scientific Justification

6.1. A(SP)A legislation places an obligation on the Secretary of State to "assess any scientific justification" as part of a harm benefit analysis of a programme of work. The legislation also obliges the Secretary of State to consider "expertise in the area of science for which it is intended that protected animals will be used". This scientific assessment is part of the project licence evaluation process undertaken by the ASRU. In addition, as discussed later (Section 7), scientific assessment should also be embedded into the local Animal Welfare Ethical Review Body (AWERB) review of potential project licence applications.

6.2. This review has found that some approved project licences include the FST as part of suite of possible tests but without a clear explanation for why the applicant believes it is necessary and justified to use the FST in order to fulfill the scientific objectives.

6.3. As with any proposed use of animals in research, relevant scientific justification would include the likely value of the data obtained and reason(s) why the scientific objectives of the programme of work could not be achieved without the use of the FST, or whether the research question could be reframed to avoid the use of animals.

6.4. When using forced swimming as a model 'stressor', a relevant justification would include how the FST (rather than more mild stressors) is likely to provoke physiological changes that affect mood, behaviour or cognition, in terms of both its qualitative features and severity.

RECOMMENDATION 1

- Formal evaluation of project licence applications should include a consideration of the specific scientific justification for use of the FST, including relevance to the diagnostic features of human illness of interest or specific induced physiological changes that are being investigated.

RECOMMENDATION 2

- The use of the FST in project applications should be rejected if it is being proposed as a 'model' of depression (including in the phenotyping of genetically altered mice) or for studies of anxiety and its treatment.

7. AWERB Governance

7.1. In the light of current debate and discussion on the validity of the forced swim test, it is essential that the processes of local project review undertaken by an AWERB can demonstrate strong governance when applications for project licences propose the use of animals in aversive behaviour tests such as the FST. In this context, the group notes that some establishments have devised a list of 'Discouraged Procedures' (which may include the FST). In such cases, the FST might for example not be permitted within the establishment as a model of depression but may be permitted when the intended purpose is to screen for candidate antidepressants (subject to adequate justification by the PPL applicant).

RECOMMENDATION 3

- The processes of local project review should ensure that the proposed purpose for using the FST has been clearly explained and assessed and opportunities for adoption of the 3Rs fully explored.

8. Replacement

8.1. In response to our questionnaire, PPL holders answered that they were not aware of any non-animal alternatives to the FST. Some researchers noted that other tests involving animals, such as tail suspension, electric shocks, and chronic restraint are all aversive, and possibly more so, than the FST. Some approaches were considered by PPL holders to be preferable to the FST, such as observing grooming, nest-building, the degree of interest in novel objects, sucrose preference, the light / dark box and paddling water-mazes, but their limitations were also noted.

8.2. PPL holders reported that non-rodent species such as zebrafish and fruit flies might potentially be used in place of the FST to screen for anti-depressant activity, in the future, but that these approaches had not yet been adequately validated. Stakeholders held a range of views on the possibility of replacing the FST with one or more non-animal approaches. (See Appendix 4 for a list of the publications submitted by stakeholders). Organisations involved in neuroscience research stated that there are no current non-animal alternatives. While the organisations opposed to the use of animals in research did not provide evidence for existing, validated alternatives, they did report on potential future developments that might replace animal studies, including artificial intelligence.

8.3. It was generally accepted that there are currently no confirmed biomarkers for either depression in humans or that predict antidepressant efficacy in animals, but that there are promising candidates under development (Sewell et al. 2021). It was also hoped that longitudinal observational studies of human patients would lead to *in silico* approaches to understanding how particular anti-depressants work. Human induced pluripotent stem cells might also lead to studies on human neurons *in vitro* or in combination with organoids or organ-on-a-chip

technologies. Such future developments need further research as they are not currently considered valid alternatives to the FST.

8.4. Overall, during our review of the responses from stakeholders and PPL holders, we have found no evidence of reliable, reproducible and accepted non-animal alternatives to the FST where it has been accepted that its use is scientifically justified.

RECOMMENDATION 4

- Further research should be conducted into non-animal methods for studying depression, antidepressants and other areas of research where the FST is currently used. Funders should consider specific funding calls in these areas.

9. Reduction

9.1. In the licences that we reviewed, the FST was usually highlighted as an optional procedure among others: e.g., in a suite of tests. However, it was rare to see any explanation of the experimental design or the number of animals that might be used specifically in the FST.

9.2. We are also unsure how potential harms and benefits can be properly assessed when considering the justification for this aspect of the work if: a) it is not clear whether the test will even be used; b) on what basis such a decision would be taken; and c) how many animals could be involved.

RECOMMENDATION 5

- When it is proposed to use the FST in a programme of work, details of the experimental design and the number of animals to be used in the test should be included in the licence.

10. Refinement

10.1. It is a requirement under A(SP)A that all animal use is as refined as possible. Refinement means acting to minimise the pain, suffering, distress or lasting harm that may be experienced by research animals, and to improve their welfare.

10.2. In the majority of the licences we reviewed, the forced swim test was included as part of a protocol with a moderate severity limit. It was frequently described as an aversive behavioural test that would be used, if considered necessary, after a suite of less aversive tests. The FST has the obvious potential to induce stress in the animals involved, and so care needs to be taken to ensure that the overall harms involved are justified and minimised as far as possible.

10.3. There are several parameters that can affect the harms and response in the FST, and this may explain some of the variability seen across laboratories. However, there was some inconsistency in the level of detail given when describing the test. Not all the licences stated the temperature of the water, nor how long the test would last, nor whether it was to be repeated. In those that did give this information, the water temperature ranged from 16 to 30 degrees Celsius with little, if any, justification as to how the optimal temperature had been determined. This is important because the relationship between the temperature of the water and animals' behaviour in this test is not straightforward (Amario, 2021).

10.4. The majority of the tests involving mice stated that they were limited to a maximum of six minutes. One licence stated that a typical time in the water for mice would be two minutes or less. Although this would be appropriate when the procedure is being used to study the physiological response to stress, when used to study antidepressants, stable assessment of

immobility usually requires tests of longer duration (e.g.,Porsolt et al., 1978 (rats); Koek et al., 2018 (mice)). When rats were being used, the maximum time was 15 minutes.

10.5. A further three factors merit consideration because they affect immobility and possibly the intensity of stress in the FST but were not mentioned in the PPLs surveyed: one is the depth of the water (Abel, 1994a); another is the diameter of the apparatus (Armario, 2021); and a third factor is changing the water and apparatus between successive tests (Abel 1994b). Failure to record and report all these and other key parameters could explain variability of results between establishments.

10.6. Several of the licences noted that hypothermia is a potential consequence of swimming in water and most included descriptions of how the animals would be dried and warmed following the test, before being returned to their home cage. In some licences, though, it appeared that only animals removed from the water prematurely (because they were struggling to swim) would be warmed, suggesting that those that completed the test without appearing to be in distress would not be warmed. Not all of the licences mentioned drying or warming the animals, so we cannot comment on whether this is standard practice or not.

10.7. In the licences we reviewed, the likelihood of either rats or mice struggling to float in the test was regarded as a rare possibility, but some researchers stated that they would monitor for that as an endpoint for the experiment. As noted above, none of the licences mentioned the possibility of the animals aspirating water.

10.8. The aftercare given to the animal will contribute to the animal's overall experience and should be carefully and critically considered. Several licences discussed monitoring the animals following the test and that if, in exceptional circumstances, normal behaviour was not observed within a specific time period, the animal would be culled.

10.9. The FST is, by its nature, a stressful experience for the animals involved. Assuming appropriate and robust justification for its use has been provided and accepted, we would expect to see all licences providing full details of how the test will be refined as far as possible. This should include the use of non-aversive handling techniques (i.e. not picking up mice by their tails) when moving animals from their home cages to the test apparatus. Water temperature is also important because hypothermia is a potential adverse outcome (and will confound the experimental results). The duration and frequency of the test are also important variables because these affect the immobility scores: these variables need to be optimised to induce immobility in untreated (control) animals, at a minimum, and to ensure that any change in immobility following an experimental intervention can be evaluated. The balance between refinement (for individual animals) versus reduction of the overall numbers required to achieve a reliable outcome in the test must always be considered.

RECOMMENDATION 6

- An optimum water temperature should be identified for both mice and rats, aiming for the water to be at a temperature that minimises the risk of hypothermia while achieving the scientific aims of the study. In addition, as a further refinement, the water should be changed between each animal and the equipment cleaned.

RECOMMENDATION 7

- Applicants should provide robust, scientific justification for the typical and maximum length of swim time, and this should be reduced to the minimum necessary, commensurate with reliable evaluation of animals' behaviour in the test.

RECOMMENDATION 8

- All protocols proposing use of the FST should provide details of how the animals will be monitored during the procedure and cared for after the test has been completed.

11. Severity

11.1. As noted above, from the licences examined it was difficult to assess the severity of the FST in isolation. Notwithstanding that limitation, the applicants variously cited it as mild, moderate or severe in the project licence holder survey responses.

RECOMMENDATION 9

- We recommend that applicants assess the severity of their intended procedure in the light of the relevant guidance note (Home Office, 2014).

12. Non-Technical Project Summary (NTS)

12.1. The potential use of the FST was not always included in the NTS. In some NTSs, reference was made to behaviours that animals may exhibit indicating discomfort, but specific tests like the FST were not mentioned. Our general impression is that the likely harms involved with the tests are not being sufficiently described in the NTS.

RECOMMENDATION 10

- The AWERB should review the NTS and ensure that the FST is included and clearly described as a potential procedure if it is listed in the protocols.

13. Summary of recommendations, including suggested primary audience for each recommendation:

1. Formal evaluation of project licence applications should include a consideration of the specific scientific justification for use of the FST, including relevance to the diagnostic features of human illness of interest or specific induced physiological changes that are being investigated. (ASRU)
2. The use of the FST in project applications should be rejected if it is being proposed as a 'model' of depression (including in the phenotyping of genetically altered mice) or for studies of anxiety and its treatment. (ASRU)
3. The processes of local project review should ensure that the proposed purpose for using the FST has been clearly explained and assessed and opportunities for adoption of the 3Rs fully explored. (AWERB)
4. Further research should be conducted into non-animal methods for studying depression, antidepressants and other areas of research where the FST is currently used. Funders should consider specific funding calls in these areas. (Funders)
5. When it is proposed to use the FST in a programme of work, details of the experimental design and the number of animals to be used in the test should be included in the licence. (Applicant)
6. An optimum water temperature should be identified for both mice and rats, aiming for the water to be at a temperature that minimises the risk of hypothermia while achieving the scientific aims

of the study. In addition, the water should be changed between each animal and the equipment cleaned. (Applicant)

7. Applicants should provide robust, scientific justification for the typical and maximum length of swim time, and this should be reduced to the minimum necessary, commensurate with reliable evaluation of animals' behaviour in the test. (Applicant)

8. All protocols proposing use of the FST should provide details of how the animals will be monitored during the procedure and cared for after the test has been completed. (Applicant)

9. Applicants should assess the severity of their intended procedure in the light of the relevant guidance note (Home Office, 2014). (Applicant)

10. The AWERB should review the NTS and ensure that the FST is included and clearly described as a potential procedure if it is listed in the protocols. (AWERB)

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Appendix 1: Examples of peer-review publications reporting reduced immobility of mice and rats in the Forced Swim Test after treatment with compounds from each of the classes of antidepressants.

DRUG / CLASS	RAT	MOUSE
MONOAMINE TARGETS		
Monoamine Oxidase Inhibitors	West CH & Weiss JM (1998) *Pharmacol Biochem Behav.* 61(1):67-79.	Bourin M et al. (2002) *J Psychiatry Neurosci*. 27(3): 178–185.
Tricyclics	D'Aquila PS & Galistu A (2019) *Behav Brain Res*. 373:112088.	de Felipe MC et al. (1989) *Eur J Pharmacol*. 159(2):175-80.
Selective Serotonin Reuptake Inhibitors (SSRIs)	De Vry J et al. (1999) *Eur Neuropsychopharmacol*. 9(6):461-8.	Yohn CN et al. (2020) *Psychopharmacol.* (Berl) 237(5):1281-1290.
Serotonin / Norepinephrine Reuptake Inhibitors (SNRIs)	Nowakowska E et al. (2003) *Arzneimittelforschung* 53(4):237-42.	Socala K et al. (2012) *Pharmacol Biochem Behav*. 103(2):273-8.
Norepinephrine Reuptake Inhibitors (NRIs)	Detke MJ et al. (1995) *Psychopharmacol*. (Berl) 119(1):47-54.	Dziedzicka-Wasylewska M et al. (2006) *Neuropsychopharmacol.* 31(11):2424-32.
Mixed Action	Dremencov E et l. (2004) *Prog Neuropsychopharmacol Biol Psychiatry*. 28(1):141-7.	Masuda Y et al. (2000) *Methods Find Exp Clin Pharmacol.* 22(9):667-70.
OTHER TARGETS		
Tianeptine	Nowakowska E. et al. (2000) *Arzneimittelforschung* 50(1):5-10.	Wlaz P et al. (2011) *Pharmacol Rep*. 63(6):1526-32.
Agomelatine	Bourin M et al. (2004) *J Psychiatry Neurosci*. 29(2):126-33.	Bourin M et al. (2004) *J Psychiatry Neurosci*. 29(2):126-33.
Ketamine (Esketamine)	Donegan JJ & Lodge DJ (2017) *Int J Neuropsychopharmacol.* 20(4):354-358.	Li Y et al. (2015) *Behav Brain Res*. 279:100-5.

Appendix 2: Questionnaire for Project Licence Holders

The ASC
Animals in Science Committee

20 September 2022

Request for information from applicants whose project licences involve the use of Porsolt (or modified Porsolt) Forced Swim Test procedures.

Dear Project Licence holder

The Animals in Science Committee (ASC) has been Commissioned by the Home Office[3] to provide strategic advice on the use of the Porsolt (or modified Porsolt) Forced Swim Test. A subgroup of the ASC is taking this work forward.

As a holder of a project licence which includes the Porsolt (or modified Porsolt) Forced Swim Test in the list of procedures the ASC would be grateful if you would assist in their evidence-gathering process by providing responses to the following questions, as appropriate.

Please submit the completed questionnaire to asc.secretariat@homeoffice.gov.uk at the latest by 14 October 2022.

Confidentiality

The Home Office shall keep confidential and not disclose to any third party any part, or the whole, of any confidential information disclosed to it under this questionnaire and to treat the confidential information with the same degree of care and with sufficient protection from unauthorised disclosure as the Home Office uses to maintain its own confidential or proprietary information. Confidential information is planned to be held only until the ASC has provided the advice and any policy based on this advice has been published and then it will be deleted. Any data in the final report will be combined and anonymised so that contributors cannot be identified.

Questions re. Porsolt (or modified Porsolt) Forced Swim Test licences (for licence holders)

1. Please explain why you included a FST in your licence application for your overall project of work.

2. To what extent is the FST an integral and essential element of your protocols (please explain)?

[3] https://www.gov.uk/government/publications/animals-in-science-committee-ministerial-commission

3. When you included the FST in your licence application, was this done as one of a battery of possible tests which might or might not be used, or did you always intend to utilise it in practice?

4. If the animals are to experience a battery of tests, what factors will you use to determine their sequence?

5. Are there further refinements that can be made to FSTs or behavioural test battery protocols that would still yield required data?

6. How many times have you used a FST in the course of this licensed project of work? How many animals have performed an individual test?

7. How many times do you estimate that you will use a FST during the course of this licensed project of work? How many animals do you estimate will have performed an individual test during this time?

8. Would you still be able to achieve your research objectives, for all your aims/goals, if you didn't specifically use a FST?

9. What alternative approaches to a FST (*in vitro*, *in silico* or *in vivo*) did you consider for obtaining the data or insights you require?

10. Are you aware of any alternatives on the future horizon and are there any barriers to their adoption?

11. How would you describe the severity of your FST?

12. What criteria have you used to assess the severity of your FST?

13. How would you rank your FST versus the other tests you use in terms of severity?

14. Do you have any other comments that you would like to share with us regarding the use of FSTs?

Appendix 3: Questionnaire for stakeholders

The ASC
Animals in Science Committee

19 October 2022

Request for information on the use of Porsolt (or modified Porsolt) Forced Swim Test procedures.

The Animals in Science Committee (ASC) has been commissioned by the Minister[4] to provide strategic advice on questions relating to the use of the Porsolt (or modified Porsolt) Forced Swim Test (FST). A subgroup of the ASC is taking this work forward.

As an organisation with an interest in this field, the ASC would be grateful if you would assist in their evidence-gathering process by providing responses to the following questions, as appropriate.

Please submit the completed questionnaire to asc.secretariat@homeoffice.gov.uk at the latest by 11 November 2022.

Confidentiality

The Home Office shall keep confidential and not disclose to any third party any part, or the whole, of any confidential information disclosed to it under this questionnaire and to treat the confidential information with the same degree of care and with sufficient protection from unauthorised disclosure as the Home Office uses to maintain its own confidential or proprietary information. Confidential information is planned to be held only until the ASC has provided the advice and any policy based on this advice has been published and then it will be deleted. Any data in the final report will be combined and anonymised so that contributors cannot be identified.

Questions: Porsolt (or modified Porsolt) Forced Swim Test (FST)

1. Does your organization endorse the use of the FST in research of psychiatric disorders, their treatment, or stress?

2. Please explain the rationale for your response to question 1 (whether you answered yes, or no)

[4] Animals in Science Committee: ministerial commission - GOV.UK (www.gov.uk)

3. Do you believe there are refinements that can be applied to the FST?

4. Would it be possible to achieve the same objectives without using the FST? Please provide evidence (e.g., literature references) for your response

5. Are there alternative approaches to the FST (*in vitro*, *in silico* or *in vivo*) that have equivalent predictive validity? Please give examples of supporting peer-reviewed literature

6. Are you aware of any alternatives on the horizon and are there any barriers to their adoption?

7. How would you describe the severity of the FST (mild, moderate or severe)? Please justify your response: i.e., what criteria have you used to assess severity

8. Do you have any other comments that you would like to share with us regarding the use of the FST?

Appendix 4: References supplied through the Stakeholder Questionnaire

The following is a list of peer-reviewed literature and other sources supplied through the Stakeholder Questionnaire responses. These have been collated alphabetically and updated to remove duplication. A number of these references have been used for our review. We have not conducted a quality check of this list for accuracy of citation or relevance.

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