CN|NC
An Coiste Náisiúnta um Chaomhnú Ainmhithe a Úsáidtear ar Mhaithe le hEolaíocht
National Committee for the Protection of Animals Used for Scientific Purposes

Report to HPRA from NCPA on the Forced Swim Test (FST)

1. Commission of this report

1.1. On 11th July 2024 the HPRA requested advice from the National Committee, **in accordance with Regulation 61 of SI No. 543 of 2012,** for the National Committee to consider whether there is a justified case for the inclusion of the Forced Swim Test (FST) in project applications in Ireland, and if so, the circumstances under which its use would be considered appropriate.

1.2. The NCPA convened a subcommittee ("*the subcommittee*") to consider this request. The subcommittee did not consider repeating the work of other bodies, e.g. Animals in Science Committee (see section 2.2 below) as worthwhile. The UK's Animals in Science Committee (ASC) identified that the FST is potentially used for a number of different purposes. They also considered that in many cases it was not clear from the project information whether or not the test would actually be used as often it was requested as part of a battery of behavioural tests each of which was optional. The subcommittee consider that a similar situation was probable in Ireland and determined that the most constructive way forward in providing advice to the HPRA was to acquire information on the use of the FST in the Irish context. With this in mind, on 26th September 2024 the subcommittee requested that the Regulator write to institutions where permission to use the FST had been authorised to request information on its use, as well as the use of a number of other tests often used in the context of models of depression. The request was sent to stakeholders on 8th October 2024 and responses were provided to the NCPA on 18th December 2024.

1.3. This document provides the recommendations of the NCPA to the HPRA with respect to the use of the FST in Ireland.

2. Introduction

2.1. Concern has been raised by welfare organisations and within the scientific and regulatory communities in Europe and the UK as to whether the FST (also known as the Porsolt test), remains an appropriate model from both a scientific and animal welfare perspective.

2.2. A review of this test was performed by the UK's Animals in Science Committee and their findings reported in July 2023 https://tinyurl.com/ASC-report-FST . This review included consideration of projects authorised under the Animals (Scientific Procedures) Act 1986 with protocols that included the FST, a questionnaire to interested parties and a review of literature. The review acknowledged the scientific and welfare concerns, however, despite these concerns, the ASC 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.

2.3 The ASC review identified ten recommendations https://tinyurl.com/ASC-FST-recommendations . A summary of the recommendations is that

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- The FST may be justifiable for the screening of potential antidepressants and when studying the neurobiology of stress but that it should not be used as a model of depression or to study "depression-like" behaviours or for studying anxiety disorders.
- There is a need to confirm its predictive validity for antidepressant compounds with novel mechanisms of action, that when the FST is used to induce a stress response justification should be made for why it is the most appropriate method for the types of stress being investigated.
- When FST is used, the parameters of the test should be 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.
- The key experimental details of how the FST is conducted should be recorded and reported in publications due to significant differences in the method used for the test between laboratories.

3. Purposes for using the FST

3.1. From the comprehensive review of the literature performed by the ASC, the main reported uses of the FST are:

- To induce a change in the behaviour of animals in studies of neurobiological processes aimed at explaining 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.

4. Harms of the FST

4.1. As the experimental conditions mean that the animal cannot escape, the FST is considered aversive. The severity may vary depending on duration of the test or other factors, such as the temperature of the water. According to Annex VIII of EU Directive 2010/63, this procedure is classified as severe if animals are made to swim to exhaustion. Where animals are removed at an earlier timepoint, a lower severity may be appropriate.

5. Request for information from stakeholders

5.1. The subcommittee sought information from establishments in Ireland that have projects with authorisation to perform the **FST.** In addition to data on FST, the subcommittee also asked for information on three additional behavioural tests**,** the **Tail Suspension test (TST)**, **Sucrose Preference test (SPT**) or the **Female Urine Sniffing test (FUST).** These tests are also potentially used to investigate depression-like behaviours. TST is used to measure immobility as a sign of depression/ despair; SPT is used to test for anhedonia; and FUST is used to test reward-seeking behaviour in male rodents. The requested information included number of animals used, what scientific purpose(s) the test(s) were used for, whether animals were used

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in multiple tests and the number of projects in which the FST was authorised. Data were requested for studies approved and performed between 2020 and 2024. The details of the letter from the NCPA Chair requesting this information are provided in Annex 1 and the covering email to establishments from HPRA is providing in Annex 2.

6. Response from stakeholders

6.1. Six institutions with authorisations for FST between 2020 and 2024 responded to the request for information. Responses included both data and narrative responses. The anonymised responses were circulated to the subcommittee.

- Two out of six establishments indicated that, despite being authorised, FST had not been used over the period in question. Both establishments commented that FST had not been performed as it was considered that there were more refined tests available to provide the data required. SPT and FUST were used in these establishments over the reporting period.

- Four out of six establishments indicated that FST had been used between 2020 and 2024.

- One of the four establishments reported that FST had been used only in 2020 and not since. One establishment reported that FST had been used only in 2022 and not in any other years over the reporting period. The remaining two establishments reported use over the years 2020 – 2024.

- Three of the four establishments that reported use of FST over the time frame 2020 – 2024 also reported the use of TST and SPT either instead of, or in conjunction with FST.

7. Analysis of the use of FST in Ireland

A "Systematic Review on The Use of the Forced Swim Test in Ireland" was conducted under the supervision of one member of the subcommittee by a final year Masters student. The review was conducted according to the PRISMA guidelines, in PubMed, Embase and Google Scholar from inception to December 2024. The bibliography of each of the included articles was reviewed for potential additional eligible studies. The following inclusion/exclusion criteria were applied (Figure 1):

- Full-Text Studies were included if they conducted any investigation in an Irish Laboratory which involved the use of rodents in the Forced Swim Test, including both pre-clinical randomized and non-randomized investigations.

- Studies were excluded if:
  - They were not full-text publications (such as Reviews Article/Journal Article/Paper Supplement etc.)
  - The were not conducted in an Irish laboratory
  - No full-text version was available

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- Could not answer > 80% of this Systematic Review Data Extraction Categories

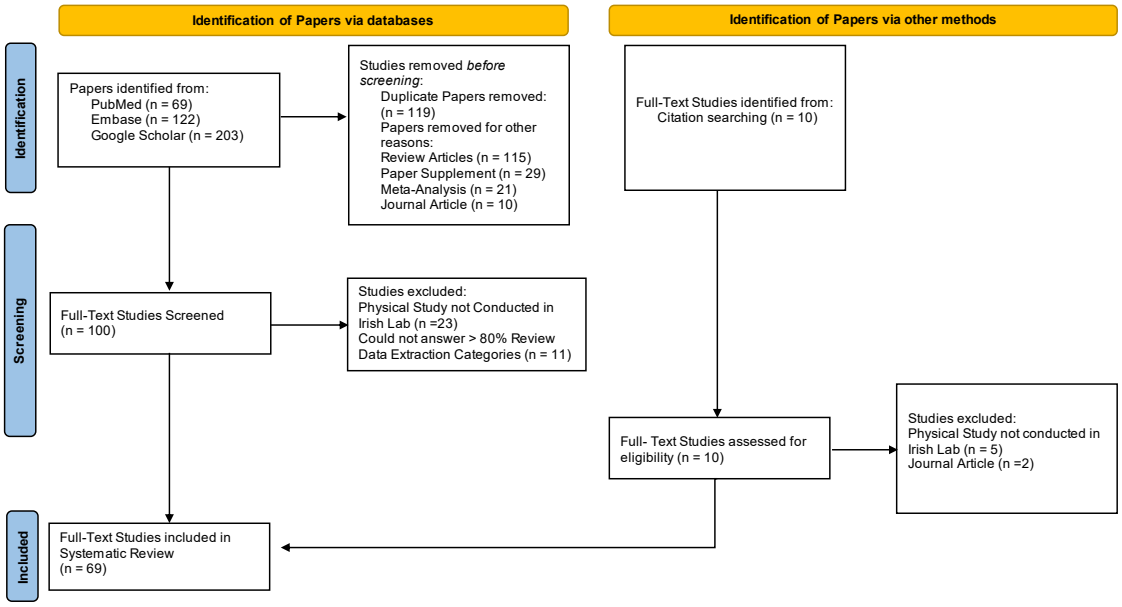

Figure 1: PRISMA flow diagram

The following data were extracted from the 69 articles included in the systematic review: Publication Date, Name of Paper, conducted in Ireland, Use of the Forced Swim Test, Was the Forced Swim Test used Alone, Was a drug treatment used, Sex of Rodent, Species of Rodent, Water Temperature, Dimensions of Container Used, Drying Method, Duration of Forced Swim Testing, Randomization, Blinding, Housing Numbers and Conditions, Testing Hours and Sequence.

7.1 Findings of the systematic review

Four academic institutions (Establishments 1, 2, 3, and 4) accounted for the 69 published articles (Figure 2). Establishment 3 was the only institution reporting the use of the FST in earlier

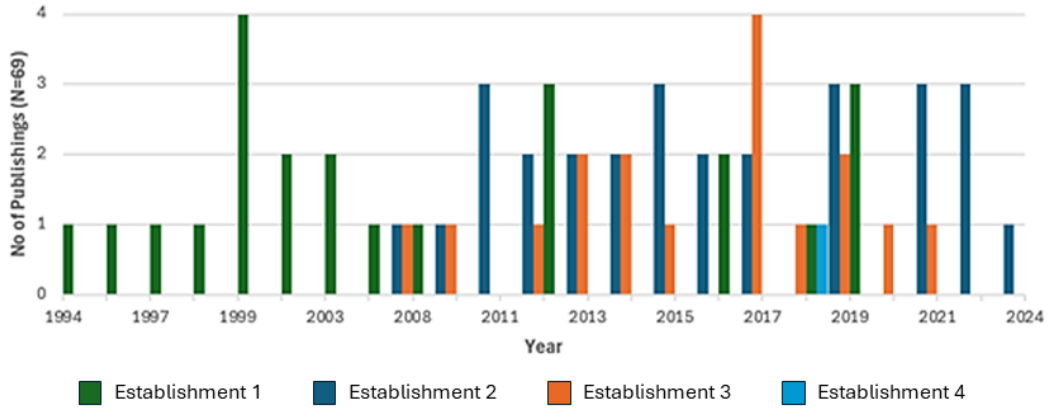

Figure 2: Number of studies reporting the use of the Forced Swim Test published by each Establishment by year.

years but had no publication since 2019. Establishments 1 and 2 increased their use of the FST

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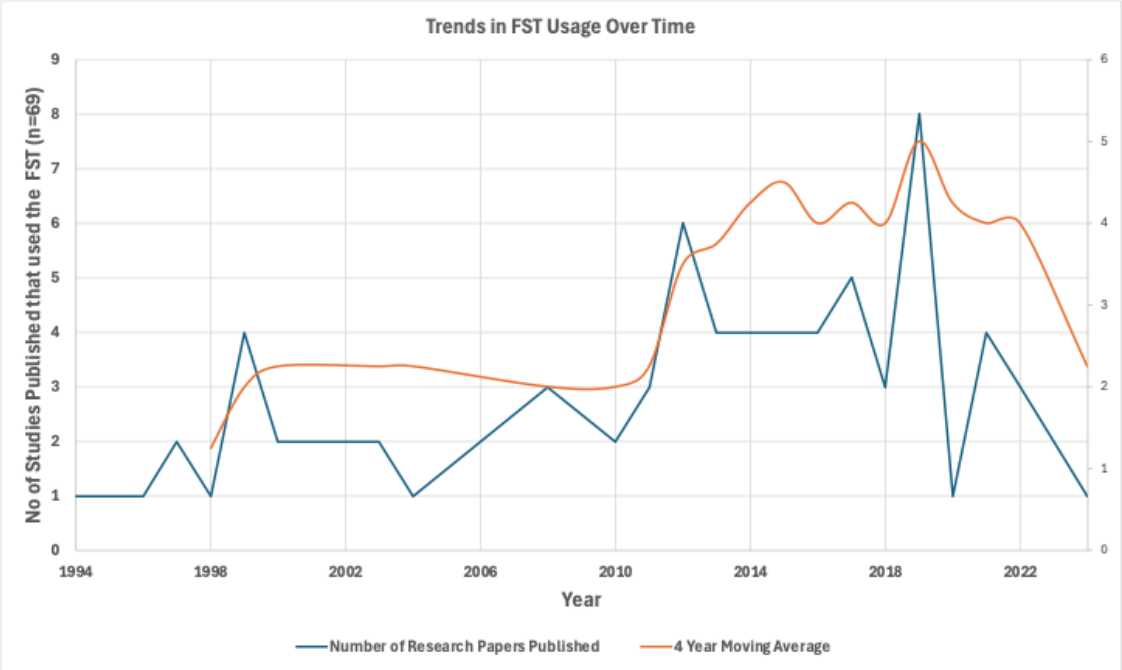
in more recent years, while Establishment 4 only published one article during the whole period. Overall, FST use was higher in Irish institutions between 2012 and 2019, with 37 articles published during this period and a peak of 8 publications in 2019 (Figure 3). There was a clear overall decline after 2019, possibly related to the COVID pandemic-related disruption, although other factors may have contributed.



For comparison, a similar search conducted in PubMed in September 2025 without restricting to the Republic of Ireland shows a similar world-wide trend, although the peak seems to have occurred before the 2020 pandemic (Figure 4), confirming that other factors may have played a role in the decline, especially considering the lag between experiments and publications.

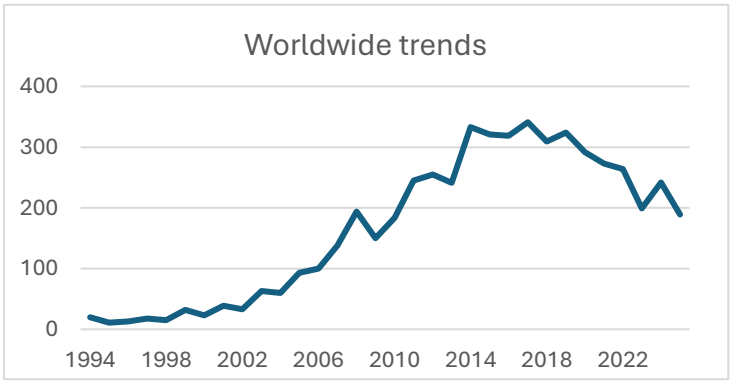


Figure 4: Total number of research papers reporting the use of the FST from 1994 to 2024 worldwide.

Figure 3: Total number of research papers published each year which reported the use of the Forced Swim Test from 1994 to 2024 (blue line). A four-year moving average plot is represented by the orange line.

Rodent species and strain - Of the 69 Irish published studies, 34 (49%) used only Sprague Dawley rats for all studies reported in the article; of these 34, 28 only used male animals, 5 used both males and females, one used only females. Sprague-Dawley and Wistar Kyoto rats were used in five studies (4 studies used males only and the other study used both males and

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females. Wistar-Kyoto rats were used in two studies (one males only and one included both males and females. Male Han-Wistar rats were used in three further studies. Eight studies used C57BL/6 mice (6 used males only, one used a mix and one did not specify sex). Less frequently used mouse strains included BALB/cOlaHsd, C57BL/6OlaHsd and d BTBR T+ Itpr3tf/J mice.

Behavioral tests - Seventeen studies (25%) used the FST as their sole method of testing. The other papers used the FST along with at least one other testing method. Of these, 11 studies (16%) used the FST and the Open Field Test (OFT) only, and 3 papers (4%) used the FST along with the Tail Suspension Test (TST) only. The remaining papers used a battery of behavioural tests which included some combination of the TST, OFT, Elevated Plus Maze, Home Cage Monitoring, Light-Dark Box, Social Interaction Test, Female Urine Sniffing Test, Water Plus Maze, Marble Burying Test, Stress-induced Hyperthermia Test, Restraint Stress, Novel Object Recognition Test, Morris Water Maze, Hot Plate Test, Sucrose Preference Test, Formalin Test, Y-Maze, Colorectal Distention, Novelty-induced Hypophagia, 3-Chamber Test and Social Fear Conditioning.

Rationale for use of the FST: In keeping with the findings of the ASC review of the literature (Section 3.1), we subdivided the reported uses of the FST in Ireland into 4 categories. Of the 69 Irish publications, 41 indicated that the FST was used to evaluate the antidepressant activity of an intervention (e.g., candidate drug, dietary modifications), 27 studies assessed depressive-like behaviour, and 7 studies used FST as a stressor. No study reported using the FST to study anxiety, but some publications focused on anxiety used FST in a battery of tests to assess the overal spectrum of activity of various interventions on mood disorders. Some studies used FST for more than one reason (e.g., to assess depressive-like behaviour and then to test the anti-depressant activity of an intervention). Some studies examined the effects of various technical parameters of the FST (e.g., rodent strain, sex differences, diurnal rhythm) but this was always in the context of its use to assess antidepressant activity. While the test was used almost exclusively to assess antidepressant activity in the first part of the systematic review period (i.e., until 2010), its use shifted to assessing depression-like behaviour after 2010 (Figure 5).

Technical parameters:

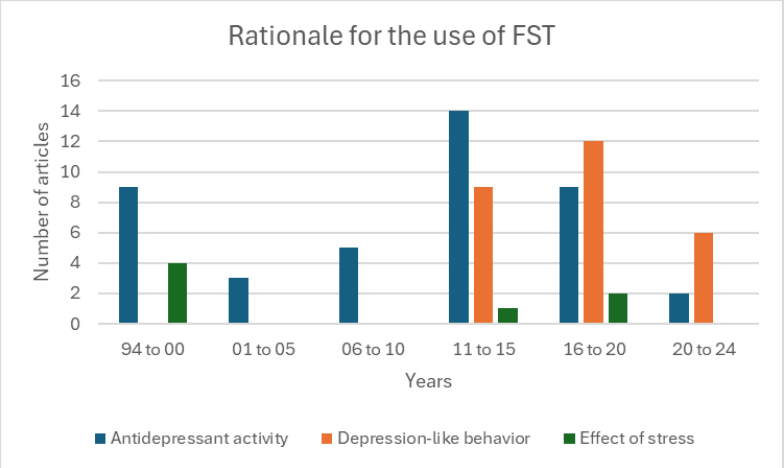

Figure 5: Reported rationale for use of the FST in the Republic of Ireland (antidepressant activity of an intervention, assessment of depressive-like behaviour, use of the FST as a stressor) from 1994 to 2024.

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Water Temperature - 20 studies (29%) explicitly reported maintaining the water temperature at 25°C throughout testing. 5 (7%) conducted testing at 23°C.
Nearly half the studies (49%) provided a temperature range rather than reporting a specific temperature (24 ± 1°C (5%), 23 ± 1°C (5%), 23-25°C (10%) or 21-25°C (1%)). Three studies did not report the water temperature.

Container Dimensions and Water Volume - Container dimensions of 40cm in height and 18cm in diameter were the most frequently reported (23%). A water depth between 15-20cm was used in 42% of the papers; a 30-cm depth was used in 33% of the paper; one study used as little as 7-8 cm of water while a further two papers specified a depth of 35cm. Three papers did not provide information. Only 12 studies reported that the water was changed between each rodent.

Frequency, duration, timing - 58% of studies conducted the FST twice with each rodent, with a 15-minute pre-FST session followed by a 5-minute testing session 24 hours later. 26% of the articles conducted the FST once with a 6-minute testing period followed by a 4-min period during which immobility was measured. 62% of articles provided no details of when the FST was performed. Eight studies (12%) stated that the FST observations were conducted in the morning; 5 reported that testing was performed in the afternoon. Ten studies (14%) broadly outlined that testing was run during daylight hours.

Drying Method - Only 10% of studies allowed the rodents to dry beside a heater after being removed from the water. A further 10% of studies towel dried the rodents. Two studies reported gently towel drying initially and then placing the rodent into a drying cage to allow them to dry fully. An additional two studies allowed the animals to dry naturally. A large proportion (n=46) did not specify how the rodents were dried.

Randomisation and Blinding - 29 articles made no reference to randomisation. 35 articles reported that the observers rating the performance of the rodents in the FST were blinded to the experimental conditions; 25 studies did not include any details on how blinding was achieved. 17 articles did not include information on either randomisation or blinding. Thirty-two studies explicitly stated that the groups and/or testing order were randomised but did not explain how this randomisation was achieved. Two studies reported the use of a computerised random number generator to allocate animals to the various interventions.

8. Validity of FST

8.1. As discussed in section 3.1 above, the ASC reviewed the validity of FST as a model of depression, as a predictive screen for anti-depressants, as a stressor and in studies of anxiety-like behaviour.

8.1.1. **Validity as a model of depression:** The ASC point out that there is an absence of scientific evidence to link any aspect of behaviour of rodents in the FST with any aspect of depression in humans, meaning that many argue that it is hard to justify the use of this procedure as a 'model' of depression or even 'depression-like' behaviour. Thus, it was considered that it was unclear for studies aimed at phenotyping of animals after experimental interventions (such as genetic alteration or drug challenge) how any behavioural change in the test can be interpreted and in particular, how, and the extent to which, any change would be interpreted as relevant to a depressive phenotype, particularly as there can be marked variation in the behavioural response according to the strain of animal used. In addition, the ASC

commented that 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.

8.1.2. **Validity as a predictive screen for antidepressant treatments:** The ASC 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 could be seen as undermining its validity when used for this purpose, although they also comment that it could be argued that these disparities do not undermine the validity of the test as a predictive (qualitative) screen for putative antidepressant treatments. Other criticisms of the FST as a screen for antidepressants include the rapidity of effect (evoked within 24 hours after treatment) where for most, but not all antidepressants, a response to treatment of depression in humans has a latency of several weeks. It was also discussed that a key limitation of the FST to screen for antidepressants is that it is not based on a mechanistic understanding (rather it is a black-box test), so 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 ('false negatives') and that it may or may not be useful for screening new classes of antidepressants.

8.1.3. **Validity as a stressor:** The rationale for the use of the FST in this context appears to be 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). The ASC literature review indicates that stressors of different types, duration and intensity provoke different profiles of biological responses and it is not clear the ways in which the features of this test are qualitatively analogous to the types of stress that are typically experienced by humans. They comment that this is also true of other aversive tests that may be used to study the neurobiology of stress. In light of this issue, the ASC indicate that projects 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, i.e. when using forced swimming as a model 'stressor', a relevant justification should always 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.

8.1.4. **Studies of anxiety-like behaviour:** There is evidence in the literature 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. The ASC concluded that there appears to be no scientific justification for inferring that a change in behaviour in the FST reflects animals' anxiety status.

8.2. The NCPA subcommittee found no reason to disagree with the ASC conclusion that the FST may be justifiable for the screening of potential antidepressants and when studying the neurobiology of stress, where appropriate specific justification is provided, but that it should not be used as a model of depression or to study depression-like behaviours or for studying anxiety disorders due to questions over its validity for these purposes.

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9. Recommendations:

Whilst this advice is provided by the National Committee to the HPRA and AWBs, in line with the Committee's remit under the Directive, we believe that it will be helpful to additional stakeholders, including individual researchers, ethics committees and funders. After each recommendation, the stakeholders that the recommendation primarily targets appear in brackets.

9.1. Use of FST as a model of depression and to investigate the mechanisms of anxiety

The Committee agrees with the conclusions for the ASC's literature review that the FST is not an appropriate model of depression or to investigate mechanisms of anxiety or its treatment. It does not appear from the Irish literature review that FST has been used to investigate anxiety or anxiolytics.

Recommendation 1 (all stakeholders): FST should not be authorised in projects as a model of depression.

Recommendation 2 (all stakeholders): FST should not be authorised in projects as a model to investigate the mechanisms of anxiety or its treatment.

9.2. Use of FST as screen for antidepressant treatments:

The information provided by establishment C makes it clear that they are investigating a number of alternative approaches that may help to minimise or (in the longer term) eliminate the use of the FST and they are commended on their progress in this area. Both regulators and sponsors have a role in reducing the use of FST, since the test is not required in order to get a new treatment to market *per se*, rather the regulators require evidence that demonstrate the effectiveness of a novel therapy.

The mechanism behind FST identifying antidepressant activity is not entirely clear. There have been attempts to classify the mechanism of action as being either nor-adrenergic or serotonergic, but it remains unclear as to whether FST will detect antidepressants that target alternative mechanisms to the drugs currently in use. As such, the validity of FST at detecting new classes of antidepressant compounds and other types of therapies is unknown.

Recommendation 3 (all stakeholders): FST is currently acceptable as a screen for anti-depressant drugs, but it cannot be considered that the FST will have good predictive validity for novel classes of antidepressants so the use of FST for this purpose should be regularly reviewed in the light of current best practice.

Recommendation 4 (researchers): Due to the nature of depression as a disease of all genders, both sexes should be used in animal studies that use the FST, in keeping with the Sex Inclusive Research Framework (SIRF); https://doi.org/10.31219/osf.io/mxg3e . The use of a single sex is only acceptable when there is robust scientific justification to support it.

Recommendation 5 (HPRA): If possible, HPRA should review the evidence provided from preclinical studies that support first in human studies to identify the current industry standard for identifying successful treatment candidates, with a view to assessing trends in use of FST for this purpose.

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9.3. Use of FST as a stressor

The FST is listed as a severe procedure in Annex VIII of EU Directive 2010/63, although it is possible that some mitigations could reduce the severity. A number of alternative stressors can be considered as more translationally relevant.

Recommendation 6 (researchers): Strong justification should be provided as to why FST is the most relevant stressor from a translational perspective. Historic use of the FST is not sufficient justification for its continued use as a stressor. Where use of the FST is proposed as a stressor the project should specifically state (i) why more refined tests cannot be used and (ii) why FST is the most translationally relevant model.

9.4. Considerations for ethical approval of use of FST

The system of grant award can lead to the difficult situation for Ethics Committees that a grant has been awarded prior to their input, meaning that models, such as FST, have been accepted as part of the proposed research programme by the grant awarding body. This can cause conflict between researchers and Ethics Committees where the Ethics Committee does not feel the procedure is the most refined option.

Recommendation 7 (researchers, institutions): Researchers are strongly advised to consult with their Ethics Committee and their Designated Veterinarian (DV) before including FST in a project proposal. Local systems should be put in place to reduce the potential for conflict between Ethics Committees, researchers and funding bodies with respect to the use of the FST. Approaches that could be considered are the approval for use of the FST prior to grant submission or that Ethics Committees highlight to researchers that the FST is a contentious procedure and so, even where funding is received for projects specifying its use, the Ethics committee may reject that aspect of that work. Therefore, researchers should understand that having the grant awarded does not mean that FST will gain ethical approval and this should be made clear to the grant awarding body at time of submission of the grant.

Recommendation 8 (researcher, local project review process): The processes of local project review should ensure that strong justification is provided for the proposed purpose for using the FST and that this has been clearly explained by the applicants to allow informed ethical assessment and opportunities for adoption of the 3Rs to be fully explored.

9.5. Standardisation

Both the ASC report and the data from the review of the use of the FST in Ireland raised concerns that both the way in which the FST is performed in different research labs and how it is reported in papers is not consistent. The Committee agree that this is a significant issue.

Recommendation 9 (National AWB Network, involving AWBs, researchers and DVs of relevant facilities where FST is performed): A standardised protocol for FST in Ireland should be developed by the AWB network, with support from local AWBs, DVs and researchers across Ireland that have experience of the FST, and agreed with HPRA and used. Deviations from the agreed standardised protocol would then have to be justified on a case-by-case basis to the Regulator. The protocol should include, at a minimum

- How the animals will be monitored during the procedure.

- A description of the Humane Endpoints (HEPs) that should be used. Objective, rather than subjective, HEPs should be developed as far as possible.
- A maximum time that animals may be kept in the water
- Details of what care will be provided after the test has been completed, specifically including drying the animal and giving warming support after it has been in the water.
- The parameters of the test, e.g. water temperature, which should be optimised to minimise the risk of hypothermia, maximum duration, that the tank water is changed between animals, etc.
- The parameters (in terms of animal behaviours) that will be assessed
- A robust scientific and statistical justification for the typical and maximal length of swim, which should be reduced to the minimum possible for reliable results.
- A standardised training approach for supporting those new to performing the procedures should be established. Videos of HEPs should be developed to aid training.
- Animals must be scored against HEPs by a person that is blind to the treatments. This person should be independent to the study.

9.6. Severity:

Recommendation 10 (all stakeholders): In line with Annex VIII of the Directive, FST to exhaustion should be considered as a severe procedure. The Committee see no justification for swimming to exhaustion and refinements in the protocol should be in place to allow authorisation at moderate.

9.7. Authorisation of projects proposing FST:

Recommendation 11 (researchers): In addition to the recommendations that use of FST should be rejected as a "model" of depression and for studies of anxiety and its treatment, the Committee recommends that project applications should include a 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 12 (researchers): When it is proposed to use the FST in a project, specific details of the experimental design and the number of animals to be used in the test must be included in the application rather than it appearing as one of a battery of behavioural tests.

Recommendation 13 (researchers): The scientific endpoint, i.e. fixed time or swimming to exhaustion, must be clearly stated in all applications

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Annex 1: Letter to HPRA requesting information from Irish establishments authorised to perform FST from NCPA Chair

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An Coiste Náisiúnta um Cheomhaú Ainmhithe a Úsáidtear ar Mhaithe le hEolaíocht
National Committee for the Protection of Animals Used for Scientific Purposes

26th September 2024

Dear Dr Beechinor,

Thank you for your letter of the 11th July 2024, detailing the **HPRA Request to the NCPA for advice in accordance with Regulation 61 of SI No. 543 of 2012** for the National Committee to consider whether there is a justified case for the inclusion of the Forced Swim Test (FST) in project applications in Ireland, and if so, the circumstances under which its use would be considered appropriate.

A sub-committee of the National Committee, comprising of the Chair and three National Committee members, met on 27th August and then again on 24th September to consider the request. The sub-committee felt that it would be helpful to seek some information from establishments in Ireland that include this test in their rodent behavioural assessment workflows. In addition, the sub-committee felt that information on the use of other tests that are considered as measures of depression and related mood disorders would be helpful in considering the utility of the FST, specifically the Tail Suspension test (TST), Sucrose Preference test (SPT) and the Female Urine Sniffing Test (FUST) for male mice.

I am writing to ask if it would be possible to request the information below for the NCPA from those establishments where the FST, TST, SPT or FUST are used.

1. **How many animals have been exposed to FST?** We would request that data be provided for each year between 2020 to 2023 (inclusive), and for 2024, to date. Please could these data be split by species and sex.
2. **What was the purpose of the use of these animals?** Specifically, how many animals, of what species and sex, were used to assess the effectiveness of anti-depressant intervention (by means of drug, dietary or other); how many as a model of depressive behaviour; and how many for a purpose that blended (combined) these two purposes. If the FST was used for any other purpose, please indicate what this was.
3. **How many animals used in FST were also used in other behavioural tests predictive of depression or other mood disorders?** Here, we wish to know whether the FST is used alone, or as part of a battery of tests, some of which may be modelling the same characteristic.
4. **How many projects submitted that year** proposed the use of each of these tests.

A suggested possible format for the data is below:

Year	1. Number of animals used in FST	2. Purpose of use				3. Number of animals used in multiple models	4. Number of projects submitted
		1. Assessing treatment effectiveness	2. Depression model	3. Blend of 1 and 2	4. Other (indicate what)		
2020	Male mice: Female mice: Male rats: Female rats:						FST: TST: SPT: FUST:
2021	Male mice: Female mice: Male rats: Female rats:						FST: TST: SPT: FUST:

Glossary

ASC - UK Animals in Science Committee
SPT - Sucrose Preference test
CMS – Chronic Mild Stress
FST - Forced swim test
FUST - Female Urine Sniffing Test
TST - Tail Suspension Test
OFT – Open Field Test

2022	Male mice: Female mice: Male rats: Female rats:						FST: TST: SPT: FUST:
2023	Male mice: Female mice: Male rats: Female rats:						FST: TST: SPT: FUST:
2024	Male mice: Female mice: Male rats: Female rats:						FST: TST: SPT: FUST:

5. **Which other tests are in use as models of depressive behaviour in the establishment?** It is important to have similar data provided as for the FST, but otherwise information on which other tests are used, and specifically whether TST, SPT or FUST are used, would be helpful. This information can be captured in a free-text field.

We would request that this data be available to the NCPA by the end of November 2024.

The NCPA understands that this request will mean additional work for researchers and AWBs, but consider the data will be very useful in allowing us to perform a fair and rigorous review of the current use of the FST and other tests of depressive state/anxiety and this will help inform the Committee so that it can provide the HPRA with an informed opinion on the inclusion of the FST in project applications in Ireland.

The Committee would like to thank institutions, researchers and the HPRA for their help in this matter.

Yours sincerely,

Dr. Ngaire Dennison, MA VetMB MRCVS

Chair, National Committee for the Protection of Animals

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Annex 2: email request for information from HPRA on behalf of the NCPA

From: HPRA SAP Scientific Animal Protection <sap@hpra.ie>
Sent: Tuesday 8 October 2024 2:36 pm
Subject: National Committee Request for Data

Dear Animal Welfare Body Chairs and Establishment Compliance Officers,

The HPRA has submitted a request for the advice of the National Committee for the Protection of Animals Used for Scientific Purposes on whether there is a justified case for the inclusion of the forced swim test (FST) in project applications in Ireland, and if so, the circumstances under which its use would be considered appropriate.

A sub-committee of the National Committee has met to consider and discuss this request. The sub-committee has concluded that it would be helpful, in considering the utility of the FST, to seek information from establishments in Ireland that perform this test, in addition to other behavioural assessment tests. Specifically, information is requested on the use of the **FST, Tail Suspension test (TST)**, **Sucrose Preference test (SPT**) and the **Female Urine Sniffing Test (FUST)**.

The National Committee has asked that the HPRA pass on this request to the relevant establishments, to allow the Committee to perform a fair and rigorous review of the test and provide advice accordingly. Please see the attached letter for full details of the information requested.

Please note that the National Committee requires that this information is submitted by the end of November, therefore please submit responses to **sap@hpra.ie** by close of business **Friday 29 November 2024,** using the subject title ***'National Committee Response: Requested Data'*.**

Kind regards,

The SAP Team

Veterinary Sciences Department – Scientific Animal Protection

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