

## Appendix 2: CoP for non-human primates

Neurocognitive research using non-human primates (NHPs) primarily use macaques, rhesus macaques in particular, and crab-eating macaques to a lesser extent. Marmosets are also occasionally used in research. This CoP is based on experiences with macaques.

If this type of research were to use another type of primate, a species-specific adaptation would be required of this CoP. Neurocognitive research conducted in the Netherlands only applies fluid restriction, not food restriction. There is no CoP for food restriction for NHPs.

### Guidelines for fluid restriction for NHPs

#### General:

1. During the drafting process of the project description and the working protocol in which fluid restriction is to take place, consultation will be required between the relevant researcher, the Animal Welfare Body (IvD) and an appointed veterinarian (and/or other relevant expert) regarding the design and the execution of the study, in particular, concerning the extent, duration and manner of fluid restriction, the training of the animals and the determination of decision points and criteria for interim adaptation, temporary suspension or premature termination of the protocol.
2. A project application must scientifically substantiate the researchers' choice for a motivation method (if fluid restriction is

selected) as well as provide the results of a Synthesis of Evidence.<sup>27</sup> It must also provide insight into the type of reward offered and method of dispensation.

3. The simultaneous application of fluid and food restriction is prohibited.
4. Pregnant animals may not be used for neurocognitive research in which fluid restriction is applied, unless explicitly permitted in the project license, following scientific substantiation and careful consideration and with an adapted degree of restriction.
5. In order to monitor the growth of the animal, the animal's weight progression is compared with the standardised growth curves for the species, sex and breeding line before and during the procedure, taking into consideration the percentile in which the animal is situated during the acclimatisation period. If the animal exhibits a sharply diverging growth curve and falls below 85% of its expected weight, then a decision must be made in consultation with the Animal Welfare Body/veterinarian regarding whether to (temporarily) remove the animal from the procedure or to limit restriction. In the event of a sharp drop in weight, a decision is made in consultation with the Animal Welfare Body and the person referred to in Article 14 (WOD) concerning what additional nutrition the animal is to receive and whether the procedure can be resumed.

6. The animals' living environment must be enriched in such a way that it encourages animals to make choices in their behaviour. This means that an animal must be housed in a social environment, unless solitary housing is permitted in the project license or is unavoidable for medical or social reasons. In such exceptional cases, the period of solitary housing must be kept to a minimum. In addition to a social environment, varying cage enrichment must also be offered to a sufficient extent.
7. An animal may carry out a task for a maximum duration per day that is outlined in the project license application, unless the animal is trained in the home cage and is free to determine when it wishes to carry out the task. If the animal is trained in a primate chair, the aim must be to have the animal seated in the chair for as short a time as possible. Each working protocol must make a reasoned case to the Animal Welfare Body regarding the maximum duration that the animal is allowed to spend on its task, also taking into account the consequent potential increase of the number of sessions required. Exceeding the length of time determined by the Animal Welfare Body (IvD) is only permitted following a revised recommendation based on an amendment to the working protocol. The Animal Welfare Body in such cases may not recommend that the animal be kept in the chair for longer than the maximum time laid down in the project license or, if not listed in the latter, in the corresponding project license application.
8. There should be enough time before the night period begins for the members of the cage to be re-introduced to one another after their "working day" (social housing). This also allows the health

and behaviour of the animals to be monitored. Sufficient time may vary for each pair. This may be determined in consultation with the responsible animal handler/behavioural expert.

#### *Training:*

9. Animals should have the opportunity to adjust to the schedule of restriction gradually and must demonstrably master their tasks before they are moved on to a higher level of training. The amount of time required for this will vary per species and per individual.
10. The use of a capture pole when training animals, for example, to ensure that the animals take their place in a primate chair, is only permitted if an agreement has been made with the Animal Welfare Body and/or external behavioural expert, and following approval by the NVWA, regarding what way the pole will be used if the animal cannot be trained in another way.

#### *Restriction protocol:*

11. The researcher responsible for the research must at all times, in consultation with the Animal Welfare Body, apply a type of restriction that is the least taxing for the animals and which still allows the research objective to be achieved. It is the task of the Central Authority for Scientific Procedures on Animals (CCD) to assess the reasons given for this choice.
12. There is a preference<sup>28</sup> for reward strategies that entail high-stimulation rewards, such as (watered down) fruit juice, which requires a minimum amount of restriction.

13. The degree of restriction is related to the number of required trials in combination with the complexity/aversiveness of the task. The lower number of trials required, the lower the degree of restriction is permitted.
14. Prior to a restriction protocol commencing, the body weight and the ad lib intake of fluid/food of the animal must be determined. Translated into metabolic weight (*please see Appendix 7*) this figure is used to determine the minimum daily fluid need of the animal. The actual supply of fluid will preferably be higher than this level and is determined on the basis of the results of the training.
15. The absolute minimum amount of fluid<sup>29</sup> that an animal must consume is 17 ml/kg<sup>30</sup> metabolic weight<sup>31</sup> per day. The running average over three days is at least 35 ml/kg metabolic body weight/day. For example: over a period of three days, an animal receives a total of 105 ml/kg metabolic weight. If an animal were to drink 17 ml/kg on Day 1 and 40 ml/kg on Day 2, then it must drink a further 48 ml/kg on Day 3 in order to arrive at a total of 105 ml/kg metabolic weight for those three days and a daily average of 35 ml/kg.

Animals that do not meet this minimum amount requirement during the procedure must be offered fluid in their home cage that same day, such that they still consume the daily minimum and the minimum running average for fluid. The aim should always be to have the animal drink more.

16. No uniform information could be obtained from the literature or consulted experts regarding young animals and the definition of “animals in a phase of growth” and the minimum volume of fluid to be provided. The fluid restriction protocols that are currently being applied lack scientific substantiation, making it impossible to provide a scientifically sound uniform opinion on the fluid needs of young animals. Due to the absence of a conclusive scientific substantiation, the everyday monitoring of the health and welfare of individual animals must be ensured by including a number of specific relevant observations in the restriction protocol, which must be reported and recorded daily. As a precaution, the minimum volume of fluid for young animals is set at 30 ml/kg of metabolic body weight per day, which is assumed to satisfy its physiological needs. The restriction protocol must be adapted as soon as the physiological, clinical and/or behavioural parameters begin to deviate.
17. Push days<sup>32</sup> are prohibited: if the animal succeeds at fewer trials than expected, the animal may not be (temporarily) subjected to more stringent restriction than stated in the working protocol. Push days constitute an infringement of animal welfare due to the fact that food and fluid is kept from the animal for too long.
18. When dispensing dry food, the fluid needs of the animal must be taken into account. As such, the combination of dry food with a type of fluid is preferred. Animals must be given fruit on a daily basis; the fluid intake from the fruit is not counted as part of the daily fluid intake.

19. As soon as it is clear that the animal has ceased carrying out a task<sup>33</sup> or is making too many errors, the procedure must be halted and this must be recorded in the welfare log. If, at that point, the animal has not yet consumed its daily minimum volume in fluid or food during the procedure, this must be provided until the animal has consumed its minimum amount. If the animal has consumed the minimum amount of fluid or food during the procedure, it can keep working as it will be possible to consume more fluid or food.
  20. If an animal begins to make too many errors, it is highly likely that the task is too difficult for the animal. In such cases, the supply of fluid may not be lowered to a level lower than is laid down in the working protocol. It may be determined, in consultation with the veterinarian or another sufficiently qualified expert and the Animal Welfare Body, whether the supply of fluid must be elevated in order to counter (initial) deviant behaviour or whether the task should be simplified.
- Monitoring health and behaviour*
21. Animals that are used in a fluid restriction protocol must be evaluated and assessed on a daily basis for potential changes in their health and behaviour. In the event of deviations, appropriate measures must be put in place to ensure the welfare of the animals. The findings must be recorded in the welfare log. The assessment, in any case, focuses on the behaviour, activity, alertness and the weight of the animals on the days that the animals are working.
  22. All information about the animals, procedure and the monitoring findings should be present or retrievable in the proximity of the animals.
  23. The duration and degree of the fluid restriction must be laid down in the working protocol, as well as any information on the training, key responsibilities and endpoints.
  24. If an animal falls ill during the procedure or is recovering from an illness or operation, or if the animal exhibits deviant behaviour, the animal will only be subjected to restriction once it has fully recovered. Whether or not the fluid restriction regime should be adapted is determined in consultation with the veterinarian. An animal that is ill at the start of the experiment or procedure, or is recovering from an illness or operation, or exhibits deviating behaviour, may only be used in the research – and be subjected to restriction – once it has fully recovered and has been released by the veterinarian or another competent person that is not involved in the research.
  25. It is undesirable to allow animals that have been subjected to a restriction protocol to suddenly have unlimited access to fluid, since this could lead to excessive intake. An animal should be offered no more than the individually determined *ad lib* intake established beforehand. In the event of extreme circumstances (e.g. dehydration) an animal must be taken out of the procedure for a long period of time. Even in the case of short-term recovery periods and periods during which the animal is not participating in training, the researchers must ensure gradual transitions; on

occasion a permanent – albeit light – restriction compared to voluntary intake is desirable (this must be assessed individually for each animal). In the case of short-term interruptions (weekends), restriction may be maintained, or the supply of fluid may be increased slightly. On both weekend days, an animal must at least receive the absolute minimum amount of food/fluid.

26. The parties responsible for the monitoring of the welfare of the animals must be demonstrably competent and qualified and must be able to recognise any deviations that indicate physical or behavioural changes.

