SUMMARY OF OPINION

Opinion of the Scientific Panel on Animal Health and Welfare on a request from the Commission related to the aspects of the biology and welfare of animals used for experimental and other scientific purposes

(Question N° EFSA-Q-2004-105)

Adopted by the AHAW Panel on 14th November 2005

EFSA was invited by the EU Commission to produce a scientific opinion concerning the "Revision of the Directive 86/609/EEC on the protection of animals used for experimental and other scientific purposes".

This scientific opinion was adopted by written procedure on the 14th November 2005, by the Scientific Panel on Animal Health and Welfare (AHAW) after its Plenary Meeting held on 12th and 13th of October.

According to the mandate of EFSA, ethical, socio-economic, cultural and religious aspects are outside the scope of this opinion.

Summary of the Scientific Opinion for each of the three parts of the Mandate from the Commission:

1. Summary of the need for protection for invertebrates and fetuses and the criteria used (Questions 1 & 2)

   The Panel was asked to consider the scientific evidence for the sentience and capacity of all invertebrate species used for experimental purposes and of fetal and embryonic forms to "experience pain, suffering, distress or lasting harm". Indicators of an animal's capacity to experience suffering include long-term memory, plasticity of behaviour, complex learning and the possibility of experiencing pain. Some invertebrate species: (i) possess short and long term memory, (ii) exhibit complex learning such as social learning, conditioned suppression, discrimination and generalisation, reversal learning, (iii) show spatial

awareness and form cognitive maps, (iv) show deception, (v) perform appropriately in operant studies to gain reinforcement or avoid punishment, (vi) possess receptors sensitive to noxious stimuli connected by nervous pathways to a central nervous system and brain centres, (vii) possess receptors for opioid substances, (viii) modify their responses to stimuli that would be painful for a human after having had analgesics, (ix) respond to stimuli that would be painful for a human in a manner so as to avoid or minimise damage to the body, (x) show an unwillingness to resubmit themselves to a painful procedure indicating that they can learn to associate apparently non-painful with apparently painful events. At a certain stage of development within an egg or the mother, the characteristics listed above may appear. Such information has been used in coming to conclusions about sentience.

Cyclostomes (lampreys and hagfish) have a pain system similar to that of other fish and brains that do not differ much from those of some other fish. There is evidence that cephalopods have adrenal and pain systems, a relatively complex brain similar to many vertebrates, significant cognitive ability including good learning ability and memory retention especially in octopuses, individual temperaments, elaborate signalling and communication systems, especially in cuttlefish and squid that can show rapid emotional colour changes, may live in social groups and have complex social relationships. Nautiloids have many characters similar to those of other cephalopods, they can track other individuals, live for a long time and are active pelagic animals. The largest of decapod crustaceans are complex in behaviour and appear to have some degree of awareness. They have a pain system and considerable learning ability. As a consequence of this evidence, it is concluded that cyclostomes, all Cephalopoda and decapod crustaceans fall into the same category of animals as those that are at present protected. Using similar arguments, the dramatic evidence of the sensory processing, analytical and prediction ability of salticid spiders provides evidence for awareness greater than in any other invertebrates except cephalopods but we have little evidence of a pain system so do not at present put these spiders in that same category. Free-swimming tunicates are also in this borderline area and social insects and amphioxus are close to it.

Whenever there is a significant risk that a mammalian fetus, or the fetus of an oviparous animal such as a bird, reptile, amphibian, fish or cephalopod, is for any length of time sufficiently aware that it will suffer or otherwise have poor welfare when a procedure is carried out on it within the uterus or egg, or after removal therefrom, such animals should be included in the list of protected animals. The stage of development at which this risk is sufficient for protection to be necessary is that at which the normal locomotion and sensory functioning of an individual independent of the egg or mother can occur. For air-breathing animals this time will not generally be later than that at which the fetus could survive unassisted outside the uterus or egg. For most vertebrate animals, the stage of development at which there is a risk of poor welfare when a procedure is carried out on them is the beginning of the last third of development within the egg or mother. For a

fish, amphibian, cephalopod, or decapod it is when it is capable of feeding independently rather than being dependent on the food supply from the egg.

Precocial oviparous species, some of which are breathing at the time of hatching present much evidence of being aware before hatching and during the last days before hatching,

Even though the mammalian fetus can show physical responses to external stimuli, in some species perhaps for as much as the last third of their development, the weight of present evidence suggests that consciousness is inhibited in the fetus until it starts to breathe air. It is possible that in a mammalian fetus there might be transient episodes of increased oxygenation above the threshold required to support some aspects of consciousness. It is clear that there is a risk, perhaps a small risk, that any mammalian fetus may on occasion be affected by some experimental procedures in such a way that their welfare is poor, sometimes because they are suffering pain. If a mammalian fetus is removed from the mother and starts to breathe, its level of awareness will change to that typical of such animals after parturition. In addition, protection may need to be given against emotional states in pregnant mothers to safeguard subsequent behavioural modification and welfare of the offspring.

When a procedure is performed on a fetus that is likely to produce pain in the newborn or newly-hatched of that species, adequate anaesthesia and analgesia should be given provided that the agents used do not significantly increase the likelihood of fetal mortality. In the circumstance where no suitable anaesthetic or analgesic agents are available, procedures should not be carried out on such fetuses. When the procedure might cause a lasting inflammatory response that persists post-natally, protection should be given against pain and suffering. A schedule of anaesthetics and analgesics that are suitable for use in pregnant animals, and fetuses should be prepared.

2. Summary of the need for purpose breeding of animals and the criteria used (Question 3)

Species listed in Annex I to Directive 86/609/EEC are those that must be 'purpose bred' when used in experiments (unless a specific exemption has been obtained). The criteria for inclusion of species in Annex I have not been clearly defined and hence no information is available on why they were originally included. Therefore, the Commission has asked the EFSA to issue a scientific opinion on the scientific criteria that could be used to determine in which cases animals to be used in experiments should be purpose-bred and, based on these criteria, determine which species currently used in experiments meet these criteria.

It is the opinion of the AHAW panel that including a species as "purpose-bred" within Annex I will confer a considerable degree of assurance that animals of that species will be provided with suitable accommodation, welfare and care practices. As a consequence of health and colony management within breeding

establishments, there can be improved confidence in the quality of the animal, resulting in improved science and a reduction in animal numbers required. Taking these factors in isolation, for the great majority of scientific investigations, there would be welfare and scientific merit in recommending that all animals used in scientific procedures be purpose-bred. However, before making such a recommendation, there are a number of other important factors that have to be considered. The consequences of inclusion of all species could, for example, result in loss of genetic diversity, the generation of large numbers of surplus animals and significant delays in scientific progress. A risk assessment approach has therefore been taken to this issue, with the group analysing the potential benefits for and the adverse consequences of the inclusion of each species in Annex I. Two issues have been considered: animal welfare and scientific quality. For each, three steps have been followed: identification of the hazards, exposure assessment and consequence assessment.

The criteria suggested by the Technical Expert Working Group (TEWG) organised by DG ENV (2003) have been considered and incorporated into an assessment process against which the inclusion of each of the commonly used laboratory species was reviewed. The criteria considered by the AHAW panel have been whether legislation already exists to protect animal welfare, genetically altered animals, health and genetic quality of animals, demand, extrapolation of results to farming or to wild populations and capture from the wild.

It is recommended that, wherever possible, animals used should be of a uniform standard so that there is good and effective control over the animals' genetic fidelity, microbial status, nutrition, socialisation to humans and other animals (e.g. ferrets, dogs and even rodents) and environment. Ideally all animals should be purpose bred but, in practice, some exceptions will be necessary. Exceptions should be made to purpose breeding when it is necessary for the research that a particular strain or breed is used, or that scientific progress would be unduly delayed providing that the scientific data resulting from such research were considered likely to be of good quality, i.e. the competent authorities should consider the potential adverse consequences for research should an exemption for the use of non-purpose bred animals be refused (86/609/EEC: Article 19(4)). Genetically altered animals (of all species) should be added to Annex I. The review of all the commonly used laboratory species has concluded that with the exception of quail (*Coturnix coturnix*) all the other species listed should continue to be purpose-bred and some further species should be added, namely: Chinese hamster (*Cricetus griseus*), Mongolian gerbils (*Meriones unguiculatus*), two *Xenopus* species (*X. laevis* and *X. tropicalis*) and two species of *Rana* (*R. temporaria* and *R. pipiens*).

3. Summary of humane methods of killing animals (Question 4)

Nearly all animals are killed at the end of a research project and it is important that this is done humanely i.e. causing as little suffering as possible for the animals concerned. The majority (85-90%) of animals used in research are small

rodents however, of necessity (as we are trying to cover all methods for all animals), much of the Report deals with the methods for large animals. The Opinion of the scientific panel on AHAW is based on the Report annexed to this Opinion that presented recent data building on the three earlier authoritative reports on the humane killing of animals i.e.: 1) the Scientific Report related to welfare aspects of animal stunning and killing methods of the main commercial species of animals (EFSA, 2004, http://www.efsa.eu.int); 2) Close et al. 1996/1997 (endorsed by the EU for the humane killing of laboratory animals); and 3) the AVMA Report (2000) dealing with methods for all animals. The Opinion does not repeat what is already dealt with in detail in those reports but we have included a section dealing with new data for each method where applicable, and some conclusions and recommendations are retained. The Scientific Report and Opinion deal with the various technical ways of killing animals starting with electrical and mechanical methods, followed by gaseous and then injectable methods. The section on the use of gaseous agents is in some considerable detail as it is the subject of much new data, with more than 20 new papers in the past 10 years, many of them dealing with the commonest laboratory animals. The interpretation of this data has been varied. The recommended methods for each species are given in Tables 7 to 14 at the end of this section but, in general, we have adopted the recommendations given in the existing EU Guidance (Close et al., 1996/97) except where stated. The AHAW panel suggested that these methods could be varied but only with a scientific justification and appropriate authority, i.e. the recommended methods represent the default position. We also address more general issues including ensuring death, training of personnel, killing animals for their tissues and oversight, the choice of method and when this might affect the scientific outcomes, and gathering information on methods used as well as their efficiency and effectiveness.

Key words

Animal research, experimental animals, animal welfare, invertebrate sentience, fetal sentience, purpose breeding, euthanasia.