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Report of the ANIMAL PROCEDURES COMMITTEE for 1989

London: HMSO

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Report of the Animal Procedures Committee for 1989

Presented pursuant to Act Eliz. II 1986 C. 14 Section 20(5) (Animals (Scientific Procedures) Act 1986)

Ordered by The House of Commons to be printed
24 July 1990

LONDON: HMSO

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MEMBERSHIP OF THE ANIMAL PROCEDURES COMMITTEE AS AT 31 DECEMBER 1989

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ANNUAL REPORT OF THE ANIMAL PROCEDURES COMMITTEE FOR 1989

Submitted to the Rt Hon David Waddington QC MP, Secretary of State for the Home Department, and to the Department of Health and Social Services, Northern Ireland; July 1990

1 Introduction

Functions of the Committee

1.1. The Animal Procedures Committee is a statutory body, appointed under sections 19 and 20 of the Animals (Scientific Procedures) Act 1986. Under section 20(1) of the Act, the duty of the Committee is to advise the Secretary of State on matters concerned with the Act and his functions under it. The Committee may itself select subjects for study and the Secretary of State may refer matters to the Committee for consideration.

1.2. The Animals (Scientific Procedures) Act 1986 regulates *any experimental or other scientific procedure* applied to a vertebrate animal which may have the effect of causing that animal *pain, suffering, distress or lasting harm.* The Act came into effect on 1 January 1987 and its provisions are now fully in force.

Composition of the Committee

1.3. The Chairman of the Committee, Professor David Williams, President of Wolfson College, Cambridge, was elected Vice-Chancellor of Cambridge University during 1989 and it was with great regret that the Committee heard that, owing to pressure on his time, he would no longer be able to continue as Chairman beyond the end of the year. Professor Williams was the first Chairman of the Committee and the last Chairman of its predecessor, the Advisory Committee on Animal Experiments; and the Committee recalls with gratitude the wisdom and understanding with which he steered their proceedings.

1.4. The membership of the Committee as at 31 December 1989 is shown on page iv. As mentioned in para 1.4 of last year's report, five members reached their maximum period of appointment early in 1989 and, in December, the Home Secretary announced the appointment of Lord Nathan as Chairman in succession to Professor Williams, and of four new members of the Committee.

1.5. Lord Nathan is a solicitor who includes among his appointments membership of the House of Lords Select Committee on the European Communities and the Royal Commission on Environmental Pollution.

Business of the Committee

1.6. The Committee met eight times during 1989. One of these meetings was a visit to an establishment carrying out licensed work, as part of its continuing review of psychological and behavioural research; and one was a visit to a breeding and supplying establishment, in anticipation of the bringing into force of section 7 of the Act (para 2.15 below). The Committee is again grateful for the welcome which was extended to its members and for the care and trouble which was taken by their hosts in making the arrangements for these visits.

2 Operation of the Animals (Scientific Procedures) Act 1986

Numbers of Certificate and Licence Holders

2.1. The Committee has continued to receive regular reports on the final stages of implementation of the Act. During 1989, 20 certificates of designation, 972 project licences and 4,605 personal licences were issued under the Act in Great Britain. Of the project licences, 441 were assessed as being of mild severity; 449 were assessed as being of moderate severity; and 14 were assessed as being of substantial severity. The remaining 68 project licences, where the work was with terminally anaesthetised or decerebrate animals, were unclassified.

2.2. In Northern Ireland, 3 certificates of designation were issued, together with 19 project licences and 42 personal licences. Of the project licences, 10 were assessed as being mild; 7 moderate; and two of substantial severity.

2.3. On 31 December 1989, in Great Britain, there were 375 certificates of designation for scientific procedures establishments; 4,193 project licences held by 3,617 project licence holders; and 17,940 current personal licences. Section 7 of the Act, bringing breeding and supplying establishments under new controls, came into effect on 1 January 1990, so these additional certificates are not included in this total.

Code of Practice on the Housing and Care of Animals used in Scientific Procedures

2.4. The Committee's Annual Report last year described how this *Code of Practice,* which was laid before Parliament on 7 February 1989, had been based on earlier guidelines produced jointly by the Royal Society and the Universities Federation for Animal Welfare. It set conditions and space requirements which conform with, and in some cases exceed, the requirements of the Council of Europe Convention and the European Community Directive 86/609/EEC.

2.5. During the year, the Committee has heard of some of the costs which designated establishments face when having to upgrade their housing facilities to bring them in line with the *Code of Practice.* In general, this upgrading will involve once and for all capital expenditure, rather than lead to increasing continuing costs. Although the *Code of Practice* was based on voluntary guidelines which were widely discussed within the scientific community long before the *Code* was published, there is nevertheless a long lead time between planning to upgrade animal house facilities and completing the building work.

2.6. The Committee has heard of the progress which has already been made in a number of establishments in implementing the *Code of Practice.* It has also been reassured by the approach taken by the Inspectorate which presses for immediate implementation of the conditions of the *Code of Practice* only where current facilities lead to a significant impairment in the welfare of animals.

2.7. One area of concern in the implementation of the *Code of Practice* has been the less than ideal conditions under which some of the larger non-human primates were housed. The Committee endorsed to the full the active steps being taken by Inspectors to urge establishments to make early progress with increasing the facilities for these animals, for instance by providing foraging, access to exercise cages and viewing panels between cages.

The ending of transitional arrangements

2.8. One of the key features of the 1986 Act is the introduction of project licences, as the means by which a programme of work is set out and justified. The previous legislation had, in the main, effectively provided only for the equivalent of personal licences, which ensure that all who carry out animal procedures are competent to do so or, if not, are appropriately supervised.

2.9. The transition to project licensing was a major task which was fully completed in the summer of 1989. All regulated work in Great Britain and Northern Ireland is now covered by project licences and this is a significant improvement in the arrangements which control scientific procedures on living animals.

2.10. This has had the effect of making the project licence into the principal method by which animal procedures are controlled. Following the publication of the *Guidance on the Operation of the Animal (Scientific Procedures) Act 1986* in February 1990, para 4.5 of which sets out in detail their responsibilities, project licence holders are becoming increasingly aware of their role in supervising and managing their programmes of work.

2.11. Under section 5 of the Act, a project licence may remain valid for up to 5 years but, in practice, licences are amended and reviewed more frequently than that in order to take into account developments as the work continues. The Home Office has endeavoured to ensure the consistent application of standards across project licences.

2.12. The transition to the controls of the 1986 Act was made with the Inspectorate remaining almost always below its full complement and, as a result, with some Inspectors having to work with an excessive workload. The Committee very much hopes that the current recruitment exercise for Inspectors will be successful and that it will be possible to maintain the Inspectorate at full strength and that it will not be overloaded in future.

The use of assessors

2.13. As part of the preparation for the transition to the 1986 Act, the Home Office established a large panel of assessors, composed of distinguished scientists in their respective fields, to whom project licence applications were referred as necessary.

2.14. However, now that the transition to project licences has been completed, it is usually necessary for each referral to be made on a case-by-case basis to the individual expert most suited to comment on specialised aspects of a particular project. There remains, therefore, no further need to maintain a general panel of assessors, although many of the assessors to whom licence applications will be referred in future will also have served on the panel of assessors.

Introduction of controls over establishments breeding and supplying animals for use in scientific procedures

2.15. Section 7 of the Act, the last to be brought into force, came into effect on 1 January 1990 and, for the first time, establishments which breed and supply animals used in scientific procedures were brought within the control of specific legislation for this purpose.

2.16. Breeding and supplying establishments fall into two main groups. The first comprises those scientific procedure establishments which keep their own breeding colonies of animals, principally for their own use. These establishments needed additional designation under section 7 and the facilities which housed these breeding colonies thereby came more directly under the control of the Act.

2.17. The other main group of breeding and supplying establishments consists of about 25 establishments which exist solely or partly to breed animals for use in scientific procedures, but at which no scientific procedures take place. These establishments were inspected prior to being designated and, in all cases, this designation is provisional for one year. The Committee is gratified that this extension of the controls on laboratory animals has been completed according to the timetable which was envisaged.

2.18. In a number of establishments, the Inspector has set a timetable for the completion of a programme of work which is judged to be necessary to bring the facilities of the establishment fully in line with those required. In common with the approach taken with scientific procedure establishments and the *Code of Practice*, a reasonable time scale is set for the implementation of these improvements, except where a significant risk to the welfare of the animals requires more urgent action.

2.19. During the year, the Committee paid a visit to a large, specialist breeding and supplying establishment and saw the way in which a range of laboratory animals was being bred, including animals which were being bred behind microbiological barriers. The Committee was most impressed by the quality of the housing and care offered by this establishment.

Statistics

2.20. The *Statistics of Scientific Procedures on Living Animals, Great Britain, 1988* were published in July 1989 and the Committee was gratified to note that all of the recommendations which it had previously made for enhancement of these statistics were implemented in this volume, in particular the increased detail about carnivores and primates. The Committee is greatly impressed by the quality of this regular statistical volume which describes fully, and in a comprehensible way, the work which is carried out in this country. The 1988 statistics are the last to be collected from personal licence holders as the 1989 statistics have been collected from project licence holders.

2.21. Currently, the statistics are presented exclusively in terms of the number of *procedures,* rather than the number of *animals.* Statistical information about the numbers of animals is required to satisfy our obligations under the Council of Europe Convention and the European Directive and some data in this form were provided for the Committee's use this year. The Committee very much hopes that, after the transition to collecting statistics from project licence holders, it will be possible for further work to be done to increase the level of published information about the numbers of animals used.

The assessment of severity

2.22. During the year, the Committee considered the basis on which severity was assessed in project licences. This is a very difficult issue, involving as it does considerable subjectivity. The severity assessment given to an individual *procedure* aims to take into account all the techniques applied to each animal or group of animals; the nature of any likely adverse effects; and the specific and general actions to be taken to mitigate these effects. This should indicate the most severe situation for animals subjected to that procedure and will set the upper limit of severity which can be allowed. Such an assessment does not take into account the number of animals expected to experience the maximum severity or the proportion of the animal's lifetime for which it is expected to experience the adverse effects.

2.23. The assessment given to the overall severity of a *project* does take into account the number of animals used in each of the procedures; the proportion of animals that may be expected to be exposed to the upper limits of severity in each procedure; and the proportion of time that they may spend at the upper limit of severity. It is in this way that an attempt is made to weigh the sum of the likely adverse effects of all the animals against the benefits likely to accrue from the scientific work. The Committee will undoubtedly wish to return to how this balance is struck from time to time.

2.24. In order to give effect to the observation of the severity limit in practice, personal licensees are being encouraged to use the protocol sheets of project licences as working documents to give them knowledge of the severity limit of each procedure; the operative details; the predictable adverse effects; and the required mitigating actions and endpoints. The Committee endorses this approach to the assessment and control of severity which has now been incorporated in the Guidance on the operation of the Act.

Infringements

2.25. During the course of the year, the Committee received reports of all infringements against the Act and against licence conditions. As in previous years, almost all of these infringements were not such as to involve danger to the welfare of the animals concerned.

2.26. During 1988 and the early part of 1989, many of the infringements arose through misunderstandings of the implications of the new legislation. In these cases, the licence holders generally received letters of admonishment from the Home Office. However, for infringements which were more than technical, the Home Office has increasingly adopted the practice of revoking licences or imposing additional and more specific or stringent licence requirements on infringers.

2.27. The licensing provisions of the 1986 Act enable detailed arrangements to be made in order to prevent the recurrence of an infringement. By the end of 1989, it was becoming uncommon for an infringement which was other than technical to be dealt with without the Home Office revoking a licence or imposing a variation in the conditions of certificates or licences. The Committee has endorsed this approach to infringements.

Guidance on the Operation of the Animals (Scientific Procedures) Act 1986

2.28. During the year, the Committee considered in some detail the drafts of the *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986* as required by section 21(3). The original draft *Guidance* was produced in advance of the Act and was principally concerned with the transition from the former legislation to the 1986 Act.

2.29. The *Guidance* which was laid before Parliament on 14 February 1990 is drawn up as a working document with detailed statements of the duties of all those who have specific responsibilities under the Act: certificate holder; project licence holder and deputy project licence holder; named veterinary surgeon; named person with responsibility for the day-to-day care of animals; and personal licensee.

2.30. The Committee endorsed the terms of the *Guidance* which it is sure will be valued by all those responsible for scientific procedures on living animals. The Committee also welcomed the detail which the Guidance gave to the assessment and control of severity; the circumstances in which animals may be re-used; the handling of infringements; the standard conditions on certificates, project and personal licences; and the maintenance of records. The *Guidance* is a major statement on the way the Act is operated in the United Kingdom.

3 Scientific work considered or reviewed by the Committee

The referral of project licences to the Committee

3.1. Throughout the transitional period, there have been a number of referrals of project licence applications to the Committee. The Home Secretary continues his practice of seeking the advice of the Committee on research work which involves novel principles where he considers that the Committee can give additional advice, in particular on the balance between adverse effects on the animals and the expected scientific benefits of the work.

3.2. In addition to these licence applications, it is the current practice that all project licence applications for microsurgery training; the testing or use of tobacco and tobacco products; and the safety testing of cosmetics are referred to the Committee for advice in recognition of the public controversy which such work can arouse. The Committee is also notified, at its own request, of applications for work involving non-human primates which include a substantially severe procedure and, had there been any, of projects in which the use of non-human Hominoidea was proposed (there have been none in the United Kingdom for at least ten years).

3.3. The Committee does not usually consider other licence applications unless a general question has been raised about the acceptability of a certain type of procedure: no precedent is set by the referral of any licence to the Committee.

Applications involving the use of tobacco

3.4. During the year, the Committee considered four applications involving the use of tobacco. One of these was intended to produce safer tobacco products and the Committee deferred consideration of the application pending further advice on the scientific merits of the project and from the Independent Scientific Committee on Smoking and Health (the Froggatt Committee). The Froggatt Committee expressed the view that it would be unjustified to use animals to test minor modifications and essentially "cosmetic" additives to smoking materials although it recognised that a stronger case might be made for animal testing of more radical changes, such as occurred with the "smokeless cigarette". Research into the mechanism of action of the harmful constituents of tobacco was one area in which the Froggatt Committee believed some animal work might continue to be justified.

3.5. In the light of this advice, the Committee decided to advise the Home Secretary that the application should be refused in its present form and it was withdrawn.

3.6. In another of these cases, the application involved the use of tobacco to induce chronic bronchitis and the research project was directed at how to treat this. Work on animals was to be carried out alongside in vitro work on human tissue cultures. Although the application clearly had some merit, the Committee expressed reservations about the scientific value of the project and the benefit which might flow from it. It therefore asked that the application be referred to an assessor for a second opinion and this is awaited.

3.7. In the third case, the aim of the project was to study the effect and mode of action of foreign chemicals on the immune system; to investigate the contribution of the immune system to pathological conditions; and to improve and refine methods for predicting and evaluating adverse effects of chemicals mediated through the immune system. The Committee considered this work on the immune system to be properly justified and recommended that the application be granted.

3.8. The fourth application concerned the connection between the presence in plasma of free radicals generated by tobacco smoke, which can damage the vascular endothelium, and the development of atherosclerosis. It was proposed that 90% of the rats would be terminally anaesthetised but some conscious animals would receive the subcutaneous administration of cigarette smoke derivatives. The Committee considered that this was a good example of fundamental research in an important area of pathology and recommended that the application should be granted.

The use of MPTP to study Parkinson's disease

3.9. As part of its developing practice of considering types of licence, rather than individual project licence applications, the Committee considered the use of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (known as MPTP) in research into idiopathic Parkinson's disease. This research, which is principally performed on primates, involves the administration of MPTP to generate the symptoms of Parkinson's disease so that research into corrective treatments, involving either surgery or drug treatment, can take place.

3.10. The Committee is in no doubt that this is valuable work, but of substantial severity. The Committee considered in detail an excellent review of the current state of MPTP research produced, at its request, by the Inspectorate. An abridged version of this paper forms appendix I of this report.

3.11. In 1988, members of the Committee had visited an establishment where MPTP research was carried out using marmosets and examined the effects on the animals concerned. Following this, and its consideration of the review paper, the Committee recommended that MPTP research should continue under the conditions which were already present on most of the MPTP project licences.

The use of strychnine in research

3.12. The Committee also considered papers on the continued use of strychnine in research. Historically, strychnine has been used as an experimental model for epilepsy because it can cause convulsions. However, it is now known that the type of convulsion produced by strychnine is unrelated to the type of convulsions which occur in epilepsy and therefore its use to simulate epilepsy cannot be justified.

3.13. Strychnine does, however, have unique properties as a glycine receptor antagonist. The Committee heard how the use of strychnine can be of value in the study of brain mechanisms and the high pressure neurological syndrome, as can be experienced by deep-sea divers. The Committee acknowledged that the use of strychnine might be continued in such research, provided that it is administered only under very strict conditions; that the animal is actively supervised; and that it is humanely killed at the first sign of the onset of any convulsion.

Project licences assessed as being of substantial severity

3.14. During the course of the year, the Committee reviewed the kinds and categories of the 80 or so (out of the total of 4,193) project licences which have been assessed as being of substantial severity. Five categories of such work have been identified.

3.15. The largest of these involved a microbiological challenge, where the principal risk is to the unprotected control, rather than to the experimental animal. This work included research on neonatal influenza; liver failure; a range of infections; and the potency testing of vaccines and anti-microbial agents. The Committee has awarded two research grants into alternatives in this area of work (see paras 5.12–5.15).

3.16. Another group was principally for the diagnosis of disease, not only in humans but also in animals and fish.

3.17. A further group was concerned with toxicity, often involving an LD50 or an LC50 test. Some of these projects were concerned with safety testing but others include research on toxins; the toxicity of industrial waste and hazardous substances, including the effect on wildlife; the mechanism of action of toxins; and research into pancreatitis.

3.18. In a further group of projects, the major impact on the animal was surgical. This included projects on organ transplant and rejection; spinal nerve growth; diseases of the colon, liver and heart; and cancer research.

3.19. In considering these projects, the Committee was satisfied that their severity was appropriately assessed. Work of substantial severity is closely monitored by Inspectors, who advise licence holders on its control and the Committee was pleased to hear of the progress which had been made with some projects to limit their severity by the introduction of earlier endpoints.

3.20. It is gratifying that a proportionately small number of projects is assessed overall as being of substantial severity. Nevertheless, the refinement of techniques in order to reduce severity remains a central concern of the Committee and, during 1990, it will be considering some of these categories of project licences in more detail.

4 Other issues of concern to the Committee

The use of animals in the safety testing of cosmetics and toiletries

4.1. Both beauty preparations and toiletries are classified as cosmetic substances under the definition used by the EC and in the United Kingdom. Beauty preparations extend beyond decorative cosmetics to include the preservatives and other substances which protect them against degradation. Examples of toiletries are toothpastes, soaps, shampoos and sunscreens, all of which may contain active ingredients to improve the health and hygiene of the user.

4.2. There are currently six project licences in force which permit the safety testing of cosmetic substances (other project licences cover the development of substances, like preservatives, which may subsequently be used in cosmetics as well as food; or substances which prevent tooth decay, which may be used in toiletries like toothpastes as well as medicines). All the cosmetic safety testing project licences have been considered by the Committee and all contain a special condition requiring that, each year, detailed statistics are submitted for its further consideration. One feature of these statistics is that it allows a distinction to be made between the safety testing of beauty preparations and toiletries.

4.3. There are a number of misunderstandings about the nature and purpose of cosmetics safety testing. The statistics which the Committee has seen from the six project licence holders makes it clear that about 90 per cent of the cosmetics testing in this country is carried out for the safety testing of toiletries and less than 10 per cent is for the safety testing of beauty preparations or their ingredients. 57 per cent of all testing is on ingredients alone and, of the remaining procedures for the testing of finished products, 65 per cent is for skin care or skin cleaning products; 20 per cent for hair products; 11 per cent for deodorants; 4 per cent for bath products (all of which are toiletries); and only 1 per cent is for the safety testing of finished beauty preparations.

4.4. As a result, skin tests account for about 75 per cent of all cosmetics safety testing procedures, about ten times more than for eye irritancy tests which are used principally for hair and skin products or their ingredients. The conclusion can be drawn that, in this country, finished beauty preparations are only very rarely tested on animals and cosmetics safety testing should be understood as predominantly the testing of toiletries and their ingredients.

4.5. It should be noted that cosmetics safety testing procedures usually have only a mild effect on the animals as no useful purpose is served by persisting with the testing of a substance which exhibits irritant effects: indeed, as a result of information already acquired about cosmetic ingredients and the use of *in vitro* screening tests, the chances of animals suffering adverse reactions are minimised.

4.6. The Committee was concerned to hear, at the end of the year, that there were preliminary draft proposals by the European Community to increase the quantity and range of cosmetic ingredients and products which mandatorily require safety testing. It understands that the Government has taken steps to limit any mandatory extension of cosmetics testing and the Committee will be conducting, in 1990, a study of the EC position.

The induction of psychological stress

4.7. For some years, the Committee, like its predecessor, the Advisory Committee on Animal Experiments, has been conducting a study into the induction of psychological stress in scientific procedures on living animals. In the course of this study, the Committee has visited a number of establishments where such procedures were being administered and this year it carried out another such visit.

4.8. The Committee has always appreciated that psychological stress can arise in a number of ways in research on living animals, even through the requirement to keep the animal in a cage. But in a small number of areas of research the induction of stress is itself an important element.

4.9. However, in the examples which the Committee has seen, most of the stresses involved have appeared to be sufficiently well controlled to be relatively minor, whilst at the same time achieving their scientific objective. Most of the examples of psychological stress which the Committee has seen have been induced for the purpose of investigating disease-related symptoms.

4.10. The deliberate induction of stress in an animal for scientific reasons raises a number of difficult issues. In some instances, the term is used to describe experiments in which the production of "stress" is the issue of the investigation, for instance where the effectiveness of pharmacological substances for the relief of stress may be studied. In other instances, the term is used to describe the application of different forms of stimulation which, while not used primarily to study "stress" could, because of their apparently unpleasant nature, nevertheless have the same undesirable effects as those stimuli deliberately introduced as stressors.

4.11. There are three principal ways of assessing what sorts of procedures are "stressful". The first, and one which may historically have been the most influential, is to define stress as a *response* and to identify patterns of physiological and psychological responses elicited by different situations. Under such a definition, stress is seen as the *non-specific response of the body to any demand made upon it.* Such a response is the mobilisation of the autonomic nervous system, the adrenomedullary and the adrenocortical systems, bringing about, for example, the secretion of adrenalin and cortisol.

4.12. The second type of definition focuses on stress as a *stimulus* or event. Such an approach has, for instance, been used particularly by psychiatrists when identifying an event in the environment, either short term or long lasting, as being the precipitant of disorder.

4.13. The third approach, which is theoretically more attractive, views stress as an *interaction* between events or stimuli in the environment and the individual, and which produces a mismatch between the environment and the resources of the organism to cope with the demands which the environment imposes. It follows that, provided the organism is able to cope, "stress" does not necessarily occur. Hidden in this definition is the issue of the length of time for which the potential "stressor" is present. A rock climber may find the position of being suspended up a vertical rock face pleasurable for a time but, should that time become extended and the resources for coping with the situation become exhausted, then "stress" is experienced.

4.14. However, just as there are individual differences in humans in the ability to cope and the extent to which what is a stressor for one person is acceptable or even pleasurable stimulation for another, so there are individual, strain and species differences among animals in the way that they react.

4.15. One issue which it is important not to overlook is that it is often not so much the apparent "nastiness" of stimulation which is stressful to an animal as the extent to which the application of that stimulation is uncertain or unexpected. Furthermore, even a mild stimulus which signals that an unpleasant stimulus may or may not occur is capable of mobilising the adrenocortical and adrenomedullary systems.

4.16. Thus, simple "nastiness" of events or stimulus situations is no clear indication that they may be stress producing, nor is the apparent innocuousness of other stimuli necessarily a guarantee that they are acceptable. Apparently mild environmental manipulations, for example, the *ad libitum* feeding of rodents under single housing conditions, can induce severe and long lasting stress responses. By contrast, within days of bilateral inferotemporal cortical lesions in the monkey, behaviour is normal, except for a highly specific impairment in visual pattern recognition, and behaviour remains normal for the life of the animal. On cursory inspection, the former procedure might be considered mild while the latter, which involves surgery, might be assessed as substantial.

4.17. This apparent paradox arises because severity tends to be assessed largely on the basis of the nature of the stimulation or operative procedure which the animal has to endure. However, the assessment of severity could perhaps be better established if it were possible (and most often it is not) to determine the animals' response to the procedure.

4.18. Another issue which has arisen in this context is the use of "animal models" of mental disorders. There is general agreement that in no case is an animal model of psychiatric disturbance a full model of that disorder in humans nor is it necessary for this to be so.

4.19. For example, one theory about schizophrenia is that it results from a excessive activation of dopamine pathways. Certain attentional disturbances are shown in schizophrenia and if these can be modelled in animals by increasing dopamine levels artificially, then a handle may be obtained on the mechanisms involved so that the therapeutic effectiveness of drugs may be improved. There is no suggestion, or evidence, that animals with increased dopamine levels are distressed or are in any way experiencing schizophrenia. (Indeed it would be difficult to conceive how an animal could experience such a disorder involving as it does, to a large extent, speech and language).

4.20. The Committee has concluded that very much the best way forward is to employ a mixture of common sense and as much knowledge as is available in order to reach satisfactory judgements about projects involving stress, but it is possible to draw up some guidelines about potentially stressful procedures that can be practicably employed and these are reproduced as the first part of appendix II of this Report. Even these need to be carefully interpreted in cases of doubt: for instance, it might be permissible to exceed the water deprivation criterion of 23 hours if the animals in question were gerbils. Equally, however, it is hoped that Inspectors and research workers would be well aware of the stressfulness of mild signals for the uncertain onset of unpleasant events and assess such experiments carefully.

4.21. As stimulus intensity is not the only, or even necessarily the most important, criterion by which to judge the potential of a stimulus environment to induce stress, it might be useful for several additional factors to be borne in mind when considering a particular procedure since they may influence the severity of a potentially stressful environment. These form the second part of appendix II of this Report. The Committee considers that, if there is any proposal which goes beyond these broad guidelines, it should be specially justified and it will often need to be referred to the Committee.

4.22. Having concluded its review of this field of research, the Committee will, in future, be concentrating its attention on specific aspects of such research.

The confidentiality of certificate and licence holders

4.23. In the autumn, a major contract house became the subject of a press campaign. The Committee considered the press reports, reports from the Inspector and other material. It was surprised that the allegations should have been made against such an establishment, not least because of the high reputation which it enjoyed. The Committee had visited the establishment in 1988 and had been impressed by the standards of care and the conduct of the scientific procedures.

4.24. However, the incident drew attention to the difficulties of confidentiality which such an incident can raise. Applications for certificates and licences are clearly marked as being confidential at all stages and this is necessary both to protect the establishment and its staff and to protect legitimate concern for scientific and commercial confidentiality. At the same time, it creates a difficulty if it is not possible for the Home Office to provide straightforward information about the establishment in response to legitimate enquiries.

4.25. Accordingly, towards the end of the year, the Home Secretary asked the Committee to consider whether there was scope for the freer provision of information in such cases. The Committee is in the course of conducting this review of confidentiality and will be calling for evidence from individuals and organisations in 1990.

The future of the LD50 Test

4.26. The LD50 test has long been a source of public concern. The Committee's predecessor, the Advisory Committee on Animal Experiments, presented a report to the Home Secretary in 1979 and the Committee has always taken an interest in the level of lethal toxicity testing published in table 14 of the *Statistics of Scientific Procedures on Living Animals, Great Britain* .

4.27. Over the last year, as the result of a Government initiative led by the Department of Health and supported by the Home Office, the European Community has been conducting a study of the continued need for formal LD50 testing for certain (but not all) regulatory purposes. During 1989 the Commission was informed that the research study, which was coordinated in the UK, indicated that an alternative to the LD50 test which uses fixed doses and does not rely upon lethality as an end point was likely to provide an acceptable replacement measure of toxicity for those regulatory purposes where a formal LD50 value was unnecessary.

4.28. The Committee has been following these developments with interest and is anxious that their implications are taken up as quickly as possible in the United Kingdom and that the Government encourages other member states of the OECD to adopt the fixed-dose procedure instead of the LD50 test. These negotiations are currently continuing and, for example, the pharmaceutical industry has made good progress in pressing successfully for the elimination of LD50 tests in the development of pharmaceuticals in Japan. Nevertheless, a considerable amount of work needs to be done at all levels to ensure that the domestic legislation of member states is brought into line with the requirements of international agreements, in order to take full advantage of the proposed new protocols.

4.29. The Committee hopes that the progress which has been made with the LD50 test might be used as a model for the replacement of the Draize Eye test, although finding an internationally accepted alternative for this test is at an earlier stage and the scientific outcome is much less certain.

Death of 79 beagles on a ferry

4.30. In common with many people in this country, the Committee was shocked to read of an incident in which 79 of a batch of beagles being exported from England to Sweden had died *en route* in the ferry. These beagles were destined for use by a Swedish pharmaceutical firm.

4.31. This incident took place before section 7 of the 1986 Act had come into force so the establishment which exported the dogs was not, at that time, subject to regulation or inspection under the Act.

4.32. In the early part of 1990, the company and one of its directors were convicted of offences under the Transit of Animals (General) Order 1973 and the Protection of Animals Act 1911. They received heavy fines, as did the transport company for the charges which it faced. The director concerned is no longer employed by the firm.

4.33. This coincided with an application for the kennels to be designated under section 7 of the Act and the Committee asked for papers on the conditions of these kennels and the background of the incident on the ferry. Having considered these papers, the Committee recommended that stringent conditions should be imposed on the certificate which was granted to these kennels and, as a result, it was agreed that the least satisfactory building in the kennels would be closed.

5 Research to reduce, refine or replace animal procedures

5.1. During 1989, the Committee's Research Sub-Committee consisted of its Chairman, Professor John Ledingham, with Professor Anthony Dayan, Mr Clive Hollands and Sir Andrew Huxley as members. An Inspector acted as adviser and the Home Office provided the Secretariat. The Sub-Committee met five times during the year.

5.2. The Research Sub-Committee is principally concerned to support practicable shorter-term research work which appears to offer the best and most tangible return for the funds available. It will not normally seek to fund basic research or compete with major research funders. It is not deterred from identifying research in industry or institutions already funded by Government, if the requisite criteria are met.

5.3. The Sub-Committee also considers funding research into the husbandry of animals used in scientific procedures but, in doing so, it pays particular attention to whether the project has clear objectives which are readily susceptible to measurement and scientific analysis.

Correspondence with the Secretary of State

5.4. In paras 4.9–4.11 of its Annual Report last year, the Committee expressed its concern that the funds available for the Committee's research programme into the reduction, refinement and replacement of the use of living animals in scientific procedures were inadequate and it called upon the Government to make a much more substantial sum available for this purpose. During October 1988, the Chairman of the Committee and Sir Andrew Huxley, representing the Research Sub-Committee, met the then Parliamentary Under-Secretary of State, Mr Douglas Hogg MP, to discuss the paucity of funding and to ask that a way be found of increasing the provision.

5.5. Following this meeting the then Home Secretary, the Right Honourable Douglas Hurd MP, wrote to Sir Andrew Huxley saying:

> *"I agree with you that the scheme got off to a disappointing start when the funds were unexpectedly cut so early on. There is never enough money to do all that we want to do, but I am glad that Douglas Hogg, in his meeting with you in October, undertook to seek to increase the money available for this year so that the Research Sub-Committee could commission approximately the same level of new work as it did last year. He went on to hold out the possibility of further funding if this could be justified to support additional work of high merit and particular value.*
>
> *We see this as a sensible way to build up a research programme which is manageable and for which we can ensure value for money. As I am sure you will understand, it is most important for the survival of the scheme that the projects we support are worthwhile and fully justify the expenditure."*

5.6. In reply to the Home Secretary, Sir Andrew Huxley wrote as follows:

> *"I do appreciate that the sum to be made available this year is an increase on that in the previous year, but it still means that the amount of new work we will be able to commission will be only on the same scale as was possible last year after the severe cut from the amount that we thought we had been promised. As a result, we have not felt able to advertise for applications for grants this year but propose to operate by inviting a few selected persons to undertake sponsored research.*

I do indeed understand that the prospects for the scheme depend on the work we support leading to useful results. If, however, this means that no increase in funds will be made until the scheme begins to show results, any extension—even towards the level that we were originally led to expect—will be several years in the future. A research programme under a scheme of this kind normally runs for something like three years and the publication of the results usually takes a year or so more."

5.7. The Committee remains concerned that it has not yet been possible to increase formally the level of funding for its research programme. This is a matter to which the Committee attaches great importance and it is a tangible way by which the conduct of animal procedures in this country can be improved. We very much hope that, before long, the Home Office will be in a position to announce an increase in the sums of money which are available to the Committee for its research programme.

Research grants awarded during the year

5.8. One of the grants awarded by the Research Sub-Committee was for a report commissioned from Professor Robin Coombs FRS on the production of monoclonal antibodies, including the circumstances in which it was reasonable for an antibody to be produced by the ascites procedure in rats and mice rather than by an *in vitro* culture method.

5.9. In an excellent report, Professor Coombs concluded that, although rapid progress was being made in the development of *in vitro* culture of monoclonal antibodies, there were still circumstances in which small scale production involving the ascites method remained justified and, indeed, essential. Professor Coombs's report is now being considered by the Committee for its wider implications on monoclonal antibody production *in vivo* and his principal findings are reported in appendix III.

5.10. Following the recommendation of the Research Sub-Committee, the Committee has also agreed three additional research grants. One is for the development of a recombinant vaccine against infectious bursal disease virus (Gumboro disease). Infectious bursal disease virus (IBDV) is an important pathogen of chickens, causing the death or necessitating the humane killing of many millions of birds in the United Kingdom each year. It causes immunosuppression in young chicks which results in greater susceptibility to other diseases and decreased immunological response to vaccination. Live attenuated vaccines grown *in vitro* are available but there have been reports of problems with their efficacy and with bursal lesions associated with their use.

5.11. The Research Sub-Committee considered that, on cost and welfare grounds, IBDV is a good candidate for the development of a new genetically-engineered vaccine. The construction of a recombinant fowlpox virus, which carries and expresses the genes coding for IBD protective antigens offers the possibility of an effective live vaccine which would drastically reduce the need for birds in the production of the vaccine. It therefore awarded a grant to Dr M E G Boursell of the AFRC Institute for Animal Health for this purpose. This is a collaborative project funded equally between the Agricultural and Food Research Council, Pitman-Moore and the Home Office.

5.12. The Research Sub-Committee also recommended a research grant for the development and validation of an *in vitro* assay for the potency of rabies vaccines. The use of mouse protection tests for the standardisation of rabies vaccine has ensured the production of safe and efficacious vaccines for many years, but the test gives highly variable results, is time consuming and requires the use of animals and infectious virus. Although mice exhibiting signs of infection by rabies virus are generally killed without reaching the stage of dying from the disease, they inevitably have to suffer to provide incontrovertible evidence of rabies. The new method, once proven, should be considered as a replacement for the in-house pharmacopoeial assays of protective potency.

5.13. The project will validate use of single radial immunodiffusion assays for the standardisation of rabies vaccines as an alternative to the currently recommended mouse protection test. Additionally, the project is aimed at reducing the numbers of mice involved in the currently accepted mouse protection test via the introduction of an *in vitro* complement fixation test. The grant for this purpose has been made to Dr M Ferguson at the National Institute for Biological Standards and Control.

5.14. The Sub-Committee has further recommended a research grant for the replacement for the Clostridium chauvoei vaccine potency test (blackleg). The standard potency test for blackleg vaccines must be performed on every batch of vaccine and this involves the inoculation of ten guinea-pigs with the vaccine and later challenging them, together with five unvaccinated controls, with a virulent culture of Clostridium chauvoei. The level of protection is assessed on the basis of survival of the vaccinated guinea-pigs and the death of unvaccinated controls.

5.15. The object of the project is to develop and validate a serological test that would specifically assay levels of antibodies that confer protection against Clostridium chauvoei. The resulting test would be carried out on sera from rabbits inoculated with multicomponent clostridial vaccines in routine potency tests in order to render the present challenge test unnecessary. A grant has been made to Mr P A Knight of Wellcome Research Laboratories for this purpose.

5.16. The Committee has also agreed a grant for the evaluation of welfare in the husbandry of laboratory rabbits. Professor David Morton, of the University of Birmingham, has expressed the view that the behavioural repertoire of rabbits and their welfare may be greatly increased by housing them in groups, in sawdust-covered floor pens. The *Code of Practice* recommends that rabbits should be group-housed wherever possible, but satisfactory standard methods for the routine group-housing of rabbits of various strains, ages and sexes have yet to be developed and the implications for the welfare of rabbits maintained in different kinds of group-housing and caged conditions have yet to be evaluated.

5.17. This project involves the use of a range of physiological and behavioural measures to evaluate various common husbandry and scientific practices in the use of rabbits. The study will look at aspects of group-housing and individual caging; strain differences; social interactions; acclimatisation to environmental parameters such as temperature; and the incidence of fighting and injury. The effect of common methods of handling and restraint and of simple procedures, such as injections, is also to be assessed.

Progress of research

5.18. During the course of the year, the Research Sub-Committee received reports on the progress of the research which it has grant-aided in this and former years. The Committee will consider including short reports of this work in its annual reports when projects are completed.

6 Forward look

6.1. In each of its two previous Annual Reports, the Committee has commented on the progress which has been made during the three-year transitional period over which the Act has been implemented.

6.2. 1989 was the final year of transition and United Kingdom legislation now fully conforms with the Council of Europe Convention for the *Protection of Vertebrate Animals used for Experimental and other Scientific Purposes* and the European Community Directive 86/609/EEC of 24 November 1986 on the *Approximation of Laws, Regulations and Administrative Provisions of the Member States regarding the Protection of Animals used for Experimental and other Scientific Purposes.*

6.3. As required by section 21(3), the Committee has also played a full part in the consultations leading to the publication of the *Code of Practice for the Housing and Care of Animals Used in Scientific Procedures* and the *Guidance on the Operation of the Animals (Scientific Procedures) Act 1986.*

6.4. During 1990, the Committee hopes to present advice to the Home Secretary on the safety testing of cosmetics; and to make recommendations on whether there is scope for relaxing the confidentiality of information relating to establishments or licence holders.

6.5. The Committee is also looking forward to further progress in the international negotiations to bring about a significant reduction in LD50 testing.

Appendix I

MPTP WORK IN PRIMATES:
BACKGROUND NOTE BY THE INSPECTORATE ON CURRENT RESEARCH

Summary

MPTP-induced Parkinsonism in the monkey is the best available model of idiopathic Parkinson's disease in man. It reproduces all the motor abnormalities of the human condition (akinesia, bradykinesia, rigidity and tremor), together with the pathological degeneration of the *pars compacta* of the *substantia nigra*, and the side effects of antiparkinsonian treatments. Idiopathic Parkinson's disease worsens progressively at rates which vary with the individual. This is the principal difference to MPTP-induced Parkinsonism.

MPTP-induced Parkinsonism has acute, chronic and compensatory phases with variable recovery. A stable residual motor deficit usually remains. There has been no formal comparison of recovery between different species of monkey.

Research with MPTP has been been conducted mostly with cynomolgus and rhesus monkeys, but also with marmosets and squirrel monkeys and infrequently with baboons, capuchin and vervet monkeys.

All species of primate begin to develop Parkinsonian signs within a few days of MPTP injection. The disorder gradually increases in severity for several weeks. The degree of motor impairment is highly variable, even within a species.

The MPTP model in the monkey may be useful to

(i) study neural mechanisms which mediate symptoms and signs of Parkinson's disease;

(ii) study long term complications associated with the treatment of Parkinson's disease;

(iii) evaluate new treatments for Parkinson's disease using novel pharmaceutical products or cell implants (foetal neurones or adrenal chromaffin cells).

Background

MPTP has a long and surprising history. It was synthesised in 1947 as part of a series of piperidine-related analgesics and underwent drug trials in the early 1950's. Rats failed to develop clinical signs when given MPTP. Two monkeys given 0.5 mg/kg developed hind limb stiffness at 24-48 hours, became rigid and immobile by about 68 hours and died after 12 and 24 days.

This was almost certainly the first example of a complete animal model for Parkinson's disease, but its significance was not appreciated. As part of the same investigation, in screening for anti-Parkinsonian agents, MPTP was given to six humans at 50-300 mg daily for three weeks. Two patients died during or shortly after the fifth day and the chemical was abandoned because of its toxicity.

Two probable cases of MPTP poisoning were reported in 1964 and 1976 in chemists synthesising products for which MPTP was an intermediate step. In 1982, seven young adults using "synthetic heroin" which contained MPTP developed Parkinsonian symptoms.

Idiopathic Parkinson's disease

James Parkinson described *paralysis agitans* in 1817, later calling it Parkinson's disease. Clinical signs include bradykinesia and rigidity with, more variably, a resting tremor. Other signs may include flat facial expression, flexed posture, loss of postural reflexes, shuffling gait, loss of digital dexterity, oily skin and dysphagia.

Parkinson's disease is progressive, at a rate which varies from one individual to another. There is extensive loss of the dopaminergic neurones of the *pars compacta* of the *substantia nigra*, with smaller variable deficits in other areas like the ventral tegmental area and *locus coeruleus*. Destruction of at least 70 per cent of the nigrostriatal dopamine system must have taken place for symptoms to appear.

Motor side effects (like dyskinesia and dystonia) often develop after long term (5 years or so) L-DOPA treatment in about 60-80 per cent of patients and may become progressively more severe. One of the most common is "peak dose" dyskinesia, the nature of which may manifest variably as choreic, athetoid, ballistic, dystonic or myoclonic.

MPTP in humans

There are several well-documented cases and each resembles time-telescoped Parkinson's disease. Principal signs are hypokinesia, bradykinesia and rigidity. There is variably resting or postural tremor. Neurones in the *pars compacta* of the *substantia nigra* are extensively lost, with smaller variable effects in other systems (dopaminergic, serotonergic and noradrenergic neurones).

Patients respond well initially to antiparkinsonian therapy with L-DOPA or bromocryptine. They later develop typical long-term complications, notably end of dose deterioration, peak dose dyskinesias and "on-off" phenomena.

MPTP in monkeys

The literature dating back to the early 1980's contains varying interpretations of the effects of MPTP, even between publications by the same authors using the same species and protocol. The following summary places relatively more weight on recent papers.

(i) MPTP-induced Parkinsonism has, broadly, three phases: acute, chronic and compensatory.

(ii) Species, age, sex, dosage, duration of treatment and other factors may be critical in determining the effects of MPTP. It is more toxic in old and female animals.

(iii) Even strictly identical MPTP administration schedules in individuals of the same species lead to differences in behavioural deficits and in the specificity of damage to various neuronal systems.

(iv) Acutely, MPTP causes within 24 hours a reduction in the concentration in the cerebrospinal fluid of metabolites of dopamine, 5HT and noradrenaline. Levels of 5HT and noradrenaline, but not dopamine metabolites, recover within three months although the exact time course is not known.

(v) The gradual onset of Parkinsonian motor deficits reflects the effects of neuronal loss. MPTP causes the selective destruction of nigrostriatal dopaminergic neurones and hypokinesia: such losses for the mildly and severely affected animal are 80 and 95 per cent respectively. Dopaminergic ventral tegmental neurones and noradrenergic neurones in the *locus coeruleus* are also lost, though in smaller numbers.

(vi) Other extrastriatal dopaminergic (e.g. midbrain, n. accumbens, frontal cortex, hypothalamus), noradrenergic (e.g. cerebral cortex, cerebellar cortex) and serotonergic (e.g. caudate, frontal cortex, spinal cord) neurones may also be damaged, and there may be changes in other neurotransmitter systems (e.g. opiate systems).

(vii) The relative lack of specificity of damage to neuronal systems, and the variable expression of rigidity and tremor, parallels the variable involvement of monoamine systems (other than nigrostriatal dopamine) in idiopathic Parkinsonism.

(viii) There is a variable degree of recovery from the effects of MPTP, even within a species and the underlying mechanisms are unclear. No systematic study comparing recovery between species has been carried out. Residual Parkinsonian features may remain essentially stable for long periods.

(ix) Motor symptoms respond readily to anti-parkinsonian drugs (e.g. L-DOPA/carbidopa or bromocryptine). Prolonged treatment with L-DOPA leads to the progressive development of choreoathetoid movements after 4-8 weeks in MPTP-lesioned (but not in normal) animals. This may be the simian equivalent of "peak-dose" dyskinesia.

(x) Individual monkeys may require tube-feeding, or anti-parkinsonian treatment, during the development of the motor deficits.

MPTP in Cynomolgus (M. fascicularis)

Dose schedules typically use 0.35–0.60 mg/kg MPTP hydrochloride intravenously once a day for four days. The course may be repeated. Alternatively, single injections may be given at 7–14 day intervals for 6–28 weeks. Cumulative doses are about 2.5–27.0 mg/kg. Three recent reports describe a unilateral Parkinsonian model developing after unilateral intracarotid MPTP injection.

Minimal overt behavioural changes, other than pupillary dilation, occur after the first and second injection. Subsequent injections may elicit agitation, laboured breathing, vocalisation and apparent hallucination (for instance, reaching for non-existent objects) for up to ten minutes.

Five to six days after the first injection, variable bradykinesia, akinesia, stooped or flexed posture, rigidity, tremor and amimia (lack of facial gesturing) develop. The maximum effects are seen about 1–2 weeks after the last dose of MPTP.

A variable degree of recovery may be observed in some, but not all, animals from 4–6 weeks after MPTP administration. The residual Parkinsonian deficit may remain stable, with no further change over 6–12 months.

MPTP in Rhesus (M. mulatta)

Rhesus monkeys exposed to MPTP may not be useful as a model of Parkinson's disease until 6–12 weeks after drug administration. The effects of MPTP in humans and rhesus monkeys are broadly similar with regard to tremor, rigidity, fixed posture, eyelid closing and drooling, although in rhesus, tremor is mostly postural. Vocalisation is decreased in rhesus and in man.

Acute effects include mydriasis, tachycardia, piloerection, and tachypnoea within two minutes of MPTP administration (agonist effects) and last for about 15 minutes (0.33–1.33 mg/kg intravenously). Repeated doses of MPTP (0.33 mg/kg daily for 3–4 days) produce abnormal movements of the facial muscles (tremor, chorea, myoclonus), grimacing, retrocolis and flexor muscle spasm. These effects are more intense with each succeeding dose and last initially for 15–30 minutes.

Chronic effects include variable hypokinesia, bradykinesia, loss of dexterity, rigidity, tremor (postural or resting), flexed posture, dysphagia, decreased vocalisation, amimia and "freezing" episodes. Severity increases over the first 10–14 days, with a moderate improvement over 2–6 weeks, after which the clinical state may be stable for up to 2 years.

MPTP in squirrel monkey (Saimiri sciureus)

The most effective schedule is a "1 day, 4 dose" schedule in which four doses of 2 mg/kg MPTP hydrochloride intraperitoneally are given at 2 hour intervals. Single doses of 4 mg/kg may be fatal within 24 hours.

The acute behavioural syndrome consists of increasing degrees of bradykinesia, salivation and "nodding off". Occasionally fasciculations of the thigh muscles occur. After the second or third dose the eyes are tightly closed, interrupted at 5–10 second intervals by abrupt opening of the eyes with shaking and extension of the limbs. Intermittent head extension and rotation may occur. These reactions continue for 30–60 minutes. During the next 24–48 hours the animals become profoundly bradykinetic and may show waxy rigidity. The animals often do not eat and require hand feeding.

The chronic behavioural syndrome consists principally of bradykinesia, at times approaching akinesia, and incoordination. Postural tremor seen in about one quarter of subjects.

MPTP in marmoset

Fixed dose schedules (1–4 mg/kg intraperitoneally, 4 days) produce variable results (from slight effects to death) and a regime using a cumulative dose of 7–10 mg/kg intraperitoneally given over 3–5 days is to be preferred.

Acute effects, seen with the second and succeeding doses, include loss of balance, rocking, head drooping, prostration, vacant gaze, limb extension and neck torsion. These last for 30–60 minutes. Subacute effects, seen after 2 or 3 doses, include akinesia, dyskinesia, limb rigidity, postural abnormality, loss of vocalisation, loss of blink reflex and postural tremor. These last for about 10 days, though there is some recovery over the next 3–5 weeks.

In the late stage, after 4–6 weeks, there is a residual stable motor deficit. Movements are clumsy and poorly coordinated. These effects persist for at least 4 months.

MPTP in other species

MPTP has been investigated in a number of other species, including the dog, cat, guinea-pig, rat, mouse, frog and leech but cats, frogs or leeches are not regarded as useful models.

Beagle dogs exhibit the same extensive and specific loss of nigral neurones at similar dose levels to monkeys but a florid Parkinsonian syndrome does not develop. In rodents, MPTP is converted mostly to metabolites other than MPP and in these species MPP is rapidly degraded enzymically.

In the rat, the LD50 of MPTP is about 20 mg/kg. 5–10 mg/kg daily for 16 days causes a temporary decrease in dopamine in the *substantia nigra* with only limited loss of nigral neurones chronically but there is no permanent locomotor effect. Behavioural effects resemble those of acute treatment with 5HT (acute immobility, retropulsion, Straub tail, piloerection, salivation, clonic forepaw movement).

In mice, the effects are strain dependent: the C57 Black is one affected strain. At high doses, small losses of nigral neurones may occur and there may be prolonged decreases in levels of striatal dopamine and noradrenaline. Acutely, locomotion may decrease and a high frequency tremor may occur for up to 30 minutes. There is no long-term motor deficit.

In the guinea-pig, MPTP causes acute immobility, ptosis, rigidity, piloerection, head jerk, flexed posture, ataxia and convulsions within 5–10 minutes. These signs last for up to 4–6 hours but there is no residual change to motor function, even at cumulative doses greater than 100 mg/kg.

Mechanism of MPTP neurotoxicity

MPTP is not itself neurotoxic, but a metabolite MPP is. MPTP is metabolised by MAO(B) in astrocytes and serotonergic (but not aminergic) neurones to MPDP. MPDP rapidly auto-oxidises to MPP. MPP is transported into dopaminergic neurones by the catecholamine uptake system. Once inside the neurone, MPP enters mitochondria via another active uptake mechanism, where it inhibits NADH-linked respiration and disrupts aerobic glycolysis.

The pattern of neuronal toxicity is time dependent: dopaminergic axons are initially affected by MPP, followed after prolonged exposure by cell bodies.

MPTP is cleared in 1–2 hours in both monkey and mouse but, whereas MPP is cleared in mice in 2 hours, in the monkey MPP has a half life about 10 days and is not cleared for at least 20 days.

There is a spectrum of toxicity within aminergic neurones, the descending rank order being *substantia nigra*, ventral tegmental area, *locus coeruleus*. Furthermore, there is differential toxicity within dopaminergic neurones, with neurones in the substantia nigra being most vulnerable. The reasons for such effects are unknown, but might involve a differential affinity of MPTP/MPP for carrier mechanisms. MPTP is selective but not specific for dopaminergic nigral neurones.

Glossary

MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
MPP	1-methyl-4-phenylpiperidine
MPDP	1-methyl-4-phenyl-2,3-dihydropyridine
L-DOPA	L-dihydroxyphenylalanine
akinesia	slowness of movement and underactivity; poverty of movement; immobility
bradykinesia	slowness of movement
hypokinesia	abnormally decreased muscular movement
MAO(B)	monoamine oxidase B

Appendix II

CONSIDERATIONS WHICH SHOULD BE TAKEN INTO ACCOUNT WHEN CONSIDERING RESEARCH INVOLVING PSYCHOLOGICAL STRESS

Part I:

Examples of potentially stressful procedures which require careful justification

Food Restriction: where the objective is to motivate the animal and the restriction is likely to result in a weight loss exceeding 20 per cent of the normal total body weight of similar animals fed *ad libitum*.

Water Restriction: where the objective is to motivate the animal and the period of water restriction is to exceed 23 hours and is to be repeated.

Footshock: where the use of direct rather than alternating current is proposed or where the individual stimulus profile exceeds 1.5 mA intensity, or one second in duration.

Chemical or surgical lesioning of brain: where the animal is required to survive with aphagia, adipsia or paralysis sufficient to prevent ambulation or sensory loss liable to result in autotomy.

Kindling experiments: where death in *status epilepticus* is the endpoint.

Self administration of addictive drugs: where animals are to be submitted to the addictive process on more than one occasion and alleviation of withdrawal symptoms is not possible.

Aggression or predation: where animals are to be exposed to more than one aggressive encounter and/or significant injury is likely.

Central or peripheral blinding: any proposal involving bilateral blinding.

Forced exercise (swimming, treadmill or equivalent): where exhaustion, however defined, is the endpoint.

Isolation/Social deprivation: any proposal to isolate an animal from all contact with others of its own or a compatible species with or without actual sensory deprivation.

General: any combination or repetition of aversive stimuli or tests likely to result in severe or prolonged stress evidenced by persistently abnormal behaviour patterns (agitation, decreased responsiveness, stereotyped repetitive movements) or by physical deterioration (progressive weight loss, gastrointestinal ulceration).

Part II:

Other factors which should be taken into account when considering any potentially stressful procedure

Stimulus qualities: intensity; method of application; and duration of individual stimuli.

Predictability of "stressful" event.

Time over which the stimulus environment is to be manipulated (days/weeks, months).

Species-specific factors: physiological differences; sensory differences; psychological predisposition.

Overt response of the organism to stimulation.

Methods available for evaluating chronic stress response of the animal: weight; general activity (UFAW report on Disturbance Index*); responsiveness to the environment; and the duration of any of these effects.

Housing conditions of animals during "stress" conditions.

Relationship between animal model and human condition encompassing both normal and abnormal psychological processes.

*Barclay, R J, Herbert W J, and Poole T B (1988) *The Disturbance Index: a behavioural method of assessing the severity of common laboratory procedures on rodents.* UFAW.

Copies can be obtained from the Universities Federation for Animal Welfare, 8 Hamilton Close, South Mimms, Potters Bar, Hertfordshire EN6 3QD.

Appendix III

THE PRODUCTION OF MONOCLONAL ANTIBODIES BY *IN VIVO* OR *IN VITRO* METHODS

From a report prepared for the Committee by Professor Robin Coombs FRS

Background

Monoclonal antibodies are antibodies of selected single specificities which are continuously secreted by "immortalised" hybridoma cells. A hybridoma is a biologically constructed hybrid between an antibody-producing mortal lymphoid cell and a malignant myeloma "immortal" immunoglobulin-secreting cell.

The discovery and development of these monoclonal antibodies within the last two decades have had profound implications for medical research, diagnosis and therapy as well as for the whole of biological research. It is now theoretically possible to obtain an unlimited supply of antibodies with single selected specificities.

At the propagation stage in the process of producing monoclonal antibodies, there are two theoretically alternative procedures. In one of these, antibody-secreting hybridoma cells are grown up or propagated in the peritoneal cavity of mice or rats in the form of an ascites tumour which gives high levels of secreted antibody in the ascitic fluid. In the other procedure, the antibody-secreting hybridoma cells are propagated in *in vitro* culture, with the monoclonal antibodies being secreted into the supernatant culture fluid, albeit in low concentration.

The *in vivo* ascites procedure is a "regulated procedure" using animals and controlled by the Animals (Scientific Procedures) Act 1986, because it may have the effect of causing pain, suffering, distress or lasting harm. If the animals are not carefully supervised and sacrificed at the appropriate time, the ascites may continue to enlarge and cause distress to the animals. In a small number of cases, when a solid tumour rather than ascites develops, there is an increased potential for adverse effects.

In the culture-procedure, the propagation of the hybridoma cells is not carried out in laboratory animals but *in vitro,* although at times "animal feeder cells" have to be added to the cultures. This latter procedure is preferable to the ascites procedure unless there are special situations which require the *in vivo* procedure.

Stages in the production of monoclonal antibodies

There are essentially two stages in the production of monoclonal antibodies: the formation and selection of the hybridoma clone and the propagation of the hybridoma clone to raise the required amount of antibody.

The first stage, the formation and selection of the hybridoma clone, is always the same and always involves the use of an animal. It is carried out in the following way:

(i) antigen is injected into mice (or rats).

(ii) after an appropriate interval (5–21 days) the mice are sacrificed and lymphoid cells (progenitor antibody-producing cells) obtained from the spleens.

(iii) these lymphoid cells are mixed *in vitro* in special media and under special conditions, in order to hybridise with culture-grown myeloma cells having special properties.

(iv) the two original cell types and the newly formed hybrids are cultured in special selective medium, inhibitory to the original myeloma cells, unsupportive of unhybridised lymphocytes, but favouring the growth of hybrids.

(v) the supernatant media from the numerous micro-cultures with recognised growth of hybridomas are screened by various immuno-assay procedures for immunoglobulin formation and for specificity of secreted antibody. The cells are then further sub-cultured.

(vi) special cloning procedures are now undertaken to ensure that hybridomas with a single antibody-specificity only are being grown in each culture.

(vii) hybridoma cells may be cryo-preserved at this stage or may be propagated (i.e. grown-up) to raise the selected monoclonal antibody in the amounts required.

The propagation of the cloned hybridoma cells may be accomplished in one of two ways, either growing up *in vivo* in the form of an ascites tumour, or continuing *in vitro* in large scale cell culture.

The *in vivo* method

The *in vivo* procedures entail having mice or rats whose peritoneal cavity is first prepared for the subsequent injection of the hybridoma cells by administering intra-peritoneally 1–2 weeks previously a substance called pristane (2,6,10,14–tetramethylpentadecane) which aids the development of an ascites. As pristane is a mild irritant, it is essential that as low a dose as is necessary is used. Subsequently, following intra-peritoneal injection of hybridoma cells, the hybridoma tumour cells multiply in the peritoneal cavity and the ascitic fluid which forms is a very rich source of the secreted hybridoma antibody.

When an adequate ascites has formed the animal is sacrificed and the ascitic fluid collected. Sometimes the ascitic fluid is "tapped" or drained off the peritoneal cavity under anaesthesia with a second and final harvest being sometimes taken once the ascites has reformed. Approximately 5 ml is obtained from a mouse and 10–40 ml from a rat. Each specific antibody requires one or more mice depending on the amount of monoclonal antibody required. The antibody level ranges between 5–20 mg/ml, which is an extremely high yield.

The *in vitro* method

The *in vitro* method is performed in a laboratory specially adapted in terms of rooms, equipment and staff for carrying out cell culture aseptically. In most cases 1–10 per cent foetal calf serum (FCS) has to be added to the culture medium for satisfactory growth of the cells. In terms of yield, 1000 ml of culture supernatant is required to give the equivalent amount of antibody obtained from 1 ml ascites. Before purification a preliminary step of concentrating the antibody level is required although for certain uses (eg some immuno-assay reagents) this may not be necessary. Concentration from large volumes has, up till now, raised many problems, but affinity-columns, where immunoglobulin is absorbed and subsequently desorbed, have greatly eased this problem and ultrafiltration can be used where protein-free media are being introduced, although this is costly.

So-called "hollow fibre culture systems", an offshoot of kidney dialysis technology, are now being introduced as an alternative to the usual static and suspension cultures in large vessels. In this apparatus, fresh culture medium is continuously circulated in hollow fibres or tubes separated by their semi-permeable membranes from the hybridoma cells and their antibody-secretion in another compartment and cells and antibody can be "drawn" from this compartment at intervals. However, hollow fibre systems are extremely expensive and require dedicated human monitoring. Finally, very large quantities can be grown up in large fermenters. These large-scale production facilities with refined downstream processing are only possible in the commercial sector.

The arguments for and against each of the two alternative procedures of propagation.

The ascites method is an animal procedure and at times may also be experimental. Should there be a perfectly satisfactory alternative, which does not involve the use of animals then clearly the alternative should be preferred. The *in vitro* culture method is considered by some to be a totally viable alternative and will undoubtedly become the ideal for the future, obviating the use of laboratory animals in the propagation stage. But difficulties still remain.

There are four principal scientific arguments in favour of the use of the *in vitro* procedures:

(i) For therapeutic use of monoclonal antibodies in man (eg *in vivo* targeting or immuno-therapy) in this and many other countries (but not in the USA) culture-grown antibody is specially recommended for fear of infection with animal-borne viruses or genomic material.

(ii) Recent and future developments with gene manipulation or so-called "reshaping" and "humanising" mouse monoclonal antibodies will, of necessity, have to be propagated in culture and this is indicative of future trends. Human monoclonal antibodies have to be grown up in culture.

(iii) Commercial organisations increasingly use culture-procedures (especially large fermenters). They are, however, often producing bulk reagents for immuno-assays and are probably confined to antibodies that grow well in culture. These organisations have specially-equipped laboratories with specially-trained staff. There is an additional possibility that they are also "investing in the future" against the time when the ascites procedure will not be permitted where possible and practicable.

(iv) The culture procedure removes the potential danger to staff of contracting dangerous zoonoses from laboratory animals such as Hantaan disease of rats.

However, although great progress is being made in large scale *in vitro* culture-procedures (static and suspension cultures, so-called cartridge and fermenter cultures), volumes have to be large, as the antibody content is approximately one thousand times less than that of the ascites method. This creates a real logistical problem if the laboratory is not specially orientated or equipped for this. Nevertheless some centres have claimed satisfaction with only small/moderate scale *in vitro* culture production.

The arguments in favour of the ascites method may be set out as follows:

(i) Ascites, with its high antibody concentration (5-20 mg/ml) and 3-5 ml per mouse, or more if the peritoneal cavity is tapped twice, and 20 ml or so from a rat, is a very plentiful source of monoclonal antibody and is also very convenient for further purification. No concentration step is required before purification. The sheer volume of the usual cell cultures may often be a real problem and it is generally agreed that in so-called "downstream processing" the advantages are clearly with the ascites method.

(ii) The immunoglobulin in the semi-purified ascites supernatant is more likely to be in its native or undernatured state than the antibody-immunoglobulin propagated in culture and subsequently concentrated and purified. Cross linking monoclonal antibodies with chromic chloride is often more satisfactory with ascites-grown antibody than with culture-grown antibody. Some consider that denaturation of the culture-grown antibody is, in part, due to release of proteolytic enzymes from dying cells and also similar enzymes in serum additives.

(iii) Many researchers consider the ascites procedure to be an essential preliminary step in the early investigation of a new battery of monoclonal antibodies. This is especially so if, over and above the simple question of specificity, the full range of their biological properties have also to be tested at this stage. Such testing may require the rapid production of an adequate quantity of the antibodies. A time span of 10 days with the ascites procedure could be equivalent to 1–2 months with the culture procedure.

(iv) It is claimed that some hybridomas will not grow satisfactorily in culture and may be maintained satisfactorily only in ascites. By contrast, other hybridomas have been claimed to grow better in culture: for instance, it is better to grow some anti-hormone hybridomas (e.g. anti-steroid) in culture than in ascites since, in the latter case, the binding site of the monoclonal antibody may be blocked by the host mouse's own steroids.

(v) Preparations of culture-grown monoclonal antibodies, both before and after purification, frequently have a significant pyrogen-content and this may on occasion preclude their therapeutic use. Pyrogens are seldom found in ascites-grown antibody.

(vi) Cultures still generally require the addition of foetal calf serum (FCS from 1–10 per cent) for sustained growth and FCS is very expensive. Special proteine-free media are being developed, but these again are expensive and protected by patents. Hybridomas grown in the presence of FCS cannot be "shipped" to the USA without elaborate and costly screening tests for Foot and Mouth disease and possibly other viruses.

(vii) Contamination of cultures (in large volumes) with either microbial organisms (especially bacteria, mycoplasma and fungi) or with other hybridoma clones (many being grown simultaneously in the laboratory) is always an anxiety, although this risk is much less in the highly sophisticated, elaborately equipped, commercial laboratory. However, contamination is a significant problem for university research departments where facilities, including space and availability of technical staff, are at a premium and loss of sterility of clones is a constant threat and hazard. These risks of contamination are much less with propagation in ascites.

(viii) With culture-grown antibody, there is an extra major step in the purification of the antibody-product, namely, that of concentration. However there have recently been considerable advances with ultrafiltration for use with protein-free media and Protein-A and affinity-columns.

(ix) Ascites, clarified by centrifugation, but unpurified, is a specially favourable state in which to exchange specimens of particular monoclonal antibodies between research groups in different laboratories. Were only culture-supernatants available, in most cases these would have to be concentrated and purified and researchers may well be less willing to respond to requests for samples. This would actively discourage the exchange of research material which is so important in scientific collaboration. Furthermore supernatant material from cultures with added FCS cannot be exported (as already mentioned) to the USA, so for this purpose the material has to be ascites-grown.

(x) It is said that "genetic drift" (mutation and overgrowth) is more likely to occur in cultures than in ascites. The drift is shown by iso-electric focusing of the antibody immunoglobulin.

It can be concluded from this that, in many cases, the *in vitro* method is a satisfactory alternative to the ascites method but, in other cases, this method is not, as yet, perfectly satisfactory and does not at present offer a totally viable alternative. Indeed, in certain situations the ascites procedure has much to recommend it.

However considerable advances are presently being made in culturing hybridomas and one can anticipate, in the not too distant future, that when the limitations associated with culture are overcome, the *in vivo* ascites procedure will to a large extent be replaced by the production of monoclonal antibodies *in vitro* .

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