

The Use of Non-Human Primates in Biological and Medical Research:

Evidence Submitted by FRAME

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A Background to the Study



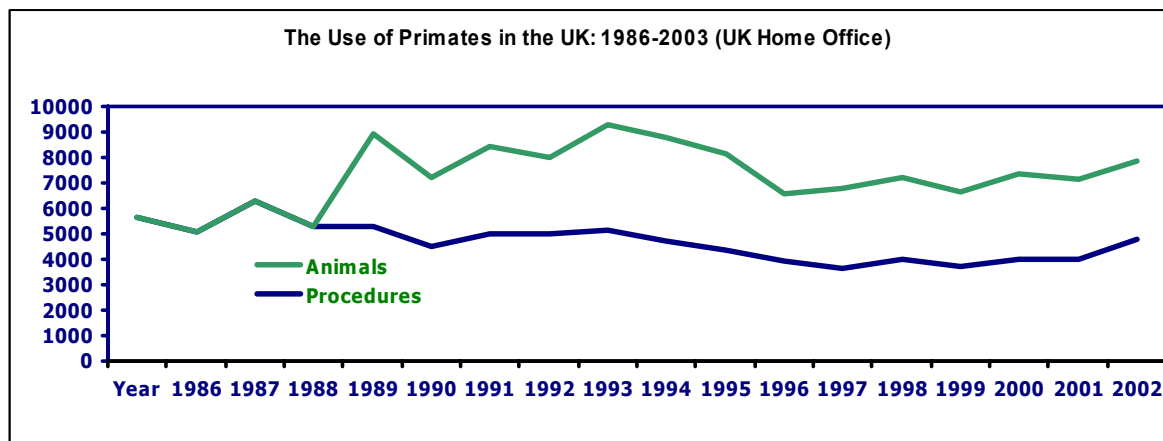
The Academy of Medical Sciences, the Medical Research Council, the Royal Society and the Wellcome Trust have recently joined forces to conduct a joint study 'to examine the scientific basis for recent, current and future use of non-human primates within biological and medical research'. An independent working group of scientific experts led by Sir David Weatherall FRS FMedSci aims to produce a report suitable for consideration by interested parties such as regulatory bodies, the legislative, scientists, animal welfare organisations and other interested parties early in 2006.

The study will examine the trends in primate research, the nature and effect

of recent and proposed changes in the worldwide use of non-human primate in research and how alternatives to non-human primates are being and could be used in the foreseeable future. The associated ethical, welfare and regulatory issues and the role and impact of the Three Rs principle of refinement, reduction and replacement will also be reviewed. As part of this study, a call for evidence was made where interested parties were invited to submit evidence for the study.

FRAME recently submitted the response summarised below.

The Main Uses of Primates in Research



Recent literature-based surveys suggest that some 100,000-200,000 non-human primates (NHPs) are used in various fields of research worldwide each year.^{1,2} Approximately 27% of the research involves studying NHPs in their natural environments for conservation and ecological reasons.¹ Most NHP research appears to be conducted in Japan, the US and the EU. It seems that around 65% of the NHPs used are old world species and a further 15% are new world species.¹ Around 10% of published research with NHPs involves the use of great apes.¹ In 2001, 50% and 28% of the articles published in 2001 which described the use of great apes originated from US and European research institutions, respectively. Furthermore, while a higher proportion of research with great apes than research with other NHPs involves conservation and ecological studies, great apes are still used for in laboratory-based research, most notably within the US.¹ In 2002, no great apes were used for laboratory-based research within the 15 Member States of the EU.³

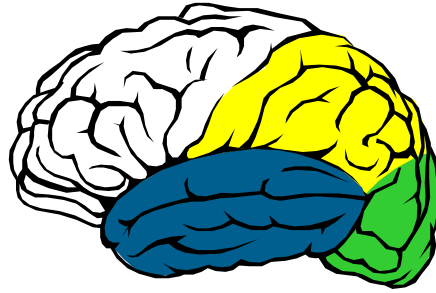
Worldwide, by far the most common research areas for which NHPs are used are microbiology and virology (in particular in AIDS and hepatitis research), the neurosciences, biochemistry or chemistry and pharmacology/physiology.¹ Primate re-use occurs most often within pharmaceutical research, immunology and toxicological and related fields.¹ Within the EU, NHPs are used

predominantly for applied research, namely, research and development, quality control and toxicity, and safety testing of medicines and medical devices, with only around 15% of all NHPs being used for fundamental studies and 7% for non-toxicological studies.³

The use of NHPs, especially macaques, in toxicological research is likely to be substantially under-represented in literature surveys, since it is often conducted to satisfy regulatory requirements and involves the production of confidential data. It is also possible that the choice of species for regulatory testing is not guided by scientific evidence, but by tradition and an implicit belief that regulators will not accept data from other species such as the ferret, dog or minipig. Admittedly, dogs, for instance, are not always appropriate, as is the case in non-steroidal anti-inflammatory drug studies, where inherently different renal excretion rates account for the finding that humans and NHPs are able to tolerate higher doses of these chemicals.

Numerous arguments, based on both ethical and scientific grounds, have been advanced in opposition to the use of NHPs in research and testing. Here, we address some of the current uses of NHPs in neurobiological and genetics research and examine the future use of NHPs within the realms of vaccine development.

The Scientific Basis for Species Selection in Neurological Research



NHPs have historically been used as models of human physiology and diseases because they are closely related phylogenetically and display many of the anatomical, physiological and behavioural features found in humans. Those who oppose primate-based research argue that it is precisely this evolutionary link that makes the use of NHPs in research objectionable, since the similarity of these animals to humans implies that they can suffer in the same ways that humans do. Hence, there have been calls for moving toward an end to experimentation on NHPs.⁴

The evolutionary links between humans and NHPs are being assessed by using the information being derived from several genomics projects. Thus far, the genomes of the great apes have been compared with that of humans, and humans have been shown to share closest genetic conservation with *Pan* species (chimpanzees and bonobos). Genetically, humans and chimpanzees are around 98.4% identical, and genetic identity is as high as 99.1% when only protein-encoding DNA is compared. Admittedly, this does indicate that chimpanzees and humans are extremely closely related. Yet the 1% or so difference between their genetic make-ups has led to dramatically different physical and physiological features. Studies suggest that the DNA sequence differences, while small, alter the sequence of protein-encoding genes and patterns of gene regulation, and cause differences in DNA structure, such that the patterns of DNA expression within specific tissues can be dramatically different in chimpanzees and humans.⁵ Furthermore, DNA re-arrangements, insertions and

deletions can substantially alter epigenetic regulation of genes such that dramatic differences in spatial expression of key functional and structural proteins are inevitable.

These findings are of particular significance to neurobiology. A recent study identified differences in the expression levels of 169 proteins from a broad spectrum of functional classes within the cerebral cortex of chimpanzees and humans. When compared to chimpanzees, humans appear to express higher levels of many brain-specific proteins; these differences in expression are not as distinct in other organs such as the heart and liver. Such variations could form the basis for the enhanced neural activity and networking, and cerebral physiology and function, seen in humans in contrast to chimpanzees.⁶ Consistent with these observations, another study has since shown that the expression of genes involved in aerobic respiration and neuronal function is higher in humans than in NHPs, including (to a limited extent) chimpanzees.⁷ There is also evidence that, while humans show distinct left-brain-sidedness with respect to the distribution of radial cells associated with speech, chimpanzees do not.⁸

These and other studies indicate that the differences between humans and NHPs are substantially greater than was originally thought, to the extent that using NHPs for fundamental behavioural, learning, association, memory and other cognitive function studies provides information which cannot be readily extrapolated to humans.

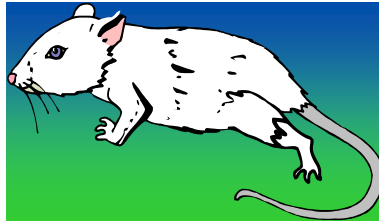
For example, implanting electrodes to monitor activity in brain regions in NHPs in response to specific stimuli might not mean that the resultant brain activity will correspond to the activity brought about by the same stimulus in humans. It should also be remembered that many of these neurological studies involve frequent handling, severe restraint and/or or fitting devices that are likely to cause NHPs considerable distress, suffering or pain. Consequently, the relevance that the results might have for human health can be substantially decreased, so such studies become unjustified when a benefit–suffering evaluation is conducted. Although their motor control is similar to that of humans, similar welfare problems arise when NHPs are used to study the nervous dysfunction that occurs in disorders such as Huntington’s chorea and other forms of dyskinesia.

The use of NHPs for visual research is based on the common features of forward facing eyes and, in some species, chromatic vision. However, there are important differences between function and neuronal organisation within the visual centres of the brains of humans and old world primates, which suggest that the use of higher primates in visual research is not scientifically justified. In addition, new world monkeys only display dichromatic vision, because they do not express one of the visual receptors that are found in the retinas of old world monkeys and apes, all of which possess trichromatic vision. By the same token, new world monkeys possess a greater number of olfactory receptor subtypes than old world monkeys and apes,⁹ as well as better-defined vomeronasal organs for olfactory perception.¹⁰ These differences almost certainly mean that there are substantial differences in the proportions and regions of the brain that regulate sensory perception in humans and new world monkeys.

NHPs are also used in neuropharmacological and neuropharmaceutical research. ICH guideline s6 states that preclinical trials on a

pharmaceutical should be performed in a species that a) expresses homologues of human proteins, and b) possesses tissues that show a similar cross-reactivity profile to that of humans. This requirement means that NHPs are considered the only suitable animals. However, similarity alone does not form a sufficient basis for assuming that NHPs are the most suitable models for humans. Single amino acid differences in protein sequences, small changes in expression levels or slight differences in biochemical pathways relating to drug action or distribution, can have dramatic consequences in terms of pharmacology. This can be illustrated by reference to the resistance of the squirrel monkey to glucocorticoids, caused by site-specific alterations in the amino acid sequence of its equivalent to the human glucocorticoid receptor protein.¹¹ Such differences in protein sequences or, indeed expression patterns, presumably account for the differences in chimpanzee and human responses to the hallucinogen phencyclidine (PCP), and are the reason why a high proportion of substances, which have been tested in NHPs, still fail in clinical trials. It seems that little regard is currently paid to the possibility of convergent evolution, which may reveal that less closely related species actually serve as better models for specific neurological diseases or as suitable systems for the testing of neuro-active substances. In some cases, the use of species with substantive functional dissimilarities provides data that are more informative of disease progression. One such example is the regulation of reproductive senescence in marmosets, which has rendered the common marmoset more useful than higher order primates for studying infertility in humans.¹² For similar reasons, owl monkeys are the best available animal model of malaria. While the use of alternative models is encouraged there are limitations. For instance, transgenic rodents can not at present supplant primate-based models of brain function, since the available imaging techniques lack sufficient resolution for use with such small animals.

Transgenic Research and Xenotransplantation



In September 2004, Australia's National Health and Medical Research Council ruled that NHPs such as baboons should never be considered as source animals for any future clinical trials of animal-to-human transplantation. The use of NHPs for xenotransplantation, first investigated in the 1960s, met with little success due to hyperacute immune responses among the experimental animals, which resulted in organ rejection. The use of immunosuppressants might alleviate this problem. It may even be possible in the future to create genetically modified NHPs, analogous to the pigs which lack porcine antigens or which express human immunosuppressant proteins.^{13,14} However, there are grave concerns about the transmission of disease, differences in organ size and the resulting fact that most primate-to-human transplants could only ever be considered a temporary measure until a human donor became available. Furthermore, there is still the argument that engineered NHPs may be sufficiently human to be afforded some form of human rights. The use of NHPs raises many other ethical issues, as

compared with using pigs, as was noted in a report of The Nuffield Council on Bioethics.¹⁵

The use of transgenic NHPs, such as rhesus macaques, has been proposed, especially with respect to examining the effect of expression of major histocompatibility complex proteins and fetal rejection. However, creating transgenic NHPs has proven extremely difficult, partly because of the inherent problems of breeding NHPs in captivity, the associated costs and the fact that gestation periods are long, few progeny are produced per gestation, and high levels of fetal death result from genetic engineering. Indeed, the first transgenic primate, created in 1991 – a rhesus macaque called ANDi, carrying, but not expressing, the gene encoding the green fluorescent protein – was the only offspring from over 200 engineered eggs.¹⁶ In 1998, an ECVAM workshop concluded that 'the use of higher order NHPs should be considered, in principle, to be unacceptable'.¹⁷ The last reported use of NHPs for genetic procedures in the UK was in 1997.

Polio Vaccine Production and Testing

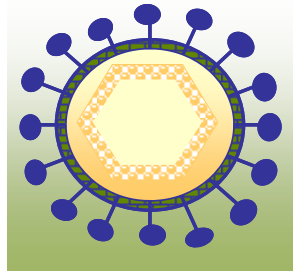


NHPs are the only experimental species that can be successfully infected with the poliomyelitis virus and are, therefore, extensively used for producing and testing polio vaccine. Since the 1960s, only captive-bred long-tailed macaques and wild-caught vervet monkeys have been used, both of which are free from the SV40 virus.¹⁸ A major polio vaccine producer based in Europe uses kidney cells derived from approximately 150 vervet monkeys per annum to produce between 600-1000 million doses of the vaccine. This is despite the fact monkey kidney (Vero) cell lines and human diploid cell cultures are acceptable production alternatives. In fact, Vero cell lines have been used to produce 440 million doses of the vaccine per annum¹⁹ and the WHO has established a Vero cell bank.²⁰

There are two types of polio vaccine, Salk (inactivated polio vaccine - IPV) and Sabin (oral polio vaccine - OPV). IPV batches can be tested for residual live virus by using cell lines rather than freshly isolated primary kidney cells from NHPs. Potency testing in Europe is conducted in chickens or guinea pigs. Under US requirements (CFR 630.3, 1993), however, potency testing must be conducted in NHPs. OPV is currently tested by intra-cerebral or spinal injection of the test

vaccine or a reference preparation into NHPs, followed by observation of the animals for 17-22 days and, finally, killing them for histological examination to ensure that it has not regained neurovirulence. The European Pharmacopoeia states that each type of the trivalent OPV must be tested separately that requires the use of a total of 110 monkeys. In 1999, the WHO Recommendations for OPV were revised, such that transgenic mice can replace primate-based neurovirulence testing for type 3 OPV, and MAPREC (Mutant Analysis by PCR and Restriction Enzyme Cleavage) can be used for DNA analysis to determine the molecular consistency of live viruses isolated from cell cultures. It has been suggested that the use of MAPREC can enable substandard batches of vaccine to be more readily identified. If this is the case, MAPREC should replace animal tests for type 1 and 2 OPV.^{21,22} In 2000, the WHO Ethical Committee on Biological Safety approved the use of the mouse neurovirulence test as a sensitive, rapid and reliable alternative to the use of NHPs for all types (1, 2 and 3) of OPV.^{23,24} Unfortunately, the primate test remains the gold standard for OPV neurovirulence testing, and the WHO Committee recommended that this test should still be used to assess new virus seed lots or changes in the manufacturing process.

SARS

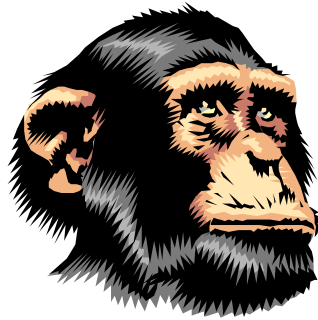


Severe Acute Respiratory Syndrome (SARS) is an emerging epidemic of the 21st century. The genetic code of coronavirus (CoV), which is responsible for SARS, has been determined and used to guide vaccine development. Unfortunately, vaccine development and testing involves the use of NHPs and other animals such as mice and ferrets.

In 2003, researchers reported that NHPs were sensitive to infection by SARS CoV.^{25,26} Macroscopic and histological lesions were found in the lungs of infected cynomolgus and rhesus monkeys. These animals developed antibodies and cytotoxic T-lymphocytes toward SARS CoV antigens, but displayed no clinical signs of infection. Furthermore, there was no evidence of monkey-to-monkey transmission. Attempts to create infection models by using guinea pigs, mice, hamsters, rats, chickens and pigeons, have also failed; mice do, however, exhibit virus replication peaks early after infection and virus-neutralising antibodies are induced. The ferret may provide the most accurate

model of human SARS, since infected animals develop a robust respiratory infection leading to disease and eventually to death, and the infection is transmitted to co-housed animals.²⁷ At a technical meeting held in February 2004, it was concluded that models for infection (mice, ferrets and monkeys), and pathology (ferrets and monkeys) were available.²⁸ Further work in this area has led to methods whereby various SARS CoV strains can be propagated in Vero cells. However, while potency testing was conducted in mice, guinea pigs and rabbits, vaccine efficacy was assessed in cynomolgus monkeys and macaques. Preliminary results indicated that vaccinated monkeys seemed to be protected against severe interstitial pneumonia, but not against viremia/viral infection.²⁴ Furthermore, although the ferret system seems to be the most suitable model for SARS vaccine development and testing,^{29,30} NHPs have yet again become the default test species, despite not being the most scientifically suitable models.

HIV and AIDS Research



Chimpanzees were used for many years in AIDS research.³¹ However, it is now known that chimpanzees do not develop AIDS, no doubt because there are crucial differences between the immune systems of chimpanzees and humans and the virus replicates slowly in these animals. Nevertheless, despite the fact that vaccine development in chimpanzees has repeatedly failed, their use in HIV and AIDS research has not been entirely abandoned. It must be questioned whether NHPs that have long life-spans, such as chimpanzees, should be infected with HIV, since there will be little opportunity for them to be re-housed outside of research institutions once they have served their experimental purpose. This problem was recently encountered by the Dutch primate rehabilitation centre, Stichting Aap, in The Netherlands. Where research has been halted, such as at the National Institutes of Health in the USA, the laboratories are faced with the problem of dealing with several hundred chimpanzees that are surplus to research requirements, but which require long-term care as it has been decided that euthanasia is not, morally, an option. In this case, the Federal Government has initiated the CHIMP (Chimpanzee Health Improvement, Maintenance and Protection) Act to establish a sanctuary system for these now-unwanted animals.³²

For over 20 years, rhesus and cynomolgus macaques have also been used in AIDS research. However, they cannot be infected with the HIV virus. Instead, research with these NHPs is primarily focused on SIV infection as a means of understanding the mechanisms of HIV transmission and AIDS development in humans. These disease models are fundamentally flawed in that the immune responses of NHPs and humans differ in such a way that HIV-1 can infect cells by using several receptors, some of which cannot be used by SIV. In fact, HIV-1 and SIV share less than 60% genetic identity,^{33,34} which is particularly important in relation to immune responses to viral coat proteins.³⁵ Even DNA-based vaccines that have produced encouraging results in NHPs have not yet been successful in humans.³⁶

In vitro studies on human cells and tissues have made possible the investigation of the immune-stimulating effects of potential vaccines and the analysis of HIV transmission.^{37,38,39} Such studies can be used to identify more suitable models of HIV infection for vaccine development and testing. Failing this, positive emission tomography can be used to safely and non-invasively examine the activated lymph nodes and spleens of patients given vaccines or to monitor viral infections in a temporal and spatial manner.⁴⁰

The Use of NHPs in Defence Research



To date, few NHPs have been used in the development of agents to counter any potential threat of biological warfare within the UK. In 2002, 42 procedures involving NHPs were conducted for defence purposes.⁴¹ However, events over the past few years have stimulated concern about the threat of bioterrorism involving agents such as Ebola, smallpox and anthrax viruses, and ricin and Botulinum toxins. Chimpanzees and rodents are host species to the Ebola virus, although the Ebola Reston strain can be transmitted through cynomolgus monkeys.⁴² Rhesus macaques have also been used in Ebola research.⁴³ NHPs are far less likely to be used in ricin research, since most species are highly susceptible to the toxin thus there are many equally suitable non-primate models which can be utilised. With regard to Botulinum toxin, a vaccine is currently available, but has not yet been fully evaluated, and should further development

be necessary, the most likely species to be used would be the mouse. Anthrax research, however, has required the use of some NHPs.⁴⁴ Similarly, NHPs are used as a source of Vero cells for smallpox production.⁴⁵ Smallpox is the only virus to have been successfully eradicated throughout the world, although stocks are held in one or two laboratories, so there is a risk that it could still be used in biological warfare. The existing vaccine is 95% effective, but has numerous side effects, some of which are potentially life threatening in 1 in 1000 people. There is no suitable primate host for smallpox. Nevertheless, recent studies indicate that high doses of the India I variola virus strain, from the Russian weapons programme, can infect cynomolgus monkeys.⁴⁶ Whether this will lead to a greater use of NHPs remains to be seen, but animals infected with this viral strain face severe suffering and an agonising death.

The Three Rs and Primate Experimentation



The Three Rs in the context of primate research has been discussed here, in relation to specific fields of research. However, there are a number of issues that warrant consideration in all fields of primate experimentation.

Refinement – While we endorse many of the refinement measures recommended by the Nuffield Council on Bioethics,⁴⁷ the Boyd Group⁴⁸ and the Animal Procedures Committee,⁴⁹ we feel that certain aspects which have been touched upon within this submission are worthy of particular emphasis. These include a consideration of how fully the ethological needs of any given primate species can be met during transport or prior to, during and following their experimental use. Where these needs cannot be met, or where the scientific objectives of a study run contrary to the general welfare of the species or of any individual animal, it may be that no amount of refinement can sufficiently ameliorate the adverse welfare consequences of using NHPs for research. In such circumstances, the use of NHPs should be restricted to those institutions that can prove their ability to cater for the welfare of the animals they propose to use.⁵⁰ The potential benefits, particularly of invasive studies or studies that are speculative in nature, must be weighed carefully against the welfare of the NHPs to be used. Where a lower order species or a species that can be captive-bred or purpose-bred can be used, there must be special justification for not doing so. This does not mean, however, that a lower order of primate or species should always be used, but instead, the choice of species must be guided by a consideration of all the evidence and welfare needs of each

species. Where no other suitable species is available, the least invasive and least harmful procedures should always be used. The use of telemetry and imaging devices, particularly during studies on neurological function, should be strongly encouraged, as they not only refine experimental endpoints, but also serve as a way to reduce the levels of primate experimentation by allowing more data to be collected from each study and from smaller numbers of animals.

Reduction – FRAME has previously advocated a progressive, determined and collaborative move toward the zero-option for primate research.^{51,52} Reduction – both in terms of the number of experiments conducted using NHPs and the number of NHPs used for each study – is a step toward this goal. Careful experimental design that takes into account the number of animals needed for each study and appropriate analytical efforts to produce statistically valid data play a key role in the strategic planning process. A thorough assessment of whether the proposed project will be able to meet its objectives or provide novel and applicable information, should be completed, taking into account existing evidence and whether similar projects have significantly advanced scientific understanding or produced relevant and reliable models of human physiology or pharmacology. The ethical review process should address the Three Rs specifically from the viewpoint of using NHPs as opposed to lower order vertebrates, invertebrates or *in vitro* systems. In addition, formal interim and retrospective assessments of licensed projects should be conducted, to ensure that the real benefits accrued are scientifically and ethically justifiable in relation to the suffering

caused. There should also be a continuous review to prevent the unnecessary duplication of research and to monitor the emergence of alternative model systems, including clinical studies in humans.

Replacement – At present, the greatest numbers of NHPs used within the EU are for research and development or the safety and toxicity testing of medicines or medical devices. Some reduction in primate use can be, and is being, achieved with non-primate animal models, and computer-based prediction techniques and *in vitro* systems could eventually replace the use of NHPs in regulatory toxicity testing and in the quality control of biologicals. However, this will be

entirely dependent on the proper development, successful independent validation and acceptance by regulatory bodies of these replacement systems. It will also be necessary to devise intelligent multi-system testing strategies, which will involve computerised pharmacotoxicological modelling and the stepwise use of sophisticated *in vitro* systems. Ideally these would be based on the ethical use of human cells and tissues and the application of modern molecular biological approaches, including genomics, proteomics and metabonomics,⁵³ and in appropriate circumstances and subject to proper controls, the use of human studies, including human volunteer studies.

Conclusions

The use of non-human primates in research and testing has given rise to extensive discussions of personhood, levels of sentience and intelligence and the surrounding ethical issues. Yet the use of primates has been accepted as a necessary evil if we are to see advances within medical science. However, it must not be forgotten that, where efficacy and safety in humans are the primary concern, the use of animal models, including primate models, will always be limited by the insuperable problem of

species differences. Furthermore, as more is understood about human diseases, admittedly partly as a result of work on animals, the emerging theme is that there are substantial differences between a disease in humans and what appeared to be a similar condition in an animal model. Aside from ethical considerations and humanity, these alone are good reasons for focusing all possible effort on moving toward the zero option as far as the use of non-human NHPs as laboratory animals is concerned.^{51,52}

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