PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS INDIA

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15 January 2026

Sh. Narendra Modi ji
Prime Minister of India
Prime Minister's Office
South Block, Raisina Hill
New Delhi-110011

Subject: US Food and Drug Administration Latest Agency to Issue Public Warnings on Misconduct by Telangana-Based Palamur Biosciences

Respected Sir:

With the utmost respect, I am writing on behalf of People for the Ethical Treatment of Animals India (PETA India) and our more than 2 million members and supporters to inform you of a disturbing culture of widespread noncompliance and malfeasance by a Telangana-based private contract laboratory and dog breeding facility, Palamur Biosciences Pvt. Ltd. (Palamur), that, if left unchecked, threatens to tarnish and undermine the international reputation of India's research enterprise.

US FDA Issues Warning Letter to Palamur

In December 2025, the **United States Food and Drug Administration (FDA)** issued a public formal warning letter against Palamur (Annexure A) through its Centre for Devices and Radiological Health following an inspection conducted by its Office of Bioresearch Monitoring Inspectorate (OBMI) Foreign Inspection Cadre in January 2025, citing "serious violations" and "systematic failures" by the company with respect to good laboratory practice (GLP) regulations. Specifically, the FDA concluded that Palamur had committed "**serious violations of Title 21, Code of Federal Regulations (CFR) Part 58 – Good Laboratory Practice for Nonclinical Laboratory Studies**." The FDA expressly stated that these violations demonstrate "**systemic failures in study director oversight of nonclinical laboratory studies and brings into question the quality and integrity of safety data collected at your testing facility**". [1]

On animal welfare, the FDA documented: poor or absent individual veterinary records; poor record-keeping during experimental processes and unsatisfactory SOPs rendering data unreliable; lack of documented veterinary examinations

[1] U.S. Food and Drug Administration. *Warning letter to Palamur Biosciences Private Limited* [Internet]. Silver Spring (MD): FDA; 2025 Dec 11 [cited 2025 Dec 23]. Available from: https://www.fda.gov/inspections-compliance-enforcement-and-criminal-investigations/warning-letters/palamur-biosciences-private-limited-708579-12112025

prior to invasive procedures; inadequate euthanasia procedures failing to ensure rapid unconsciousness and minimal distress; failure to ensure personnel clearly understand their duties; unapproved deviations from protocols that were neither detected nor documented by Palamur's Quality Assurance Unit; and unsanitary facility conditions including dirt, debris, animal droppings and "pest harborage".

The FDA further found Palamur's responses to be "inadequate," stating they **"do not provide assurance that similar violations would not occur again"** and that deficiencies yield "questionable study results" . The FDA warned, **"The unreliable data raises concerns about the quality and integrity of associated premarket submissions, which may put public health and safety at risk."**

US EPA and Health Canada Temporarily Stop Accepting Palamur's Data

Crucially, the FDA's warning letter does not arise in isolation, but rather is indicative of a troubling pattern at Palamur. The U.S. Environmental Protection Agency (EPA) on 24 May 2024 temporarily "halted the acceptance of studies generated by Palamur Biosciences Lab (Palamur) in Telangana, India, **due to the possible falsification of data following an inspection by the Indian National Good Laboratory Practice Compliance Monitoring Authority (NGCMA)**," which "conducted an inspection of Palamur and confirmed that **data were falsified for most of those 58 studies**, which were conducted between January 2020 and July 2023," resulting in "NGCMA issu[ing] a 'Not in Compliance' status for the laboratory …"[2] On 13 June 2024, Health Canada's Pest Management Regulatory Agency (PMRA) also temporarily halted acceptance of data from Palamur for this reason as well.[3] Despite these earlier signals, the facility continued operations evidently without demonstrable systemic reform.

Palamur's History of Regulatory Noncompliance

The FDA warning represents the latest **culmination of unresolved deficiencies at Palamur**, rather than the first notice. A comparative analysis (Annexure B) demonstrates that the deficiencies cited by the FDA align closely with those documented through a statutory inspection conducted on 11 June 2025 and recorded in the signed Committee for Control and Supervision of Experiments

[2] U.S. Environmental Protection Agency. (2024, May 24). EPA Halts Acceptance of Data for Pesticide Registration from a Non-compliant Laboratory. Retrieved January 6, 2026, from https://www.epa.gov/pesticides/epa-halts-acceptance-data-pesticide-registration-non-compliant-laboratory

[3] Health Canada. (2024, June 13). Memo: Not Accepting Pesticide Registration Data from Non-Compliant Laboratory. Retrieved January 6, 2026, from https://www.canada.ca/en/health-canada/services/consumer-product-safety/pesticides-pest-management/registrants-applicants/product-application/memo/not-accepting-pesticide-registration-data-from-non-compliant-laboratory.html

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on Animals (CCSEA) inspection report (Annexure C), submitted by a team of multidisciplinary experts on 17 June 2025, as well as whistleblower findings brought to PETA India's notice and submitted to the CCSEA on 10 June 2025.

Findings of 17 June 2025 CCSEA-Appointed Inspection Report at Palamur

The 17 June 2025 report was prepared after an extensive inspection of Palamur in the days following whistleblower complaints against the company that PETA India brought to CCSEA's notice (Annexure D). The signatories are those who were government-appointed to carry out the inspection: Dr Mukesh Kumar Gupta, (CCSEA Member and Director of Indian Council for Medical Research-National Animal Resource Facility for Biomedical Research; Dr Vivek Tyagi Senior Consultant CCSEA; a member of the Animal Welfare Board of India; two nominees of the Institutional Animal Ethics Committee; and the Managing Director of Humane World for Animals.

The report recommends "**immediate regulatory action…including the removal and rehabilitation of animals in order to prevent further pain and suffering**" as well as a review of Palamur Biosciences' registration and breeding license status. The report details extensive and serious violations of CCSEA guidelines and basic standards and care at Palamur Biosciences for over 1,200 across species—including beagle dogs, cows, pigs, monkeys and others. This includes poor-record keeping, improper euthanasia methods, a lack of pain-relief even during invasive procedures, poor housing, substandard veterinary care, use of unapproved species, rough handling, more than the number of approved animals, a refusal to fully comply with the inspection and other systemic lapses.

The report concludes, "**The operational deficiencies observed at PBPL [Palamur Biosciences] are not isolated incidents but indicative of entrenched structural, procedural and ethical failures.** The scale and severity of non-compliances documented during the inspection raise significant concerns regarding the facility's adherence to established standards of animal welfare and regulatory accountability. **The situation demands urgent attention—particularly with respect to the removal and rehabilitation of animals to prevent further pain, distress or suffering.** The findings also call for a critical review of the facility's registration and breeding license in view of the serious and repeated deviations from prescribed norms."

Subsequent inspection reports, including those submitted by a Court-appointed Local Commissioner during the pendency of W.P. (C) 9350 of 2025 before the Hon'ble Delhi High Court, documented serious deficiencies such as poor hygiene, bleak housing, animals in poor condition and the need to rehabilitate

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73 dogs properly (and not only performatively) who are not used for either breeding or experiments.

Palamur Resists Meaningful Reforms

Despite the findings from the 17 June 2025 report submitted to CCSEA and warnings from the FDA and other agencies, Palamur has demonstrated a **consistent pattern of resistance to meaningful reform**. Instead of undertaking genuine corrective action, as the FDA observes, the facility has implemented only superficial changes and provided unconvincing excuses, failing to address core welfare and governance issues. Rather, Palamur has actively resisted scrutiny and cooperation during and after inspections and attempted to minimise concerns. This approach reflects an attitude of doing the **bare minimum necessary to continue operations**, rather than achieving compliance, good animal welfare governance and scientific integrity. Indeed, the FDA chided Palamur, repeatedly stating that the company's "**responses do not provide assurance that similar violations would not occur again**."[1]

CCSEA's Lack of Meaningful Regulatory Inaction is Enabling Malfeasance

We respectfully submit that Palamur's culture of noncompliance and resistance to implement meaningful reforms is being enabled by CCSEA's regulatory inaction. Despite the 17 June 2025 inspection report confirming grave violations seven months ago, the CCSEA has not acted on the report's recommendations and has instead taken steps appearing to protect Palamur from genuine oversight and consequence including appointing other animal experimenters for subsequent inspections of Palamur that the December 2025 FDA warning letter exposes as rubber-stamp and grossly insufficient. This inaction is resulting in an embarrassing situation where foreign agencies like FDA, EPA and Health Canada and whistleblowers are repeatedly left to point out concerns.

CCSEA is statutorily mandated under the PCA Act, 1960, to ensure the ethical treatment of animals used for scientific and educational purposes, however, its present structure and operational practices raise serious questions about impartiality and credibility that undermine its intended purpose. This is concerning for everyone, as poor animal welfare also generally translates to unreliable experimentation outcomes, which puts public health at risk.

There is currently a significant overrepresentation of individuals who are active proponents or practitioners of animal experimentation within the CCSEA's Core Committee (Annexure E), which means that animal experimenters are expected to police other animal experimenters. This

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presents conflicts of interests including because animal experimenters may extend professional courtesies (e.g., overlooking a regulatory violation) to other animal experimenters practicing in the same field to maintain rapport and goodwill amongst colleagues.

With so much corroborated evidence of misconduct by Palamur including by different national and international agencies and experts and whistleblower complaints, what remains absent is not information, but action.

Request for Action to Protect and Advance India's Research Enterprise

We respectfully request your kind intervention to direct the Ministry of Fisheries, Animal Husbandry and Dairying to:

- Pursue the implementation of the recommendations of the report titled "Report on the Inspection of Palamur Biosciences" submitted to Committee for Control and Supervision of Experiments on Animals (CCSEA) on 17 June 2025 including the rescue and rehabilitation of animals at Palamur. NGOs stand ready to play a supportive role.
- Terminate Palamur's CCSEA registration to conduct animal experiments and close the company's animal breeding centre in response to egregious problems found spanning years. In the US, beagle breeder Envigo which supplied animals to laboratories was closed, fined USD $35 million for misconduct, and 4000 beagles were rescued from it with the help of NGOs—a move that was highly celebrated by its public.[4]
- Initiate a formal review of the structure and functioning of the CCSEA to ensure genuine oversight by animal welfare experts, action where required and to eliminate the conflict of interest posed by animal experimenters as inspectors.

We also respectfully request your kind intervention to direct the Ministry of Science and Technology and Ministry of Health and Family Welfare to:

- Consider and implement the recommendations in PETA India's Research Modernisation Deal (Annexure F) in favour of modern non-animal technologies. This would be in line with trends abroad where UK, US an Europeans governments are fast transitioning from outdated animal experiments to modern, non-animal methods.

[4] Treisman R. *A beleaguered breeder faces a record $35 million fine for mistreating its beagles.* NPR [Internet]. 2024 Jun 4 [cited 2026 Jan 14]. Available from: https://www.npr.org/2024/06/04/nx-s1-4991678/envigo-beagle-breeder-35-million-fine-animal-welfare

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We submit this representation with the highest respect in your leadership and in the interest of India's reputation as a leader of humane, modern science.

Thank you for your time and attention to this important matter. We kindly request for information on any action taken in this regard. I can be reached at aaggarwal@petaindia.org or +91-9958840994.

Sincerely,

Dr. Anjana Aggarwal
Scientist and Research Policy Advisor

Enclosures:

1. Annexure A: FDA warning letter to the Palamur Biosciences Pvt. Ltd.
2. Annexure B: Comparative analysis between the findings of the 17 June 2025 CCSEA inspection report with the FDA's 11 December 2025 Warning Letter
3. Annexure C: 17 June 2025 Inspection Report by Government appointed Inspectors
4. Annexure D: First Complaint letter dated 10 June 2025 to CCSEA
5. Annexure E: Updated list of Core committee members of CCSEA
6. Annexure F: PETA India's Research Modernisation Deal

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Annexure A:

FDA warning letter to the Palamur Biosciences Pvt. Ltd.

WARNING LETTER

Palamur Biosciences Private Limited

MARCS-CMS 708579 — DECEMBER 11, 2025

More Warning Letters (/inspections-compliance-enforcement-and-criminal-investigations/compliance-actions-and-activities/warning-letters)

Product:

Medical Devices

Recipient:

S. Ramamoorthy, Ph.D.
Chief Executive Officer (CEO)
Palamur Biosciences Private Limited
Boothpur Mandal
Mahabubnagar 509382 Telangana
India

ram.murthy@palamurbio.com (mailto:ram.murthy@palamurbio.com)

Issuing Office:

Center for Devices and Radiological Health
United States

WARNING LETTER

December 11, 2025

Dear Dr. Ramamoorthy:

This Warning Letter is to inform you of objectionable conditions observed during the United States Food and Drug Administration (FDA) inspection conducted at Palamur Biosciences Private Limited (PBS) from January 20, 2025, to January 27, 2025, by investigators from the FDA's Office of Bioresearch Monitoring Inspectorate (OBMI) Foreign Inspection Cadre. This inspection was conducted to determine whether activities and procedures related to your participation in Good Laboratory Practice (GLP) nonclinical studies complied with applicable federal regulations. Specifically, FDA investigators focused on the list of studies below including, but not limited to, implantation, acute systemic toxicity, material mediated pyrogenicity (MMP), and guinea pig maximization tests conducted at your facility.

The list of studies below is not an all-inclusive list of studies impacted by the inspection or by the violations cited in this letter.

Study number	Test

231918	Implantation Study
24636	Implantation Study
231192	Acute Systemic Toxicity
231165	Acute Systemic Toxicity
231809	Acute Systemic Toxicity
231868	Acute Systemic Toxicity
24085	Acute Systemic Toxicity
24110	Acute Systemic Toxicity
24005	MMP
24084	MMP
24378	MMP
231866	Guinea Pig Maximization Test
231586	Guinea Pig Maximization Test
231191	Intracutaneous Reactivity
231196	In Vitro Cytotoxicity

These tests are used in nonclinical studies for the development of devices as that term is defined in section 201(h)(1) of the Federal Food, Drug, and Cosmetic Act (the Act), 21 U.S.C. § 321(h)(1), because they are intended for use in the diagnosis of disease or other conditions, or in the cure, mitigation, treatment, or prevention of disease, or to affect the structure or function of the body.

The inspection was conducted under a program designed to ensure that data and information contained in requests for Investigational Device Exemption, Premarket Approval applications, and Premarket Notification submissions are scientifically valid and accurate. Another objective of the program is to ensure that human subjects are protected from undue hazard or risk during the course of scientific investigations.

Our review of the inspection report prepared by the OBMI revealed serious violations of Title 21, Code of Federal Regulations (CFR) Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies, which concerns, among other things, requirements prescribed under section 520(g) of the Act, 21 U.S.C. § 360j(g). Compliance with Part 58 is intended to assure the quality and integrity of the safety data filed in a premarket submission. At the close of the inspection, the FDA investigators presented an inspectional observations Form FDA-483 for your review and discussed the observations listed on the form with you.

We received the initial response from your firm dated February 17, 2025, concerning our investigators' observations noted on the Form FDA-483. We have since received follow-up responses to the initial response from your firm on the following dates:

- April 13, 2025
- May 12, 2025
- July 8, 2025
- August 12, 2025
- September 16, 2025

This letter discusses your written responses to the noted investigators' observations and requests prompt corrective action to address violations of 21 CFR Part 58. These violations include, but are not limited to, the following:

1. Failure of the study director to assure that all experimental data, including observations of unanticipated responses of the test system are accurately recorded and verified [21 CFR 58.33(b)].

The study director has overall responsibility for the technical conduct of the study, as well as for the interpretation, analysis, documentation and reporting of results, and represents the single point of study control. The study director's responsibilities include ensuring that all experimental data are accurately recorded and verified. Examples of the study director's failures to adhere to this requirement include, but are not limited to, the following:

a. For study 231918, the Animal/Bird Requisition and Issuance Record shows that the rabbits were 7-8 months old, but the breeding records do not include birth dates. Additionally, the animals were only given identification (R1 through R12) when they were enrolled in and throughout the study, and this identification cannot be traced back to the Animal Issue Record maintained by the Animal Breeding and Husbandry (ABH) department. The Animal Issue Record lists species/strain, sex, age range and body weight range for a group of rabbits issued for a particular study. However, there are no medical records associated with the individual rabbits and no way of verifying the accurate age of each animal. Because animal age can impact the analysis and interpretability of test results, it is important that the age of each animal in a study is accurately recorded and can be verified.

b. The raw study data for the acute systemic toxicity studies (231192, 231165, 231809, 231868, 24085, and 24110) and MMP studies (24005, 24084, and 24378) did not have the rate of intravenous administration of the bolus in each animal recorded. Additionally, the start and end time of intravenous injection are not recorded. Study plans for all acute systemic toxicity and MMP studies require that intravenous injections be administered consistent with the guidelines outlined in the International Organization for Standardization (ISO) 10993, Biological Evaluation of Medical Devices, and/or the United States Pharmacopeia (USP) Chapter 151, Biological Tests – Pyrogen Test, as applicable. The rates of intravenous injections were not recorded; therefore, it cannot be verified whether these tests were conducted according to the testing guidelines.

c. For study 24636, there were no records of physical examinations of Guinea Pigs 1 through 12 upon receipt of the animals from the vendor, upon release from quarantine, or before the surgical procedure. The health of the animals was not comprehensively examined and recorded at the start of the study and anesthesia status was not assessed before the surgical procedure. Therefore, it cannot be confirmed whether the animals were healthy or showing any abnormal clinical signs. Clinical observations are important to record for the reliability of the subsequent analysis of the responses of the animal to the test article, as well as for evaluating whether the animal needs any treatment or intervention.

As the principal point of study control, the study director did not ensure that all experimental data were accurately recorded and verified, which in turn yields questionable study results. Based on this failure, the FDA has concerns about the quality and integrity of data generated from the nonclinical laboratory studies conducted at your testing facility. Complete and

accurate study data are necessary to allow FDA to fully assess the overall safety and risk of a device with an associated premarket submission. The unreliable data raises concerns about the quality and integrity of associated premarket submissions, which may put public health and safety at risk.

Your written responses are inadequate. The written responses provided revised standard operating procedures (SOPs), forms, and staff training records, but they do not include any planned preventive actions such as frequency (e.g., quarterly, annual) of audits to check for compliance or future training for new staff and/or new procedures. Additionally, while you provided a copy of the form, PRM/PBS/TOX/014, “Animal/Bird Requisition and Issuance Record” as evidence of the health status of the animals, the form lacks detailed documentation of physical examinations conducted by qualified personnel (e.g. veterinarian). Furthermore, while your responses propose corrections for the specific observations noted, you have not indicated whether any systematic reviews of your procedures will be conducted to identify and correct any systemic issues. Your explanation, when taken into consideration with the violations described above, which occurred in multiple studies, suggests systemic failures in study director oversight of nonclinical laboratory studies and brings into question the quality and integrity of safety data collected at your testing facility. Thus, your responses do not provide assurance that similar violations would not occur again.

2. Failure of the testing facility to have standard operating procedures in writing setting forth nonclinical laboratory study methods that management is satisfied are adequate to ensure the quality and integrity of the data generated in the course of a study. [21 CFR 58.81(a)].

SOPs should be adequate to ensure the quality and integrity of data generated in a study. However, not all SOPs appear to be adequate. Examples of your failures to adhere to this requirement include, but are not limited to, the following:

a. SOP/PBS/TOX/001, Recording of Body Weight and Procedure for Feed and Water Consumption, does not include procedures for calibration of the balance used for weighing animals. Section 5.1 of the SOP states that, “[t]he balance will be set and calibrated as specific to the balance in use.” However, there is no specific information as to who is responsible for calibration (e.g., study personnel assigned to each study) or when calibration should be performed (e.g., whether the calibration should be performed prior to body weight measurement or whether it should be performed at specific dates/intervals).

b. SOP/PBS/GEN/047, Sample and Reference Material Preparation for Biological Evaluation of Medical Devices, does not include detailed procedures for extraction depending on the test article (e.g., test articles indicated for prolonged or long-term tissue contact may require different extraction conditions than those with limited contact). Additionally, the SOP does not provide detailed instructions for monitoring of changes in the color of the test extract. Sample preparation is one of the crucial steps in biocompatibility testing of medical devices. Conditions of medical device extraction, such as extraction temperature, duration of extraction, and vehicle use can significantly impact the outcomes of a biocompatibility study.

c. SOP/PBS/PAT/012, Euthanasia of Laboratory Animals/Birds, does not contain sufficiently detailed procedures for administration of carbon dioxide (CO2) for inhalation euthanasia for rodents to fulfill the objective of rapid unconsciousness with minimal distress to the animals. For example, the SOP does not contain information about the settings of the flowmeter for the delivery of the CO2 gas or include specific procedures for using CO2 inhalation in rodents.

Failure of a testing facility to have adequate SOPs raises questions about the reliability and accuracy of the data and does not ensure the quality and integrity of data generated in a study. Inadequate SOPs yield inadequate protocols that introduce ambiguity and uncertainty as to how study requirements are to be followed, as well as inconsistent execution of studies and unreliable study results. Inadequate SOPs could result in study data with a high level of variability that challenges the ability to effectively interpret the study results associated with a device. This in turn adversely impacts a manufacturer’s and FDA’s ability to assess the overall safety and risk of the subject device prior to use in humans as a legally marketed device or for purposes of beginning clinical trials.

Your written responses are inadequate. The responses provided revised SOPs, forms, and staff training records, but they do not: (1) detail how your testing facility will ensure that applicable SOPs will be followed to ensure the quality and integrity of data generated in a study; and (2) address any planned preventive actions, such as frequency (e.g., quarterly, annual) of audits to check for compliance or future training for new staff and/or new procedures. Additionally, while your responses propose corrections for the specific observations noted, you have not indicated whether any systematic reviews of your procedures will be conducted to identify and correct any systemic issues, including ensuring that there are existing SOPs that cover all relevant functions, as well as ensuring that all SOPs are sufficiently detailed to allow personnel to correctly perform these functions. Thus, your written responses do not provide assurance that similar violations would not occur again.

3. Failure of the testing facility management to assure that personnel clearly understand the functions they are to perform [21 CFR 58.31(f)].

An example of the testing facility management's failure to adhere to this requirement includes, but is not limited to, the following:

a. For Guinea Pig Maximization Test (GPMT) study 24838, the study personnel failed to identify and record adverse tissue responses to the injected adjuvant and clinical observations (e.g., difficulty breathing). SOP/PBS/TOX/008 indicates that after intradermal induction, skin reactions such as edema, erythema, and necrosis, along with other clinical signs will be recorded. However, the records that were reviewed did not indicate that clinical signs were recorded. In addition, there is no procedure that describes what clinical signs should be assessed to determine the health of the animal or how the technician would recognize skin reactions and distinguish between similar responses (e.g., between a "discrete" and "moderate" skin reaction). Furthermore, it was observed that study personnel training does not include species-specific in-life observations and when veterinarian oversight should be requested.

Failure of testing facility management to assure that all personnel clearly understand the functions they are to perform and are adequately qualified and trained creates a high level of variability that does not ensure the validity and quality of the data. Personnel that do not clearly understand the functions they are to perform cannot consistently perform tasks according to the SOPs. This can have a negative impact on a study and calls into question the quality and integrity of studies conducted at your testing facility.

Your written responses are inadequate. Your responses included revised SOPs, training records, and creating a new division for Ethology & Animal Welfare to focus on clinical sign observation, ethology, animal welfare training, and enrichment, in addition to appointing a technical consultant to assist with personnel training. However, it is not clear what oversight for the new head of Ethology and Animal Welfare will entail or what technical expertise will be provided by the technical consultant. Furthermore, your responses do not address planned preventive actions, such as frequency (e.g., quarterly, annual) of audits to check for compliance or future training for new staff and/or new procedures. Additionally, while your responses propose corrections for the specific observations noted, you have not indicated whether any systematic reviews of your training program will be conducted to identify and correct any gaps in personnel training. Furthermore, while you have provided training records containing quizzes that test personnel's recollection of the training received, you have not described how personnel's ability to perform the tasks in question will be assessed to ensure that the training achieves its intended goal. Thus, your written responses do not provide assurance that similar violations would not occur again.

4. Failure of the Quality Assurance Unit (QAU) to determine that no deviations from approved protocols or SOPs were made without proper authorization and documentation [21 CFR 58.35(b)(5)].

The QAU is responsible for determining that no deviations from approved protocols or SOPs were made without proper authorization and documentation. An example of the QAU's failures to adhere to this requirement includes, but is not limited to, the following:

a. For study 231918, the study plan stated that the rabbits should have a supraglottic airway device inserted; however, the surgical records showed that an endotracheal tube was used for delivery of inhalant anesthesia. There is no record to indicate that this deviation was identified by the QAU, and there is no documentation to indicate that this deviation was made with proper authorization.

A reliable QAU is integral to the successful understanding and completion of any GLP study. Without appropriate QAU oversight, neither the sponsor nor FDA reviewers have assurance that the data in the final study report is accurate and valid. Failure to perform QAU functions can have a negative impact on a study and calls into question the quality and integrity of studies conducted at your testing facility.

Your written responses are inadequate. Your written responses include generating a record of an SOP deviation, performing a root cause analysis, and opening a corrective and preventative actions plan. However, your responses do not detail planned preventive actions, such as frequency (e.g., quarterly, annual) of audits to check for compliance of the QAU or future training for new staff and/or new procedures. Additionally, while your responses propose corrections for the specific observations noted, you have not indicated whether any systematic reviews of your QAU will be conducted to identify and correct any systemic issues.

In addition to the device studies described above, FDA investigators also observed that your facility performs studies intended to support the approval of new animal drugs. The overall conditions and practices at your facility, as exemplified above, may impact the validity and integrity of the data obtained to support new animal drug applications. For example, in study 19278, your records indicate you centrifuged the majority of blood samples before they were actually collected. In your response, you indicated you were unable to reconstruct what happened. This calls into question the quality and integrity of data generated from the nonclinical laboratory studies conducted at your testing facility. You should ensure that you consider animal drug studies as part of your corrective and preventive actions.

FDA investigators also observed that the exterior of the testing facility had significant accumulations of dirt, animal droppings, and potential pest harborage. Upon inspection of Block F, the HVAC equipment on the outside of the building was found to be surrounded by a large amount of dirt and debris that could attract and provide harborage for various pests and could potentially be caught up in the HVAC equipment, possibly causing failure. Such conditions may impact nonclinical studies conducted at your facility.

The violations described above are not intended to be an all-inclusive list of problems that may exist with your facility. It is your responsibility as a nonclinical laboratory to ensure compliance with the Act and applicable regulations.

Within 15 working days of receiving this letter, please provide documentation of the additional corrective and preventive actions that you have taken or will take to correct these violations and to prevent the recurrence of similar violations in current or future studies for which you are the testing facility. Any submitted corrective action plan should include projected completion dates for each action to be accomplished as well as a plan for monitoring the effectiveness of your corrective actions. In addition, please provide a complete list of all nonclinical laboratory studies of FDAregulated devices for the last five years, including the name of the study, the test article, the name of the study director and sponsor, and the current status of the study. Failure to respond to this letter and take appropriate corrective action could result in the FDA taking regulatory action without further notice to you. In addition, FDA could initiate disqualification proceedings against you in accordance with 21 CFR 58.202. If you believe that you have complied with the Act and FDA regulations, please include your reasoning and any supporting information for our consideration.

Your response should reference “CTS# EC250128/E001” and be sent via email to: Irfan.Khan@fda.hhs.gov.

A copy of this letter has been sent to FDA’s OBMI Foreign Inspection Cadre via email at FDAInternationalBIMO@fda.hhs.gov. Please send a copy of your response to that office.

If you have any questions, please contact Amrin Chowdhury by phone at (240) 402-8318 or email at Amrin.Chowdhury@fda.hhs.gov.

Sincerely yours,
/S/

Ouided Rouabhi, MS
Acting Director
DCEA1: Division of Clinical Policy and Quality
Office of Clinical Evidence & Analysis
Office of Product Evaluation and Quality
Center for Devices and Radiological Health

Annexure B:

Comparative analysis between the findings of the 17 June 2025 CCSEA inspection report with the FDA's 11 December 2025 Warning Letter

Comparison Matrix:

U.S. Food and Drug Administration (FDA) Warning Letter (11 Dec 2025) vs Committee for the Purpose of Control And Supervision of Experiments on (CCSEA) Inspection Report (Submitted 17 June 2025) on Palamur Biosciences Private Limited (PBPL)

The US FDA's warnings are in relation to the reliability and integrity of experimental data, while CCSEA's commissioned expert report is largely focused on animal welfare, but as the below chart shows, there is considerable corroboration of findings and overlap as poor animal welfare also generally translates to unreliable experimentation outcomes, which puts public health at risk. The quotes are verbatim from the US FDA warning letter dated 11 December 2025 and the CCSEA-commissioned inspection report submitted on 17 June 2025.

Key summary language has been bolded by PETA India for ease of the reader.

CCSEA 17th June report: "**The inspection team concluded that many of the allegations raised by PETA India's whistle-blower-including overcrowding, veterinary neglect, inappropriate handling, and euthanasia violations-were substantiated** or could not be conclusively refuted due to the absence of required documentation."

Grounds	FDA warning letter-verbatim	CCSEA 17th June 2025 report-Verbatim
Systemic Nature of Violations	"**The violations described above… occurred in multiple studies, suggests systemic failures** in study director oversight of nonclinical laboratory studies and brings into question the quality and integrity of safety data collected at your testing facility." "As the principal point of study control, the study director did not ensure that all experimental data were accurately recorded and verified, which in turn yields questionable study results. Based on this failure, the FDA has concerns about the quality and integrity of data generated from the nonclinical laboratory studies conducted at your testing facility…**The unreliable data raises concerns about the quality and integrity of associated premarket submissions, which**	"**The inspection revealed serious and widespread non-compliance with CCSEA regulations.** Key welfare violations included **overcrowded and barren kennels, lack of environmental enrichment, feeding practices not aligned with the animals' physiological needs and body weight requirements, untrained and rough handling practices, and an alarming absence of protocols for pain management, sedation, and euthanasia. Veterinary infrastructure was critically inadequate**, with poor medical coverage, minimal drug availability, and no functioning isolation or quarantine facilities."

	may put public health and safety at risk." "Thus, **your responses do not provide assurance that similar violations would not occur again**."	"**The operational deficiencies observed at PBPL are not isolated incidents but indicative of entrenched structural, procedural, and ethical failures.** The scale and severity of non-compliances documented during the inspection raise significant concerns regarding the facility's adherence to established standards of animal welfare and regulatory accountability. **The situation demands urgent attention—particularly with respect to the removal and rehabilitation of animals** to prevent further pain, distress, or suffering. **The findings also call for a critical review of the facility's registration and breeding licence**, in view of the serious and repeated deviations from prescribed norms." "Overall, **the findings reflect a systemic and ongoing disregard for regulatory compliance, ethical responsibility, and animal welfare.**" "[T]he conditions observed at PBPL point to **substantial deficiencies in veterinary access, preventive healthcare, and critical welfare infrastructure**. These shortcomings compromise both the physical well-being and dignity of the animals and present serious ethical and regulatory concerns that warrant urgent attention."

Violation of Law, Procedure and Guidelines	"Our review of the inspection report prepared by the OBMI revealed **serious violations of Title 21, Code of Federal Regulations (CFR) Part 58 - Good Laboratory Practice for Nonclinical Laboratory Studies**, which concerns, among other things, requirements prescribed under section 520(g) of the Act, 21 U.S.C. § 360j(g)."	"The comprehensive inspection of PBPL highlights **systemic failures at multiple levels of its operations to uphold animal ethics and welfare as per CCSEA guidelines**. PBPL's approach to animal research demonstrates an operational model that prioritizes experimental output over welfare, compliance, and ethical responsibility. Despite its extensive use of dogs, non-human primates, pigs, and other species, **PBPL has failed to implement even the most basic standards of care mandated by CCSEA**."
Failure of Adequate Record-Keeping—Creating a Lack of Reliability on Compliance, Animal Welfare and Quality of Experiments	"[T]here are no medical records associated with the individual rabbits and no way of verifying the accurate age of each animal. Because animal age can impact the analysis and interpretability of test results, it is important that the age of each animal in a study is accurately recorded and can be verified." "The raw study data for the acute systemic toxicity studies (231192, 231165, 231809, 231868, 24085, and 24110) and MMP studies (24005, 24084, and 24378) did not have the rate of intravenous administration of the bolus in each animal recorded. Additionally, the start and end time of intravenous injection are not recorded…The rates of intravenous injections were not recorded; therefore, it cannot be verified whether these tests were conducted according to the testing guidelines."	"The animal record-keeping system at PBPL is virtually non-functional, with key regulatory documentation either missing or grossly insufficient. Without breeding records, reuse data, health histories, or procedural logs, PBPL operates in opaque conditions that obstruct regulatory oversight." "PBPL failed to provide any documentation detailing the number, age, sex or species-wise inventory of animals held or used at the facility, despite repeated requests." "The absence of on-site veterinary records further prevented verification of health screenings, disease status, or any actions taken regarding zoonotic diseases." "[Records] fail to provide any comprehensive overview of essential information such as the

	“For study 24636, there were no records of physical examinations of Guinea Pigs 1 through 12 upon receipt of the animals from the vendor, upon release from quarantine, or before the surgical procedure. The health of the animals was not comprehensively examined and recorded at the start of the study and anesthesia status was not assessed before the surgical procedure. Therefore, it cannot be confirmed whether the animals were healthy or showing any abnormal clinical signs.”	total number of animals used, the frequency of their use in experiments, clinical conditions identified, or the preventive and therapeutic care administered-whether at the breeding facility or the experimentation centre. This fragmented and superficial record-keeping reflects a seriously negligent approach to both regulatory compliance and animal welfare standards. Moreover, veterinary records were not available on-site, significantly hampering the ability to conduct thorough inspections or continuous assessments of animal health and well-being. Without access to these records, it is impossible to monitor medical histories, vaccination status, or previous treatments--elements that are vital to ensuring timely and appropriate veterinary care. The absence of a structured, accessible veterinary documentation system undermines the facility's responsibility to safeguard the animals in its custody.” “A critical gap lies in the absence of a functional system for recording preventive healthcare and treatment interventions. No accessible, structured on-site veterinary documentation was available, and existing loose case sheets are reportedly stored in a separate building-severely limiting timely medical assessments and ongoing veterinary oversight. This lack of accessible records undermines

		the ability to monitor animal health, track vaccination and treatment histories, or assess compliance with humane care and regulatory norms."
Inadequate SOPs and Lack of Discipline in Experimental Procedures Resulting in Cruelty to Animals and Unreliable Experiments	"[N]not all SOPs appear to be adequate." "SOP/PBS/TOX/001, Recording of Body Weight and Procedure for Feed and Water Consumption, does not include procedures for calibration of the balance used for weighing animals. Section 5.1 of the SOP states that, '[t]he balance will be set and calibrated as specific to the balance in use.' However, there is no specific information as to who is responsible for calibration (e.g., study personnel assigned to each study) or when calibration should be performed (e.g., whether the calibration should be performed prior to body weight measurement or whether it should be performed at specific dates/intervals)." "b. SOP/PBS/GEN/047, Sample and Reference Material Preparation for Biological Evaluation of Medical Devices, does not include detailed procedures for extraction depending on the test article (e.g., test articles indicated for prolonged or long-term tissue contact may require different extraction conditions than those with limited contact). Additionally, the SOP does not provide detailed instructions for monitoring of changes in the color of the test extract. Sample preparation is one of the crucial steps in biocompatibility testing of medical devices. Conditions of medical device extraction, such as	"[A] clear inconsistency was observed between the number of CCSEA-approved research protocols-reported to be 87 over the past three months-and the actual number of dogs, minipigs and monkeys present at the facility. This discrepancy suggests possible non-compliance with approved study limits or underreporting of animal populations." "Crucially, the mandatory three-month washout period-required to ensure complete elimination of substances from animals' systems before reuse-was reportedly not being followed, particularly for rninipigs. No documentary evidence was produced during the inspection to verify compliance with this requirement. This lapse not only violates standard ethical and scientific guidelines but also compromises the validity of subsequent research and the welfare of the animals involved." "Contrary to the mandates set forth by CCSEA regulations and guidelines, a case study based on data from the software application used at PBPL's experimentation facility revealed serious lapses in animal welfare. In one instance, a dog exhibiting mild to moderate tremors was not withdrawn from the

	extraction temperature, duration of extraction, and vehicle use can significantly impact the outcomes of a biocompatibility study." "Failure of a testing facility to have adequate SOPs raises questions about the reliability and accuracy of the data and does not ensure the quality and integrity of data generated in a study. Inadequate SOPs yield inadequate protocols that introduce ambiguity and uncertainty as to how study requirements are to be followed, as well as inconsistent execution of studies and unreliable study results. Inadequate SOPs could result in study data with a high level of variability that challenges the ability to effectively interpret the study results associated with a device. This in turn adversely impacts a manufacturer's and FDA's ability to assess the overall safety and risk of the subject device prior to use in humans as a legally marketed device or for purposes of beginning clinical trials."	experiment. The symptoms reportedly progressed to severity and became severe by the tenth day. The animal was then marked as 'removed' and 'killed-moribund' on the twelfth day. The terminology used in the software-'removal' and 'killing-moribund'-is ambiguous and fails to clarify whether any action was taken to alleviate the animal's suffering during this period. As per regulatory guidelines, an animal exhibiting significant neurological symptoms such as tremors, indicative of high drug toxicity, should be promptly removed from the study and provided with appropriate medical intervention. In this case, both the researcher and the clinical veterinarian failed to take timely action. Moreover, the software did not provide any detailed account of the animal's clinical parameters, additional symptoms, or medications administered. Although the clinical veterinarian claimed that such records were maintained on loose sheets using a fixed format, she was unable to produce the relevant documentation even after an extensive search. This raises serious concerns about the absence of evidence-based health monitoring or treatment interventions at PBPL."

		"The veterinary logs, maintained as loose case sheets, lack essential clinical details-such as observed clinical signs, diagnostic assessments, and medications administered. This incomplete and inconsistent documentation renders the recording system ineffective, offering no tangible benefit to the animals' health, treatment, or ongoing care."
Unauthorised Frequent Reuse of Animals for Experiments		"PBPL failed to demonstrate how many times individual animals were reused-a practice that requires specific approval from the CCSEA" "The inspection team was informed that animals across all species are reused in multiple experiments, including pharmacokinetic and toxicological studies. In the case of dogs and minipigs, it was claimed that a three-year usage period is followed, with intermittent 'washout period' of one month between experimental uses. However, no written policy documents, institutional protocols, or Standard Operating Procedures (SOPs) were provided to substantiate this claim. Practising a one-month 'washout period' is a violation of CCSEA guidelines for reuse/rehabilitation of large animals post experimentation (2020), which mandates a minimum of three months as a 'washout period'.

		It was conveyed that pharmacokinetic studies generally involve repeated use of the same animal, whereas toxicological studies are usually conducted only once per animal. The CCSEA guidelines for reuse/rehabilitation of large animals post experimentation (2020) mandate that animals showing liver or kidney impairment, within the three-year period, cannot be reused, and the detailed health status of all such animals shall be maintained in a prescribed format. However, in the absence of accessible records, there was no way to independently verify these practices."
Lack of Pain and Anxiety Management		"There was no protocol in place to address anxiety, fear, or psychological distress in animals-highlighting a grave neglect of mental welfare, and a veterinary protocol grossly failing to meet even the minimum required standards for the prevention of unnecessary suffering." "In a recent invasive experiment on two monkeys involving surgical implantation and daily wound care, only analgesics were used post procedure, with physical restraint applied without sedatives-indicating serious neglect of psychological welfare. Similarly, dogs euthanised at the end of research studies were not sedated prior to the administration of thiopentone sodium. These practices highlight critical flaws in the

		veterinary protocol, failing to meet even the basic standards for preventing unnecessary pain and suffering."
Lack of Suitable Veterinary Care, Infrastructure & Medicinal Stock		"[T]here was a complete absence of essential infrastructure-no dedicated quarantine areas, no isolation wards for sick animals, and no grooming or exercise facilities. This was consistent across all large animal species, including monkeys, dogs, sheep, minipigs, and pigs, and represents a systemic failure to uphold even the minimum standards of animal welfare." "The overall approach to animal welfare and veterinary care at PBPL, reflects a deeply troubling lack of commitment to the health and well-being of the animals in its custodv. The organisation appears to function primarily as a client-facing entity, with minimal regard for fundamental animal welfare principles, including the prevention of unnecessary pain, suffering, and distress." "Veterinary care infrastructure was deeply inadequate. The facility maintained minimal medical supplies, lacked essential analgesics, sedatives, and anaesthetics, and failed to maintain proper treatment records. Notably, no protocol was in place to manage anxiety, fear, or distress-an essential component of humane animal care. Painful and invasive procedures, such as those performed on monkeys involving surgical implantation,

		were conducted using only analgesics post procedure, with animals physically restrained without sedatives. Similarly, dogs euthanised at the conclusion of research were not sedated before the administration of thiopentone sodium. These practices reflect glaring omissions in veterinary planning and a disregard for psychological well-being." "Despite conducting procedures that are invasive or likely to cause physical and psychological distress, the clinical examination areas adjacent to the experimentation rooms were found to be unequipped with basic medical kits. Furthermore, the medical inventory lacked essential sedatives, analgesics, and anaesthetics-key components for preventing unnecessary pain and suffering in animals. No consolidated treatment records were maintained to document either pain recognition or pain management." "The medical inventory maintained by PBPL is grossly inadequate for a facility housing over 1,500 animals across various species. The central store contained only limited quantities of basic medications such as dewormers, multivitamins, and mineral supplements. Critically, there was no stock of essential medications such as sedatives, analgesics, or anaesthetics, raising grave concerns about the

		facility's ability to manage anxiety, fear, distress, pain, perform safe medical procedures, or carry out ethical clinical care. While the experimentation room includes a clinical veterinarian and an examination table, there were no emergency or pain-management medicines available at the site for immediate intervention. This further reinforces the perception that PBPL's role is largely confined to conducting studies that culminate in euthanasia, necropsy, and histopathological examination, rather than ensuring ongoing health and welfare." "Critically, both the breeding and experimentation centres lack essential veterinary medicines, including those necessary for emergency care, pain relief, or disease prevention. In the absence of these fundamental medical supplies, veterinarians are effectively unable to provide any meaningful treatment or alleviate unnecessary pain and suffering. As a result, there is no 24x7 functional veterinary system in place to safeguard the health and welfare of the large number of animals currently housed at PBPL."
Poor Quarantine, Disease Screening and Separation of Healthy and Sick Animals		"There was a total absence of dedicated quarantine rooms and isolation rooms for sick animals, which critically compromises biosecurity and disease management."

		“Across all facilities, it was reported that individual cages within shared housing rooms are being used as makeshift quarantine and isolation spaces. This practice falls far short of accepted quarantine protocols and fails to provide the critical separation needed to prevent cross-contamination. The absence of proper quarantine infrastructure in a facility housing over 1,500 animals reflects a serious disregard for both animal and human health and welfare. This concern is further exacerbated by the lack of on-site veterinary records, making it impossible to verify health screenings, disease surveillance, or any measures taken to address zoonotic risks.” “Several dogs from the breeding stock were found housed in two experimental facilities due to insufficient space in the designated breeding area- highlighting poor planning and inadequate resource management. Critically, these animals had not been screened for disease conditions prior to relocation, despite such screening being a mandatory prerequisite before introducing animals into experimental zones. This oversight raises serious concerns regarding contamination risks and compromised sterilisation standards. Additionally, some dogs were reportedly transferred for experimental procedures without visible tags or

		identifiers, making it impossible to trace individual histories or monitor their use--constituting a serious violation of standard compliance protocols." "PBPL informed that the current screening protocol for monkeys does not include Kyasanur Forest Disease (KFD)-a zoonotic infection known to be prevalent among monkeys in India. Considering that the monkeys are wild-caught, and in view of the potential biosecurity implications and associated health risks for researchers and staff, including KFD in the screening process would be a prudent and proactive measure."
Cruelty During Euthanasia Procedures	"SOP/PBS/PAT/012, Euthanasia of Laboratory Animals/Birds, does not contain sufficiently detailed procedures for administration of carbon dioxide (CO2) for inhalation euthanasia for rodents to fulfill the objective of rapid unconsciousness with minimal distress to the animals. For example, the SOP does not contain information about the settings of the flowmeter for the delivery of the CO2 gas or include specific procedures for using CO2 inhalation in rodents."	"The inspection also uncovered troubling deviations from approved euthanasia protocols. Animals were euthanised without sedation, relying solely on physical restraint-a practice incompatible with ethical norms of humane care." "The attending veterinarian confirmed that no sedatives are administered prior to euthanasia to mitigate fear, anxiety, or distress. Instead, thiopentone is injected slowly while an assistant physically restrains the animal-an approach the veterinarian himself acknowledged he would not use if the procedure were a routine surgery such as spaying or castration, or if the breed were less docile, such as a Bulldog,

		Dobermann, or Rottweiler. This underscores a troubling reliance on the naturally gentle and submissive temperament of Beagle dogs, which makes them easier to handle and restrain, even under distressing conditions, without adequate measures to reduce suffering." "Accidental pregnancy led to euthanasia of 8-I 0 piglets via intracardiac injection without prior sedation."
High Kill Rate & Only Performative Rehabilitation		"According to both records and the veterinarian in charge, approximately 30-40 dogs are euthanized each month." "The sheer number of euthanasia cases also suggests that a significant proportion of the animal population is being killed as part of experimental protocols. This may further explain why only 73 dogs were found in the rehabilitation section-a number that appears disproportionately low relative to the reported usage and turnover." "It was observed that the so-called 'rehabilitation area' appeared to be a makeshift arrangement, with a fresh paper label affixed to the door designating it as such. The space itself was evidently an experimental room repurposed as a rehabilitation unit, with no meaningful changes made to accommodate the specific needs of animals undergoing recovery. The environmental conditions

		infrastructure, routine practices, and personnel remained consistent with those of a laboratory setting raising serious concerns about the adequacy, appropriateness and sincerity of the rehabilitation process." "The environment was entirely artificial, with no access to natural light and fully temperature-controlled conditions. The flooring consisted of hard perforated polymer flooring with integrated drainage, which may cause discomfort, offering no physical comfort for the animals to stand, sit, or lie down for long periods. Critically, there was no provision for socialisation, environmental enrichment, or access to outdoor spaces-elements essential for the physical and psychological recovery of rehabilitating animals. The facility, as observed, fell significantly short of providing a conducive, humane, and restorative environment-undermining the very essence of what true rehabilitation should represent." "Dogs are currently rehabilitated within PBPL's own facility. No records were made available to the inspection team indicating that animals had been transferred to AWEI-recognised animal welfare organisations. Additionally, there was no documentation provided regarding any Memoranda of Understanding (MoUs) or financial support extended to

		such organisations for the long-term care of the animals." "The high euthanasia rate suggests an unsustainable use pattern where large numbers of animals are systematically killed after experimental use, with limited rehabilitation or rehoming efforts."
Untrained, Uncaring or Incompetent Personnel Negatively Impacting Animal Welfare & Experimentation Results & Poor Veterinary Oversight	"For Guinea Pig Maximization Test (GPMT) study 24838, the study personnel failed to identify and record adverse tissue responses to the injected adjuvant and clinical observations (e.g., difficulty breathing). SOP/PBS/TOX/008 indicates that after intradermal induction, skin reactions such as edema, erythema, and necrosis, along with other clinical signs will be recorded. However, the records that were reviewed did not indicate that clinical signs were recorded. In addition, there is no procedure that describes what clinical signs should be assessed to determine the health of the animal or how the technician would recognize skin reactions and distinguish between similar responses (e.g., between a "discrete" and "moderate" skin reaction). Furthermore, it was observed that study personnel training does not include species-specific in-life observations and when veterinarian oversight should be requested. Failure of testing facility management to assure that all personnel clearly understand the functions they are to perform and are adequately qualified and trained creates a high level of variability that does not ensure the validity and	"A serious welfare concern was observed when an animal handler lifted a heavy dog by the scruff and used a wiper to move the animal-an act carried out openly in front of the inspection team. The casual manner in which this was done suggests that such rough handling is a routine and accepted practice at PBPL. These actions are inappropriate and raise grave concerns about staff training, supervision, and basic regard for animal welfare." "Of the four veterinarians reportedly assigned to 13 experimental facilities, only two were present at the time of inspection-raising serious concerns about the adequacy of veterinary coverage and timely access to care. In the absence of regular veterinary supervision, dedicated treatment spaces, and structured welfare protocols, animals remain at significant risk of untreated medical issues and unnecessary suffering." "Veterinary care at PBPL is available only between 9:00 a.m. and 5:30 p.m., with no

	quality of the data. Personnel that do not clearly understand the functions they are to perform cannot consistently perform tasks according to the SOPs. This can have a negative impact on a study and calls into question the quality and integrity of studies conducted at your testing facility."	veterinarian coverage during night hours."
Unapproved Deviations from SOPs and Failures of the Quality Assurance Unit (QAU)	"For study 231918, the study plan stated that the rabbits should have a supraglottic airway device inserted; however, the surgical records showed that an endotracheal tube was used for delivery of inhalant anesthesia. There is no record to indicate that this deviation was identified by the QAU, and there is no documentation to indicate that this deviation was made with proper authorization. A reliable QAU is integral to the successful understanding and completion of any GLP study. Without appropriate QAU oversight, neither the sponsor nor FDA reviewers have assurance that the data in the final study report is accurate and valid. Failure to perform QAU functions can have a negative impact on a study and calls into question the quality and integrity of studies conducted at your testing facility." "The overall conditions and practices at your facility, as exemplified above, may impact the validity and integrity of the data obtained to support new animal drug applications."	
Poor Animal Housing, Lack of Enrichment & Filth	"FDA investigators also observed that the exterior of the testing facility had significant accumulations of dirt, animal droppings, and potential pest	"Housing conditions were consistently found to be overcrowded, barren, and inadequate, leading to significant welfare concerns such as

	harborage. Upon inspection of Block F, the HVAC equipment on the outside of the building was found to be surrounded by a large amount of dirt and debris that could attract and provide harborage for various pests and could potentially be caught up in the HVAC equipment, possibly causing failure. Such conditions may impact nonclinical studies conducted at your facility."	elevated stress, noise, poor body condition, and heightened risk of infectious diseases. Essential aspects such as environmental enrichment, social interaction, and proper bedding were either entirely absent or grossly insufficient across all species. The breeding facilities were particularly concerning, with overproduction of animals resulting in unauthorized repurposing of experimental spaces as stock rooms, unscreened animal transfers, and potential biosecurity risks." "Hygrometers installed in the breeding areas showed excessively high relative humidity levels, ranging from 80% to 97%, which can pose serious health risks to the animals."
Unapproved & Unauthorised Animals		"[T]he number of animals observed during the inspection did not align with the facility's declared housing capacity or the volume of CCSEA-approved experimental protocols. The presence of surplus, unscreened stock animals in experimentation rooms points to serious gaps in documentation and oversight." "The headcount and placement of dogs housed at PBPL indicate that the facility is exceeding the number approved by CCSEA, in direct violation of regulatory limits, which is 1000 dogs. This overpopulation appears to stem from breeding activities surpassing the number of animals required for ongoing

		experiments. As a result, two rooms--originally designated for experimentation and located in close proximity to active experimental areas-were repurposed as stock rooms to accommodate the surplus animals. Notably, this was done without screening the dogs for infectious diseases. Veterinarians at the facility stated that the rooms would be fumigated and sterilised before being returned to experimental use, however, even if this is outlined in the organisation's SOPs, reliance on such reactive measures raises concerns regarding the robustness of biosafety protocols." "The overall population of dogs far exceeds CCSEA-approved limits, with multiple species present without adequate disclosure or accurate record-keeping. Critical documentation - including consolidated animal inventories, veterinary treatment records, and breeding logs - was consistently absent, incomplete, or untraceable." "Company purchased Gottingen minipigs but lacked a license to breed them." "An overall high housing density of dogs was observed in the breeding modules, and excess breeding stocks were found to be housed in dog experimental areas." "A noticeable inconsistency was observed between the number of

		CCSEA-approved research protocols and the actual population of minipigs housed, which raises questions regarding breeding."
Animals in Poor Condition and Poor Handling		"None of the dogs in the breeding modules were provided with any form of bedding and were left to lie directly on slippery tiled floors-an inappropriate and uncomfortable surface that fails to meet even the most basic animal welfare requirements." "Dogs in poor body condition, including several exhibiting cherry eye, were observed at the breeding modules. However, due to the absence of consolidated medical records or documentation, there was no evidence of any treatment history or supportive interventions provided for these animals." "General body condition of minipigs appeared poor. However, due to absence of medical records on-site, the health status of minipigs could not be ascertained." "The body condition score of the cows was generally poor, with most animals appearing underweight and below the average standard." "During cleaning, nursing mothers and puppies were reportedly transferred to crates, some of which were found to be

		damaged-posing both hygiene and injury risks to the animals."
Inadequate Nutrition		"[A] single daily feeding is not aligned with standard welfare practices for laboratory-housed dogs, particularly Beagles, which benefit from multiple feedings and enrichment. Thus, the current feeding regime may contribute to nutritional imbalance and does not reflect best practices in animal nutrition and welfare management."
High Noise Levels		"The constant noise from continuous barking created an environment with dangerously high noise levels, indicative of widespread stress and discomfort among the animals. Alarmingly, the facility manager was unable to provide the exact number of dogs housed in the breeding section, and no records were available for verification."
Elaboration of Poor Housing Conditions & Filth		"The kennels in the dog breeding section were generally dirty, soiled with faeces, and poorly maintained. The overall environment of the dog breeding units was uninviting and clearly neglected, reflecting a troubling disregard for the basic care, hygiene, and welfare needs of the animals." "As outlined in the section on housing conditions for animals bred and used in experiments, while the space provided is generally inadequate, the breeding facilities also Jacked proper ventilation and were marked by poor hygiene

		standards. In contrast, the experimental facility showed marginal improvements in air-conditioning and cleanliness; however, fundamental welfare concerns persisted across both settings."
Elaboration of Poor Enrichment & Performative Socialisation Facilities		"[The dogs] were not provided with any outdoor access or designated free time. While facility staff claimed that animals were let out during cleaning, a review of CCTV footage did not show dogs being allowed out for play or exercise, raising doubts about the accuracy of these claims." "No environmental enrichment was provided, except few plastic bones-there were no toys, stimulation objects, or oppmtunities for social interaction. According to staff, the dogs were only let out of their cages during cleaning, indicating a highly restrictive and unstimulating environment with extremely limited chances for exercise or socialisation." "The designated socialisation area for dogs measured approximately 550 square metres, was barren, and had a hard concrete surface. Given that the facility houses over 1,000 dogs, each individual may have to wait weeks or even months for a single opportunity to access this limited space-rendering it

		functionally ineffective in promoting socialisation or improving welfare." "PBPL housed a range of animals-including dogs, monkeys, minipigs, pigs, and sheep-but all were confined exclusively to cages, with no access to open or enriched environments even when they were housed for more than three months, sometimes exceeding nine months. While some dogs and monkeys were housed in same-sex pairs, these arrangements are insufficient to support the natural social behaviours characteristic of these species." "Critically, there were no dedicated outdoor enclosures or exercise facilities for non-human primates. This deprived the monkeys of any oppo1iunity for natural movement, physical exercise, or cognitive stimulation. The lack of outdoor access and meaningful enrichment across species poses a significant risk to both the psychological welfare and behavioural health of the animals in PBPL's care." "Environmental enrichment across all animal housing areas was grossly inadequate. In the experimental rooms for dogs, a few plastic bones were loosely scattered in the corridors. These rigid and repetitive items lacked the novelty or functionality to effectively engage the animals. No other enrichment tools or

		activities were present. Similarly, only a few minipigs were provided with enrichment in the form of cut PVC pipe sections-simple items that failed to sustain their interest or encourage exploratory behaviour. In the monkey enclosures, circular rings were suspended as the only form of enrichment. However, these minimal features were clearly insufficient to meet the cognitive and physical needs of the primates, particularly given their confinement to small cages, either alone or in same-sex pairs." "The housing for minipigs featured polymer flooring with rectangular drainage openings, which are unsuitable for 24 X 7 housing of cloven-footed animals to stand or lie down comfortably. No meaningful environmental enrichment was provided…A few minipigs were offered minimal enrichment in the form of cut PVC pipe sections, including L-shaped bends. However, these were significantly undersized relative to the pigs' body dimensions, and the design posed a clear risk of choking or injury, as the openings were small enough that animals could potentially attempt to insert their heads. This highlights a lack of considered design and a failure to meet even the most basic behavioural and welfare needs of the animals."

		“Each sheep was individually caged without any form of environmental enrichment. The complete absence of social interaction or sensory stimulation raised significant concerns about their welfare. They were kept on polymer flooring with drainage openings, which is inappropriate for 24 X 7 housing of cloven hooves and overall physiology.” “The enclosures housing the monkeys offered little room for natural movement or social interaction. There were no dedicated outdoor enclosures or exercise facilities. The narrow metal platforms inside the cage made it difficult for the animals to sit or lie down comfortably, raising serious concerns about their physical comfort and overall welfare. The only form of enrichment observed was a single coloured ring suspended in each enclosure-an effort that proved grossly inadequate, failing to provide any meaningful cognitive stimulation or physical engagement.” “Twelve cows were housed in a makeshift cattle shed with minimal infrastructure and inadequate protection from the elements. Continuous rainfall had led to water accumulation in parts of the shed, creating damp and unsanitary conditions.”

Dishonesty		"Pigs (White Yorkshire Mixed Breeds): These animals were not initially disclosed to the inspection team, despite repeated inquiries. Their presence came to light only incidentally during a meeting, when scientists-while discussing ongoing cardioiogy-related studies such as pacemaker development-unincentionally acknowledged their use." "Contrary to the facility's initial claim that no sheep were present, seven sheep were discovered by the inspection team in the experimental section during a visit to the mixed-breed pigs. This unreported presence reflects a serious disregard for regulatory compliance and a failure to meet the basic norms prescribed by CCSEA." "CCTV footage was not made available to the CCSEA inspection team despite multiple formal .and verbal requests on the day of the visit, as well as prior intimation through an official CCSEA letter…[D]despite repeated and specific requests, the team was not shown any recordings from the corridors of the dog breeding stock areas. Staff claimed that no cameras were installed in those particular locations, leaving a critical gap in visual documentation. Similarly, when the team requested footage from the

		rehabilitation area, animal entry, and the dirty corridors of the experimental housing zones, they were again informed that no CCTV cameras had been installed in those areas either." "The inspection team is of the opinion that this lack of access to key CCTV footage, combined with the absence of camera coverage in critical areas, indicates a deliberate attempt to withhold or tamper with evidence related to potential animal welfare violations."
Acknowledgement of Likelihood of Other Problems	"The violations described above are not intended to be an all-inclusive list of problems that may exist with your facility."	"While the report highlights key gaps and deficiencies observed during the inspection, it is not intended to serve as an exhaustive documentation of all operational procedures or standard practices at PBPL."

Annexure C:

17 June 2025 Inspection Report by Government appointed Inspectors

Report on the Inspection of Palamur Biosciences Private Limited

Submitted on 17th June 2025

Disclaimer

This report has been prepared by the inspection team tasked with conducting a fact-based verification into allegations of cruelty to animals and serious non-compliance with regulatory requirements at Palamur Biosciences Pvt. Ltd. (PBPL). The inspection was undertaken with the objective of assessing the facility's adherence to applicable animal welfare laws, guidelines, and standards.

The observations and findings recorded herein are focused on areas of concern that warrant immediate attention and remedial action. While the report highlights key gaps and deficiencies observed during the inspection, it is not intended to serve as an exhaustive documentation of all operational procedures or standard practices at PBPL.

This report represents the considered findings of the inspection team, based on direct observations, review of documents, information made available by PBPL representatives, and other relevant evidence gathered during the course of the visit. It is submitted to the CCSEA for further examination and appropriate action under the relevant regulatory provisions.

I. Executive Summary

The inspection of Palamur Biosciences Pvt. Ltd. (PBPL) on June 11, 2025 and June 12, 2025, conducted by a multidisciplinary team comprising members from the Committee for the Control and Supervision of Experiments on Animals (CCSEA), the Animal Welfare Board of India (AWBI), the Institutional Animal Ethics Committee (IAEC), and Humane World for Animals India Foundation, was initiated to verify recent allegations of animal welfare violations involving dogs, pigs, and, monkeys used in research during the period 2021–2023.

PBPL currently houses a substantial number of animals: approximately 1,169 dogs, along with monkeys, pigs (including minipigs and mixed breeds), sheep, cattle, and an undetermined number of rodents and lagomorphs (rabbits). The overall population of dogs far exceeds CCSEA-approved limits, with multiple species present without adequate disclosure or accurate record-keeping. Critical documentation - including consolidated animal inventories, veterinary treatment records, and breeding logs - was consistently absent, incomplete, or untraceable.

The inspection revealed serious and widespread non-compliance with CCSEA regulations. Key welfare violations included overcrowded and barren kennels, lack of environmental enrichment, feeding practices not aligned with the animals' physiological needs and body weight requirements, untrained and rough handling practices, and an alarming absence of protocols for pain management, sedation, and euthanasia. Veterinary infrastructure was critically inadequate, with poor medical coverage, minimal drug availability, and no functioning isolation or quarantine facilities.

Particularly disturbing were the euthanasia practices observed: beagle dogs were euthanised using thiopentone sodium without prior sedation, and monkeys subjected to invasive surgical procedures involving implantation and daily wound care were physically restrained using gloves, with only analgesics administered post-procedure and dressing, and no sedatives provided. There was no protocol in place to address anxiety, fear, or psychological distress in animals—highlighting a grave neglect of mental welfare, and a veterinary protocol grossly failing to meet even the minimum required standards for the prevention of unnecessary suffering.

Furthermore, deliberate obfuscation was evident in PBPL's failure to provide CCTV footage from critical areas and in the non-disclosure of certain species during the inspection. Inconsistencies between reported study approvals and the actual number of animals on-site strongly suggest potential regulatory breaches.

The inspection team concluded that many of the allegations raised by PETA India's whistle-blower—including overcrowding, veterinary neglect, inappropriate handling, and euthanasia violations—were substantiated or could not be conclusively refuted due to the absence of required documentation.

Overall, the findings reflect a systemic and ongoing disregard for regulatory compliance, ethical responsibility, and animal welfare. Immediate regulatory action is warranted, including the removal and rehabilitation of animals in order to prevent further unnecessary pain and suffering, as well as a review of PBPL's registration and breeding licence status.

II. Introduction

1. **Date of Inspection:** 11th and 12th June 2025

2. **Time of Inspection:** On-site from 2:30 PM to 11:30 PM on 11th June; remote inspection from 10:00 AM to 12:00 PM on 12th June

3. **Name of Institution:** Palamur Biosciences Pvt. Ltd.

4. **Type of Institution:** Private

5. **Location:** Karvina, Madigattla Village, Bhoothpur Mandal, Mahabubnagar 509 382, Telangana, India

6. **Purpose of Inspection:**
   To verify complaints regarding the alleged abuse and neglect of dogs, pigs, and monkeys used in research and testing at Palamur Biosciences Pvt. Ltd. (*hereinafter referred to as PBPL*), Mahabubnagar, Telangana, during the period 2021–2023. The inspection also aimed to assess the overall conditions and practices related to animal care and use at the facility.

7. **Inspectors:**
   - Dr. Mukesh Kumar Gupta – Member, CCSEA & Director, ICMR-NARFBR, Hyderabad
   - Dr. Manilal Valliyate – Member, AWBI
   - Dr. Vivek Tyagi – Senior Consultant, CCSEA
   - Dr. B.D.P. Kala Kumar – Main Nominee, IAEC
   - Shri A. Madhava Rao – Socially Aware Nominee, IAEC
   - Ms. Alokparna Sengupta – Managing Director, Humane World for Animals India Foundation (formerly known as Humane Society International/India)

III. Animal Use Details

1. **Species Used in Experiments:**
The facility reportedly uses dogs, pigs (minipigs and mixed breed pigs), sheep, cattle, monkeys, and other species for experimental purposes.

Species-wise Distribution of Animals Housed at the Time of Inspection
It is physically not feasible for the inspection team to individually count the animals or determine their sex and age in the absence of any records provided by the facility. However, this exercise was carried out for the dogs and cattle at the housing facilities that were shown to the inspectors, though the numbers observed may not accurately represent the total number of dogs being housed or used by the facility.

Detail of animals	Species	Number	Sex	Age	Remarks
Monkeys	*Macaca mulata*	No record	No record	No record	Not counted by inspectors.
Pigs	*Sus scrofa domestica* and mixed species	No record	No record	No record	Not counted by inspectors.
Dogs	*Canis lupus familiaris*	No record	No record	No record	1169 numbers based on the headcount conducted by the inspectors at the facilities that were shown.
Cattle	*Bos Indicus*	No record	No record	No record	12 numbers based on head count.
Sheep	*Ovis aries*	No record	No record	No record	Not counted by inspectors.
Rodents [Mice, rats, rabbits]	No records shown, and no animals were presented for inspection	-	-	-	No inspection was carried out, as it was not a part of the mandate given to the inspection team by CCSEA.

PBPL failed to provide any documentation detailing the number, age, sex, or species-wise inventory of animals held or used at the facility, despite repeated requests. Although staff acknowledged the existence of an internal Excel spreadsheet containing this information, it was never shared with the inspection team.

Headcount and Observations from the Inspection

Facility	Facility Name	Number of kennels/ cages/ enclosures	No of animals	Remarks
Dogs				Headcount done by inspectors
Breeding Facilities	Module - A	40	85	
	Module - B		200	30 adults + 170 pups
	Module - C		122	36 adults + 86 pups
	Module - D	36	95	
	Maternity (MAT-1)	44	132	Pups 2-4 months
	Maternity (MAT- II)	45	126	Pups above 4 months
	Stock	42	61	Pups above 5 months
Experimentation Facilities	Experiment Room 2		32	
	Experiment Room 3		39	Stock animals accommodated without prior screening
	Experiment Room 6		40	
	Experiment Room 7		62	
	Experiment Room 10		40	
	Experiment Room 11		16	
	Experiment Room 13		46	Stock animals accommodated without Prior screening
	Rehabilitation		73	62 male + 11 female
		Total	**1169**	**594 adults + 575 pups**
Minipigs			14	9 male + 5 female
Non-Humane Primate			17	13 male + 4 female
Mixed-Breed Pig			13	As informed verbally
Sheep			7	As informed verbally
Cattle			12	Headcount done
Rodents & Others			Unknown	
		Grand Total	**1232 +**	Unknown number of rodents and others.

2. **Non-compliances in Animal Use**
The headcount and placement of dogs housed at PBPL indicate that the facility is exceeding the number approved by CCSEA, in direct violation of regulatory limits, which is 1000 dogs. This overpopulation appears to stem from breeding activities surpassing the number of animals required for ongoing experiments. As a result, two rooms—originally designated for experimentation and located in close proximity to active experimental areas—were repurposed as stock rooms to accommodate the surplus animals. Notably, this was done without screening the dogs for infectious diseases. Veterinarians at the facility stated that the rooms would be fumigated and sterilised before being returned to experimental use; however, even if this is outlined in the organisation's SOPs, reliance on such reactive measures raises concerns regarding the robustness of biosafety protocols.

3. **Number of Animals Currently Under Rehabilitation**
At the time of inspection, 73 dogs-comprising 62 males and 11 females-were reported to be under rehabilitation. This information was provided verbally, with no supporting written documentation shared by the facility. However, a headcount conducted by the inspectors confirmed the reported total.

It was observed that the so-called "rehabilitation area" appeared to be a makeshift arrangement, with a fresh paper label affixed to the door designating it as such. The space itself was evidently an experimental room repurposed as a rehabilitation unit, with no meaningful changes made to accommodate the specific needs of animals undergoing recovery. The environmental conditions, infrastructure, routine practices, and personnel remained consistent with those of a laboratory setting, raising serious concerns about the adequacy, appropriateness and sincerity of the rehabilitation process.

4. **Number of Animals Reused for Experimentation**
The inspection team was informed that animals across all species are reused in multiple experiments, including pharmacokinetic and toxicological studies. In the case of dogs and minipigs, it was claimed that a three-year usage period is followed, with intermittent "washout period" of one month between experimental uses. However, no written policy documents, institutional protocols, or Standard Operating Procedures (SOPs) were provided to substantiate this claim. Practising a one-month "washout period" is a violation of CCSEA guidelines for reuse/rehabilitation of large animals post experimentation (2020), which mandates a minimum of three months as a "washout period".

It was conveyed that pharmacokinetic studies generally involve repeated use of the same animal, whereas toxicological studies are usually conducted only once per animal. The CCSEA guidelines for reuse/rehabilitation of large animals post experimentation (2020) mandate that animals showing liver or kidney impairment, within the three-year period, cannot be reused, and the detailed health status of all such animals shall be maintained in a prescribed format. However, in the absence of accessible records, there was no way to independently verify these practices.

IV. Compliance to CCSEA Mandates

Sl. No.	Particulars	Yes/ No	Remarks
1.	Registration with CPCSEA for experimentation	Yes	
2.	Registration with CPCSEA for breeding animals for experimentation	Yes	
3.	Whether 3R principles followed	No	Reduction in animal use is a principle overseen by the CCSEA during the review and approval of research proposals. However, PBPL failed to demonstrate how many times individual animals were reused—a practice that requires specific approval from the CCSEA. The absence of such consolidated records strongly suggests non-compliance with regulatory requirements. As for the principle of Refinement, there appears to be a complete disregard. Despite conducting procedures that are invasive or likely to cause physical and psychological distress, the clinical examination areas adjacent to the experimentation rooms were found to be unequipped with basic medical kits. Furthermore, the medical inventory lacked essential sedatives, analgesics, and anaesthetics—key components for preventing unnecessary pain and suffering in animals. No consolidated treatment records were maintained to document either pain recognition or pain management.
4.	Housing facilities for animals being bred	No	During the inspection of the dog breeding facilities at PBPL, several serious violations of housing and welfare standards were noted.

			None of the dogs in the breeding modules were provided with any form of bedding and were left to lie directly on slippery tiled floors—an inappropriate and uncomfortable surface that fails to meet even the most basic animal welfare requirements. No environmental enrichment was provided, except few plastic bones—there were no toys, stimulation objects, or opportunities for social interaction. According to staff, the dogs were only let out of their cages during cleaning, indicating a highly restrictive and unstimulating environment with extremely limited chances for exercise or socialisation. The constant noise from continuous barking created an environment with dangerously high noise levels, indicative of widespread stress and discomfort among the animals. Alarmingly, the facility manager was unable to provide the exact number of dogs housed in the breeding section, and no records were available for verification. The kennels in the dog breeding section were generally dirty, soiled with faeces, and poorly maintained. The overall environment of the dog breeding units was uninviting and clearly neglected, reflecting a troubling disregard for the basic care, hygiene, and welfare needs of the animals. Hygrometers installed in the breeding areas showed excessively high relative humidity levels, ranging from 80% to 97%, which can pose serious health risks to the animals. The designated socialisation area for dogs measured approximately 550 square metres, was barren,

			and had a hard concrete surface. Given that the facility houses over 1,000 dogs, each individual may have to wait weeks or even months for a single opportunity to access this limited space—rendering it functionally ineffective in promoting socialisation or improving welfare. In summary, the breeding modules for dogs at PBPL were found to be uncomfortable, poorly enriched, inadequately managed, and not aligned with the minimum standards of care expected for breeding animals.
5.	Housing facilities for animals being experimented upon	No	During the inspection of the experimental housing areas at PBPL, several serious concerns were identified regarding housing conditions, animal allocation, and the absence of species-appropriate enrichment. PBPL housed a range of animals—including dogs, monkeys, minipigs, pigs, and sheep—but all were confined exclusively to cages, with no access to open or enriched environments even when they were housed for more than three months, sometimes exceeding nine months. While some dogs and monkeys were housed in same-sex pairs, these arrangements are insufficient to support the natural social behaviours characteristic of these species. Critically, there were no dedicated outdoor enclosures or exercise facilities for non-human primates. This deprived the monkeys of any opportunity for natural movement, physical exercise, or cognitive stimulation. The lack of outdoor access and meaningful enrichment across species poses a significant risk to both the psychological welfare and behavioural health of the animals in PBPL's care.

			Environmental enrichment across all animal housing areas was grossly inadequate. In the experimental rooms for dogs, a few plastic bones were loosely scattered in the corridors. These rigid and repetitive items lacked the novelty or functionality to effectively engage the animals. No other enrichment tools or activities were present. Similarly, only a few minipigs were provided with enrichment in the form of cut PVC pipe sections—simple items that failed to sustain their interest or encourage exploratory behaviour. In the monkey enclosures, circular rings were suspended as the only form of enrichment. However, these minimal features were clearly insufficient to meet the cognitive and physical needs of the primates, particularly given their confinement to small cages, either alone or in same-sex pairs. **Dogs:** The dog housing units in the experimental section were equipped with artificial lighting and temperature-controlled environments; however, there was a complete absence of meaningful environmental enrichment. Plastic bones were haphazardly placed in the corridors but offered no meaningful engagement or stimulation for the animals. Several dogs from the breeding stock were found housed in two experimental facilities due to insufficient space in the designated breeding area—highlighting poor planning and inadequate resource management. Critically, these animals had not been screened for disease conditions prior to relocation, despite such screening being a mandatory prerequisite before introducing animals into experimental zones. This oversight raises serious concerns regarding contamination risks and compromised sterilisation

			standards. Additionally, some dogs were reportedly transferred for experimental procedures without visible tags or identifiers, making it impossible to trace individual histories or monitor their use—constituting a serious violation of standard compliance protocols. **Minipigs:** The housing for minipigs featured polymer flooring with rectangular drainage openings, which are unsuitable for 24 X 7 housing of cloven-footed animals to stand or lie down comfortably. No meaningful environmental enrichment was provided. Although it was unclear whether these pigs were actively being used for experimental procedures, facility staff informed the inspection team that they had been imported from Denmark. A few minipigs were offered minimal enrichment in the form of cut PVC pipe sections, including L-shaped bends. However, these were significantly undersized relative to the pigs' body dimensions, and the design posed a clear risk of choking or injury, as the openings were small enough that animals could potentially attempt to insert their heads. This highlights a lack of considered design and a failure to meet even the most basic behavioural and welfare needs of the animals. **Pigs (White Yorkshire Mixed Breeds):** These animals were not initially disclosed to the inspection team, despite repeated inquiries. Their presence came to light only incidentally during a meeting, when scientists—while discussing ongoing cardiology-related studies such as pacemaker development—unintentionally acknowledged their use. The pigs were housed in enclosures similar in design to those used for dogs, albeit larger

			in size. They were kept in isolated conditions on polymer flooring with drainage openings, which is inappropriate for 24 X 7 housing of cloven hooves and overall physiology. Furthermore, the absence of species-specific environmental enrichment highlighted a broader disregard for basic welfare standards. **Sheep:** Contrary to the facility's initial claim that no sheep were present, seven sheep were discovered by the inspection team in the experimental section during a visit to the mixed-breed pigs. This unreported presence reflects a serious disregard for regulatory compliance and a failure to meet the basic norms prescribed by CCSEA. Each sheep was individually caged without any form of environmental enrichment. The complete absence of social interaction or sensory stimulation raised significant concerns about their welfare. They were kept on polymer flooring with drainage openings, which is inappropriate for 24 X 7 housing of cloven hooves and overall physiology. **Monkeys:** The enclosures housing the monkeys offered little room for natural movement or social interaction. There were no dedicated outdoor enclosures or exercise facilities. The narrow metal platforms inside the cage made it difficult for the animals to sit or lie down comfortably, raising serious concerns about their physical comfort and overall welfare. The only form of enrichment observed was a single coloured ring suspended in each enclosure—an effort that proved grossly inadequate, failing to provide any meaningful cognitive stimulation or physical engagement.

			Cattle Twelve cows were housed in a makeshift cattle shed with minimal infrastructure and inadequate protection from the elements. Continuous rainfall had led to water accumulation in parts of the shed, creating damp and unsanitary conditions.
6.	Housing facilities for animals being rehabilitated	No	**Dogs:** The rehabilitation facility for dogs was found to be a small, corner room located on the first floor of the building. It was observed that the so-called "rehabilitation area" appeared to be a makeshift arrangement, with a fresh paper label affixed to the door designating it as such. The room was evidently an experimental space that had been repurposed, with no meaningful changes made to support the functional and welfare needs of animals in recovery. The environment was entirely artificial, with no access to natural light and fully temperature-controlled conditions. The flooring consisted of hard perforated polymer flooring with integrated drainage, which may cause discomfort, offering no physical comfort for the animals to stand, sit, or lie down for long periods. Critically, there was no provision for socialisation, environmental enrichment, or access to outdoor spaces—elements essential for the physical and psychological recovery of rehabilitating animals. The facility, as observed, fell significantly short of providing a conducive, humane, and restorative environment—undermining the very essence of what true rehabilitation should represent.
7.	Identification of animals	Yes	Dogs at the breeding centre were reported to be microchipped and tagged with neck chains; pigs and

			sheep were reported to be tagged with RFID devices, while monkeys were reported to be microchipped.
8.	General condition of animals observed	No	Dogs in poor body condition, including several exhibiting cherry eye, were observed at the breeding modules. However, due to the absence of consolidated medical records or documentation, there was no evidence of any treatment history or supportive interventions provided for these animals. General body condition of minipigs appeared poor. However, due to absence of medical records on-site, the health status of minipigs could not be ascertained. The body condition score of the cows was generally poor, with most animals appearing underweight and below the average standard.
9.	Trained staff/handlers	No	A serious welfare concern was observed when an animal handler lifted a heavy dog by the scruff and used a wiper to move the animal—an act carried out openly in front of the inspection team. The casual manner in which this was done suggests that such rough handling is a routine and accepted practice at PBPL. These actions are inappropriate and raise grave concerns about staff training, supervision, and basic regard for animal welfare. While a few dogs appeared fearful, most seemed relatively at ease around humans, indicating inconsistent and largely unmonitored handling practices across the facility.
10.	Restraint	No	All dogs at the facility were housed in individual cages with no visible form of physical restraint within the enclosures. However, they were not provided with any outdoor access or designated free time. While facility staff claimed

			that animals were let out during cleaning, a review of CCTV footage did not show dogs being allowed out for play or exercise, raising doubts about the accuracy of these claims. During cleaning, nursing mothers and puppies were reportedly transferred to crates, some of which were found to be damaged—posing both hygiene and injury risks to the animals. Of particular concern was the procedure followed during euthanasia. The veterinarian responsible openly stated that sedatives were not used prior to administering thiopentone sodium to dogs. Instead, the dogs were manually restrained before injection. This approach fails to account for the fear, anxiety, and distress experienced by the animals during the procedure and demonstrates a serious violation of accepted veterinary protocols and ethical standards set by CCSEA for euthanasia.
11.	Record	No	There is a glaring absence of a proper record-keeping system to ensure the health and welfare of animals in the custody of PBPL.
	a. Permits (breeding, use and reuse)	Yes	PBCL shared the approval orders.
	b. Procurement records	No	PBPL failed to furnish any documentation or records to substantiate otherwise.
	c. Breeding record	No	PBPL failed to furnish any documentation or records to substantiate otherwise.
	d. Health records (for each animal)	No	The available documentation at PBPL consists of loose paper sheets—standard forms filled out seemingly to meet the requirements of the contracting client. These documents capture isolated cases or incidents and are submitted to the record room immediately after data entry.

			Critically, they fail to provide any comprehensive overview of essential information such as the total number of animals used, the frequency of their use in experiments, clinical conditions identified, or the preventive and therapeutic care administered—whether at the breeding facility or the experimentation centre. This fragmented and superficial record-keeping reflects a seriously negligent approach to both regulatory compliance and animal welfare standards. Moreover, veterinary records were not available on-site, significantly hampering the ability to conduct thorough inspections or continuous assessments of animal health and well-being. Without access to these records, it is impossible to monitor medical histories, vaccination status, or previous treatments—elements that are vital to ensuring timely and appropriate veterinary care. The absence of a structured, accessible veterinary documentation system undermines the facility's responsibility to safeguard the animals in its custody.
	e. Sale & transfer records	No	PBPL failed to furnish any documentation or records to substantiate otherwise.
	f. Surveillance records	No	PBPL failed to furnish any documentation or records to substantiate otherwise
	g. Rehabilitation cost records (if any; please state if no cost is undertaken)	No	PBPL failed to furnish any documentation or records to substantiate otherwise. However, this may be verified through the CCSEA, as such reports are mandatorily required to be submitted to them.
12.	Quarantine protocols	No	There is a complete absence of dedicated quarantine facilities across all animal housing units at PBPL, including those for monkeys, dogs, sheep, minipigs,

			and mixed-breed pigs. No separate rooms or designated areas have been established for quarantining new arrivals or isolating potentially sick animals, posing a significant risk to animal health, biosecurity, and disease containment. Primates (*Macaca mulatta*) are sourced from CCSEA-approved vendors and are wild-caught. PBPL informed that the current screening protocol for monkeys does not include Kyasanur Forest Disease (KFD)—a zoonotic infection known to be prevalent among monkeys in India. Considering that the monkeys are wild-caught, and in view of the potential biosecurity implications and associated health risks for researchers and staff, including KFD in the screening process would be a prudent and proactive measure. Across all facilities, it was reported that individual cages within shared housing rooms are being used as makeshift quarantine and isolation spaces. This practice falls far short of accepted quarantine protocols and fails to provide the critical separation needed to prevent cross-contamination. The absence of proper quarantine infrastructure in a facility housing over 1,500 animals reflects a serious disregard for both animal and human health and welfare. This concern is further exacerbated by the lack of on-site veterinary records, making it impossible to verify health screenings, disease surveillance, or any measures taken to address zoonotic risks.
13.	Welfare, care & veterinary access	No	The overall approach to animal welfare and veterinary care at PBPL reflects a deeply troubling lack of commitment to the health and well-being of the animals in its custody. The organisation

			appears to function primarily as a client-facing entity, with minimal regard for fundamental animal welfare principles, including the prevention of unnecessary pain, suffering, and distress. An anxiety, fear, and distress management protocol is not in place. The experiment conducted on two monkeys—involving an incision near the scapula and insertion of a medicinal repository—is a painful and invasive procedure requiring ongoing wound management. Despite this, the treatment protocol includes only the use of analgesics post procedure completion, while the animals are physically restrained by staff using protective gloves, without the administration of sedatives. This represents a serious lapse in addressing the psychological well-being of animals used in experimentation. Similarly, as reported by the facility's veterinarians, dogs euthanised at the conclusion of research studies are not sedated prior to the administration of thiopentone sodium. Taken together, these practices point to a poorly designed veterinary protocol that fails to adequately safeguard animal welfare during both research procedures and routine veterinary interventions. A critical gap lies in the absence of a functional system for recording preventive healthcare and treatment interventions. No accessible, structured on-site veterinary documentation was available, and existing loose case sheets are reportedly stored in a separate building—severely limiting timely medical assessments and ongoing veterinary oversight. This lack of accessible records undermines the ability to monitor animal health, track vaccination and treatment

			histories, or assess compliance with humane care and regulatory norms. The medical inventory maintained by PBPL is grossly inadequate for a facility housing over 1,500 animals across various species. The central store contained only limited quantities of basic medications such as dewormers, multivitamins, and mineral supplements. Critically, there was no stock of essential medications such as sedatives, analgesics, or anaesthetics, raising grave concerns about the facility's ability to manage anxiety, fear, distress, pain, perform safe medical procedures, or carry out ethical clinical care. While the experimentation room includes a clinical veterinarian and an examination table, there were no emergency or pain-management medicines available at the site for immediate intervention. This further reinforces the perception that PBPL's role is largely confined to conducting studies that culminate in euthanasia, necropsy, and histopathological examination, rather than ensuring ongoing health and welfare. Environmental conditions within the facility were also suboptimal. The breeding facility recorded elevated humidity across all areas remained around 86%. Moreover, there was a complete absence of essential infrastructure—no dedicated quarantine areas, no isolation wards for sick animals, and no grooming or exercise facilities. This was consistent across all large animal species, including monkeys, dogs, sheep, minipigs, and pigs, and represents a systemic failure to uphold even the minimum standards of animal welfare. Of the four veterinarians reportedly assigned to 13

			experimental facilities, only two were present at the time of inspection—raising serious concerns about the adequacy of veterinary coverage and timely access to care. In the absence of regular veterinary supervision, dedicated treatment spaces, and structured welfare protocols, animals remain at significant risk of untreated medical issues and unnecessary suffering. In conclusion, the conditions observed at PBPL point to substantial deficiencies in veterinary access, preventive healthcare, and critical welfare infrastructure. These shortcomings compromise both the physical well-being and dignity of the animals and present serious ethical and regulatory concerns that warrant urgent attention.
14.	All experiments conducted/being conducted are approved?	Inconclusive	PBPL failed to furnish any documentation or records to substantiate otherwise.
15.	Segregated housing for rehabilitated vs experimental animals?	Yes	Although the rehabilitation facility is separate from the experimental units, the housing conditions in both are virtually identical—enclosed, temperature-controlled rooms where animals are confined to cages without any form of environmental enrichment. Consequently, the quality of life in the so-called rehabilitation setting is indistinguishable from that of the experimental facility and falls significantly short of the fundamental principles and intended goals of true rehabilitation.
16.	Adequate shelter (space, ventilation, hygiene)?	No	As outlined in the section on housing conditions for animals bred and used in experiments, while the space provided is generally inadequate, the breeding facilities also lacked proper ventilation and were marked by poor hygiene standards. In contrast, the experimental facility

			showed marginal improvements in air-conditioning and cleanliness; however, fundamental welfare concerns persisted across both settings.
17.	Clean water and species-appropriate food available?	No	Water was provided via a drip pipe system. All species were offered packaged dry commercial feed: dogs received 300 grams once daily; minipigs were provided 500 grams per day; and monkeys were fed 150 grams of pellets along with fruits and a bun each day. In the case of dogs—specifically adult Beagles—the fixed ration of 300 grams of dry commercial pellets per day is likely insufficient to meet their daily caloric and nutritional requirements. Moreover, a single daily feeding is not aligned with standard welfare practices for laboratory-housed dogs, particularly Beagles, which benefit from multiple feedings and enrichment. Thus, the current feeding regime may contribute to nutritional imbalance and does not reflect best practices in animal nutrition and welfare management.
18.	Veterinary care accessible at all times?	Inadequate	Veterinary care at PBPL is available only between 9:00 a.m. and 5:30 p.m., with no veterinarian coverage during night hours. Although technicians are reportedly present on campus overnight, they are not stationed on the animal floors. Critically, both the breeding and experimentation centres lack essential veterinary medicines, including those necessary for emergency care, pain relief, or disease prevention. In the absence of these fundamental medical supplies, veterinarians are effectively unable to provide any meaningful treatment or alleviate unnecessary pain and suffering. As a result, there is no 24x7 functional veterinary system in place to safeguard the health and

			welfare of the large number of animals currently housed at PBPL.
19.	Daily monitoring and health logs maintained?	No	The veterinary logs, maintained as loose case sheets, lack essential clinical details—such as observed clinical signs, diagnostic assessments, and medications administered. This incomplete and inconsistent documentation renders the recording system ineffective, offering no tangible benefit to the animals' health, treatment, or ongoing care.
20.	Animals being reused are healthy and with approved?	No	PBPL failed to furnish any documentation or records to substantiate otherwise. A detailed micro-audit is necessary to determine the frequency of reuse of individual animals and to assess whether such practices are in compliance with the specific permissions granted by the CCSEA.
21.	Was CCTV footage made available and accessible during the inspection, and were there any notable findings or issues observed?	No	CCTV footage was not made available to the CCSEA inspection team despite multiple formal and verbal requests on the day of the visit, as well as prior intimation through an official CCSEA letter. The team was later informed that the designated custodian of the CCTV system was unavailable, and therefore, recordings could not be accessed during the inspection—even though senior management was present and expressed helplessness in resolving the issue. Management subsequently assured the team that online access to the CCTV footage would be facilitated the following morning, once the operator was on duty at 9 a.m. During a Microsoft Teams meeting held the next day, the dashboard monitor displaying live CCTV camera feeds was shared with the inspectors. However, despite repeated and specific requests, the team was not shown any recordings from the corridors

			of the dog breeding stock areas. Staff claimed that no cameras were installed in those particular locations, leaving a critical gap in visual documentation. Similarly, when the team requested footage from the rehabilitation area, animal entry, and the dirty corridors of the experimental housing zones, they were again informed that no CCTV cameras had been installed in those areas either. Notably, only one camera was found to be recording the presence of AWBI inspectors near the corridor of the rehabilitation centre—despite the fact that the inspection team was present there from 2:30 p.m. until late at night. The inspection team is of the opinion that this lack of access to key CCTV footage, combined with the absence of camera coverage in critical areas, indicates a deliberate attempt to withhold or tamper with evidence related to potential animal welfare violations. For a facility housing thousands of animals, CCTV should be a primary tool for monitoring and preventing cruelty. In this case, the system appeared to be non-functional—or at least non-operational—for the CCSEA inspectors, raising serious concerns about transparency and accountability at PBPL.

V. Non-Compliances Related to the Prevention of Unnecessary Pain and Suffering in Animals

Contrary to the mandates set forth by CCSEA regulations and guidelines, a case study based on data from the software application used at PBPL's experimentation facility revealed serious lapses in animal welfare. In one instance, a dog exhibiting mild to moderate tremors was not withdrawn from the experiment. The symptoms reportedly progressed to severity and became severe by the tenth day. The animal was then marked as "removed" and "killed-moribund" on the twelfth day.

The terminology used in the software—"removal" and "killing-moribund"—is ambiguous and fails to clarify whether any action was taken to alleviate the animal's suffering during this period. As per regulatory guidelines, an animal exhibiting significant neurological symptoms

such as tremors, indicative of high drug toxicity, should be promptly removed from the study and provided with appropriate medical intervention. In this case, both the researcher and the clinical veterinarian failed to take timely action.

Moreover, the software did not provide any detailed account of the animal's clinical parameters, additional symptoms, or medications administered. Although the clinical veterinarian claimed that such records were maintained on loose sheets using a fixed format, she was unable to produce the relevant documentation even after an extensive search. This raises serious concerns about the absence of evidence-based health monitoring or treatment interventions at PBPL.

The lack of a minimum stock inventory of drugs, instruments, and surgical supplies at the examination areas of each experimentation room further compounds the situation, underscoring the extent of cruelty to animals, compromise of animal welfare, and potential regulatory violations at the facility.

There is no protocol to address anxiety, fear, and distress in animals at the facility. In a recent invasive experiment on two monkeys involving surgical implantation and daily wound care, only analgesics were used post procedure, with physical restraint applied without sedatives—indicating serious neglect of psychological welfare. Similarly, dogs euthanised at the end of research studies were not sedated prior to the administration of thiopentone sodium. These practices highlight critical flaws in the veterinary protocol, failing to meet even the basic standards for preventing unnecessary pain and suffering.

Moreover, the use of nomenclature such as "removal" and "killed" in official records reflects a troubling level of insensitivity by the establishment and its personnel toward animals as sentient beings. This choice of language stands in stark contrast to the terminology mandated by CCSEA regulations, which explicitly call for the use of the term "euthanasia"—denoting a "good death" that is humane, compassionate, and ethically conducted. The terminology employed at PBPL not only lacks clarity but also suggests a disregard for the ethical principles embedded in the regulatory framework.

VI. Euthanasia

It was reported that euthanasia is a routine and significant procedure at PBPL, primarily overseen by the pathology department. According to both records and the veterinarian in charge, approximately 30–40 dogs are euthanized each month. These procedures are followed by gross pathological and histopathological examinations, the findings of which are appended to the respective research data.

However, several deeply concerning observations emerged during the inspection. The attending veterinarian confirmed that no sedatives are administered prior to euthanasia to mitigate fear, anxiety, or distress. Instead, thiopentone is injected slowly while an assistant physically restrains the animal—an approach the veterinarian himself acknowledged he would not use if the procedure were a routine surgery such as spaying or castration, or if the breed were less docile, such as a Bulldog, Dobermann, or Rottweiler. This underscores a troubling reliance on the naturally gentle and submissive temperament of Beagle dogs, which makes them easier to handle and restrain, even under distressing conditions, without adequate measures to reduce suffering.

Alarmingly, despite the high frequency of euthanasia, only 20 vials of thiopentone were available at the pathology department, with no stock visible in the central store. This raises serious concerns about whether euthanasia procedures are being conducted consistently with proper dosing and humane practices.

The sheer number of euthanasia cases also suggests that a significant proportion of the animal population is being killed as part of experimental protocols. This may further explain why only 73 dogs were found in the rehabilitation section—a number that appears disproportionately low relative to the reported usage and turnover.

VII. NGO Involvement in Rehabilitation:

Dogs are currently rehabilitated within PBPL's own facility. No records were made available to the inspection team indicating that animals had been transferred to AWBI-recognised animal welfare organisations. Additionally, there was no documentation provided regarding any Memoranda of Understanding (MoUs) or financial support extended to such organisations for the long-term care of the animals.

VIII. Lack of Transparency in Animal Use and Research Practices

During the inspection, the presence of mixed-breed pigs and sheep was repeatedly denied by the facility staff, despite direct and repeated queries from the inspection team. This immediate lack of disclosure raised serious concerns about transparency and the intent to obscure critical information.

Further compounding these concerns, the presence of mixed-breed pigs was inadvertently confirmed when a researcher referenced their use in cardiological studies, including pacemaker development. This was followed by the inspection team observing a separate room housing sheep—despite earlier denials. When committee members proceeded to assess these areas, staff at both the breeding and experimental facilities refused to switch on the lights. They cited adherence to Standard Operating Procedures (SOPs) governing lighting schedules. However, this explanation was inadequate in the context of a formal regulatory inspection and effectively obstructed visibility, thereby preventing a thorough evaluation of the animals' housing conditions.

In addition, a clear inconsistency was observed between the number of CCSEA-approved research protocols—reported to be 87 over the past three months—and the actual number of dogs, minipigs and monkeys present at the facility. This discrepancy suggests possible non-compliance with approved study limits or underreporting of animal populations.

Crucially, the mandatory three-month washout period—required to ensure complete elimination of substances from animals' systems before reuse—was reportedly not being followed, particularly for minipigs. No documentary evidence was produced during the inspection to verify compliance with this requirement. This lapse not only violates standard ethical and scientific guidelines but also compromises the validity of subsequent research and the welfare of the animals involved.

Overall, the number of animals observed during the inspection did not align with the facility's declared housing capacity or the volume of CCSEA-approved experimental protocols. The presence of surplus, unscreened stock animals in experimentation rooms points to serious gaps in documentation and oversight. These findings underscore the urgent need for a detailed review and reconciliation of animal usage records to ensure compliance with regulatory requirements and to uphold fundamental animal welfare standards.

IX. CCSEA Inspection Team's Observations in Relation to Specific Allegations Raised by PETA-India's Alleged Whistle-blower

Animal Species	Complaint Category	Specific Allegations by PETA	CCSEA Inspection Team's Observation
Confirmation of Location of Reported Incidents	**General**	Allegations of cruelty to animals and violations of animal protection laws occurring on the premises of PBPL.	The visuals presented in PETA-India's investigation video were found to match the premises of PBPL. The PBPL management acknowledged that certain footage—such as holding a dog by the scruff—was taken from a training video and claimed it is not representative of routine practice. However, they disputed some visuals, asserting that these either originated from another facility or had been manipulated.
Beagles (Dogs)	**Overcrowding & Housing**	Approximately 1,500 dogs housed in a space designed for 800, forcing 3-4 dogs into cages meant for two. Breeding facility reportedly concealed from various auditors.	At the time of inspection, 2-3 dogs per kennel were noted in each of the breeding stock modules; the whelping mothers were housed with their puppies. An overall high housing density of dogs was observed in the breeding modules, and excess breeding stocks were found to be housed in dog experimental areas. The facility's manager was unable to determine the accurate number of total dogs present, indicating potential overcrowding possibilities. Furthermore, CCTV footage from the corridors of the dog breeding stock areas was not made available, with staff asserting that no cameras were installed in those specific areas, which

			could impede verification of housing conditions.
	Breeding Practices	Dogs bred twice a year, often exceeding the company's stated policy of a maximum of five breeding cycles. Dogs as old as 13 years allegedly used for breeding, causing immense physical strain and increasing risk of difficult labor.	Consolidated breeding records and veterinary care of dogs were not available on-site within the dog breeding area. The overall high housing density of dogs in the breeding modules and the facility manager's inability to determine the correct number of dogs present suggest practices that could lead to overbreeding and exceed capacity.
	Lack of Care & Handling	Overcrowding led to frustration, food aggression, and frequent fights, causing serious injuries (especially to ears). Company allegedly failed to provide basic care, including proper wound cleaning and pain management. Workers observed handling dogs roughly, kicking them, and carelessly closing cage doors on their legs. Dogs picked up by the scruff of the neck or skin on their backs.	At the time of inspection, no seriously injured animals were observed. However, the inspection team noted an overall high housing density of dogs in breeding modules, accompanied by extreme decibel levels of barking. A few dog kennels in the breeding stock area were observed to house animals in poor body condition, some showing signs of cherry eye, with dirty conditions and an overall uninviting environment. The veterinary records for the animals were not present on-site, which significantly hindered inspectors ability to monitor medical history or verify proper wound care and pain management. No CCTV is installed at an angle that would allow visualization or recording of individual dogs.
	Medical Neglect & Suffering	Dogs developed abscesses, ulcers, and signs of severe pain following subcutaneous injections of	The complete absence of on-site veterinary records for the animals precluded the inspection team from

		test compounds. Injection sites became inflamed or developed open wounds, with infections potentially spreading. In some studies, dogs became very ill, with one reportedly vomiting excessive quantities of blood before dying. Some suffered ulcers in mouth and intestine from oral dosing.	verifying specific claims of abscesses, ulcers, severe pain, or other medical conditions and their treatment. However, the team observed a few dirty kennels and very high relative humidity levels (80-97%) in nearly all rooms, which can create an environment conducive to health problems and infections. Furthermore, the recording of adverse reactions in study-based software were poorly recorded or, apparently, not recorded.
	Euthanasia Protocol Violations	"Humane endpoints" existed only on paper; management instructed veterinarians to delay euthanasia for suffering animals until sponsor permission was granted. Dogs allegedly killed using thiopentone without prior sedation.	The euthanasia is performed by the veterinary pathologist using approved drugs. However, in the case of dogs, sedation or tranquilization prior to euthanasia is not practiced.
Minipigs	**Unlicensed Breeding & Euthanasia**	Company purchased Göttingen minipigs but lacked a license to breed them. Accidental pregnancy led to euthanasia of 8-10 piglets via intracardiac injection without prior sedation.	Minipigs were present at the facility. A noticeable inconsistency was observed between the number of CCSEA-approved research protocols and the actual population of minipigs housed, which raises questions regarding breeding. The absence of on-site veterinary records further prevented verification of euthanasia for piglets, if any.
	Lack of Enrichment	Despite a written policy requiring playtime and social enrichment for pigs, the Company routinely failed to provide either. Enrichment only provided when external visitors were present.	A notable deficiency in environmental enrichment was observed for minipigs, with only a few provided with cut sections of PVC pipes, which failed to engage them and left them visibly uninspired and bored.

			Provision for playtime outside cages is not available.
	Improper Housing	Representatives from the Danish supplier observed pigs' feet getting injured due to improper flooring.	All animals, including minipigs, were confined exclusively in cages with fibber-reinforced polymer flooring. However, the absence of on-site veterinary records for the animals precluded the inspection team from verifying specific claims of pigs' feet getting injured and their treatment.
Monkeys (Rhesus Macaques)	**Illegal Capture & Transport**	Company allegedly captured 14 rhesus macaques from the forest in Rajasthan, exceeding government permission for 12. Monkeys (approx. 1.5 years old) were sedated and placed in plastic bags, up to five per bag, for transport.	Monkeys were present at the facility and were procured from a CCSEA-registered vendor.
	Zoonotic Disease Risk & Concealment	Two monkeys tested positive for monkeypox in Rajasthan. All monkeys transported together to Telangana facility. The two positive monkeys were killed upon arrival, but others were kept alive despite transport with infected animals. Subsequent re-testing occurred only one week after arrival, despite potentially longer incubation period, as company needed monkeys for client-sponsored test. Company allegedly kept the monkeypox matter quiet, killing infected monkeys without broader disclosure, despite public health risks.	There was a total absence of dedicated quarantine rooms and isolation rooms for sick animals, which critically compromises biosecurity and disease management. The absence of on-site veterinary records further prevented verification of health screenings, disease status, or any actions taken regarding zoonotic diseases.

X. Discussion

The comprehensive inspection of PBPL highlights systemic failures at multiple levels of its operations to uphold animal ethics and welfare as per CCSEA guidelines. PBPL's approach to animal research demonstrates an operational model that prioritizes experimental output over welfare, compliance, and ethical responsibility. Despite its extensive use of dogs, non-human primates, pigs, and other species, PBPL has failed to implement even the most basic standards of care mandated by CCSEA.

Housing conditions were consistently found to be overcrowded, barren, and inadequate, leading to significant welfare concerns such as elevated stress, noise, poor body condition, and heightened risk of infectious diseases. Essential aspects such as environmental enrichment, social interaction, and proper bedding were either entirely absent or grossly insufficient across all species. The breeding facilities were particularly concerning, with overproduction of animals resulting in unauthorized repurposing of experimental spaces as stock rooms, unscreened animal transfers, and potential biosecurity risks.

Veterinary care infrastructure was deeply inadequate. The facility maintained minimal medical supplies, lacked essential analgesics, sedatives, and anaesthetics, and failed to maintain proper treatment records. Notably, no protocol was in place to manage anxiety, fear, or distress—an essential component of humane animal care. Painful and invasive procedures, such as those performed on monkeys involving surgical implantation, were conducted using only analgesics post procedure, with animals physically restrained without sedatives. Similarly, dogs euthanised at the conclusion of research were not sedated before the administration of thiopentone sodium. These practices reflect glaring omissions in veterinary planning and a disregard for psychological well-being.

The animal record-keeping system at PBPL is virtually non-functional, with key regulatory documentation either missing or grossly insufficient. Without breeding records, reuse data, health histories, or procedural logs, PBPL operates in opaque conditions that obstruct regulatory oversight. The deliberate non-cooperation during inspection — notably the failure to provide CCTV footage from critical areas — raises serious questions about transparency and intent.

The inspection also uncovered troubling deviations from approved euthanasia protocols. Animals were euthanised without sedation, relying solely on physical restraint—a practice incompatible with ethical norms of humane care. The high euthanasia rate suggests an unsustainable use pattern where large numbers of animals are systematically killed after experimental use, with limited rehabilitation or rehoming efforts.

XI. Conclusion

The operational deficiencies observed at PBPL are not isolated incidents but indicative of entrenched structural, procedural, and ethical failures. The scale and severity of non-compliances documented during the inspection raise significant concerns regarding the facility's adherence to established standards of animal welfare and regulatory accountability.

The situation demands urgent attention—particularly with respect to the removal and rehabilitation of animals to prevent further pain, distress, or suffering. The findings also call for a critical review of the facility's registration and breeding licence. in view of the serious and repeated deviations from prescribed norms. A detailed micro-audit of PBPL's Institutional Animal Ethics Committee (IAEC) is imperative, including a comprehensive reconciliation of records relating to breeding, procurement, experimentation, reuse, rehabilitation, transfer, euthanasia, and disposal. Such scrutiny is essential to evaluate compliance with approved protocols and to verify the accuracy and integrity of reported data.

XII. Photographic Evidences

Health Condition:

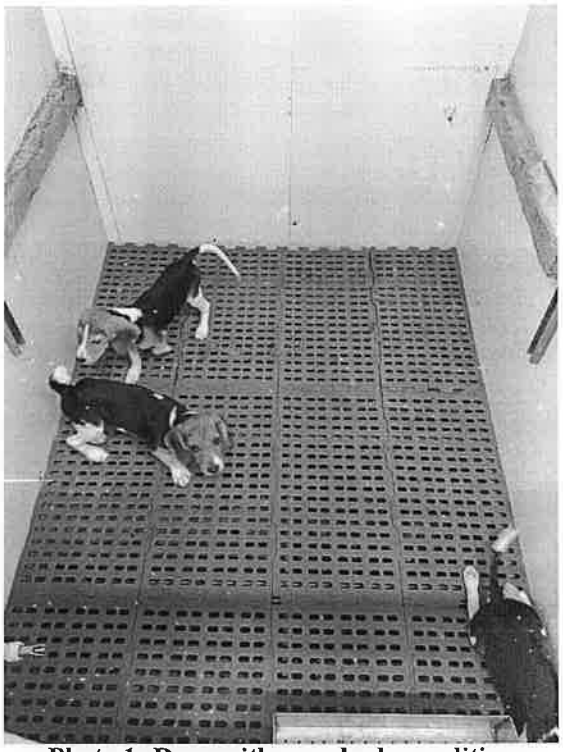


Photo 1: Dogs with poor body condition

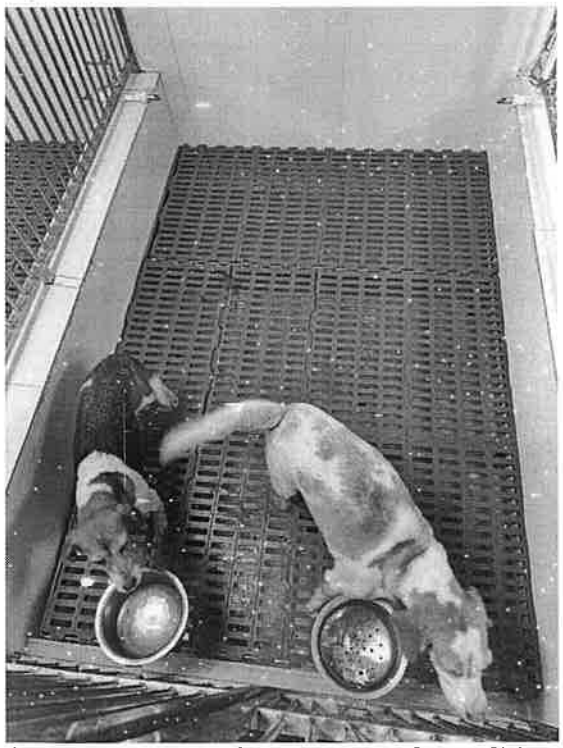


Photo 2: Dog on left with poor body condition

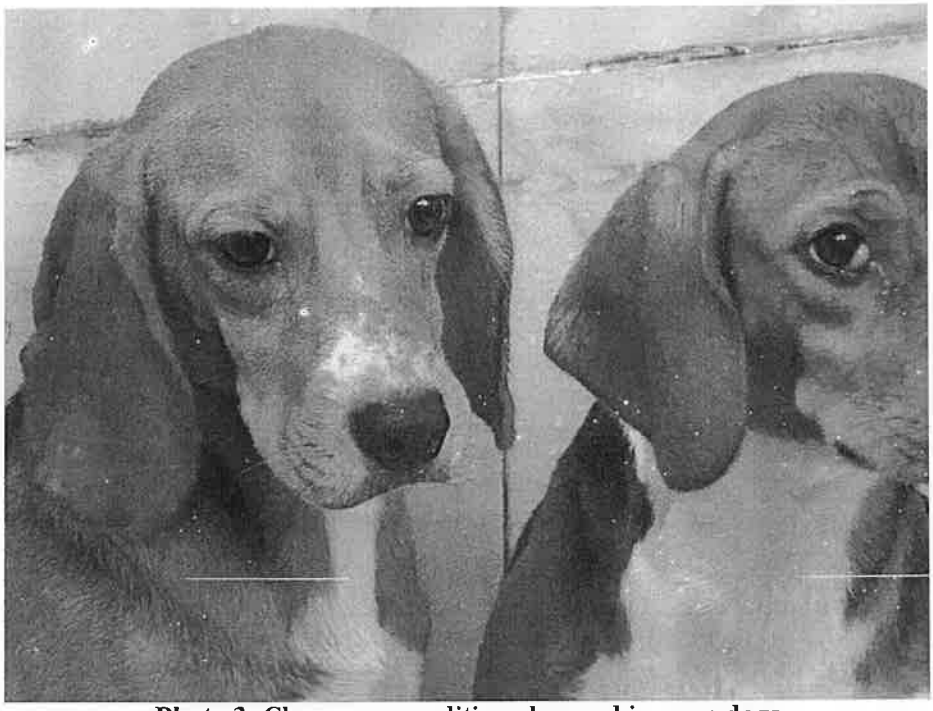


Photo 3: Cherry eye condition observed in some dogs

Photo 4: Cherry eye condition observed in some dogs

Housing Conditions:

Photo 5: No bedding and no enrichment

Photo 6: No bedding and no enrichment

Photo 7: Floor soiled with faeces and in an unclean condition

Photo 8: Floor soiled with faeces and in an unclean condition

Photo 9: Stained and poorly maintained flooring

Photo 10: Stained and poorly maintained flooring

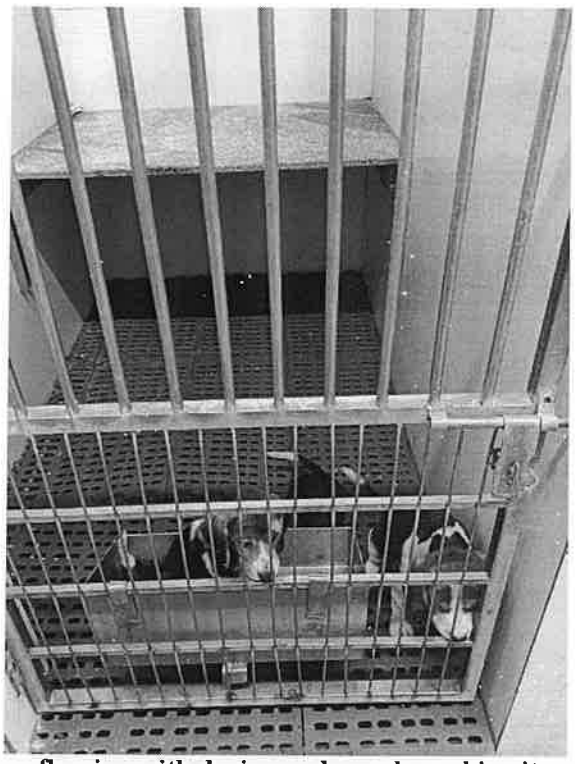


Photo 11: Polymer flooring with drainage channels, making it uncomfortable for animals to stand or lie down

Photo 12: Polymer flooring with drainage channels, making it uncomfortable for animals to stand or lie down

Photo 13: Poorly enriched living conditions with a slippery floor

Photo 14: Poorly enriched living conditions with a slippery floor

Photo 15: Polymer flooring with drains, uncomfortable for animals to stand or lie down

Photo 16: Polymer flooring with drains, uncomfortable for animals to stand or lie down

Photo 17: Enclosed space lacking natural light and enrichment

Photo 18: Enclosed space lacking natural light and enrichment

Photo 19: Restricted movement and lack of socialisation causing significant distress

Photo 20: Poorly designed enrichment tools with limited or no utility

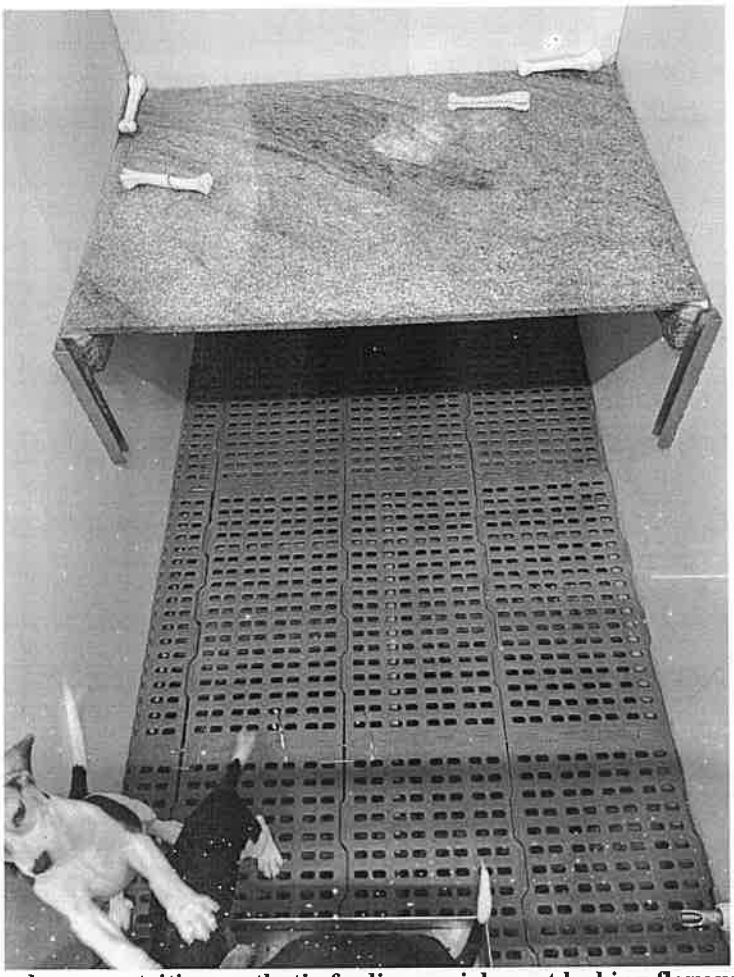


Photo 21: Bland, non-nutritive synthetic feeding enrichment lacking flavour and value

Photo 22: Bland, non-nutritive synthetic feeding enrichment lacking flavour and value

Photo 23: Enclosed space lacking natural light and enrichment

Photo 24: Enclosed space lacking natural light and enrichment

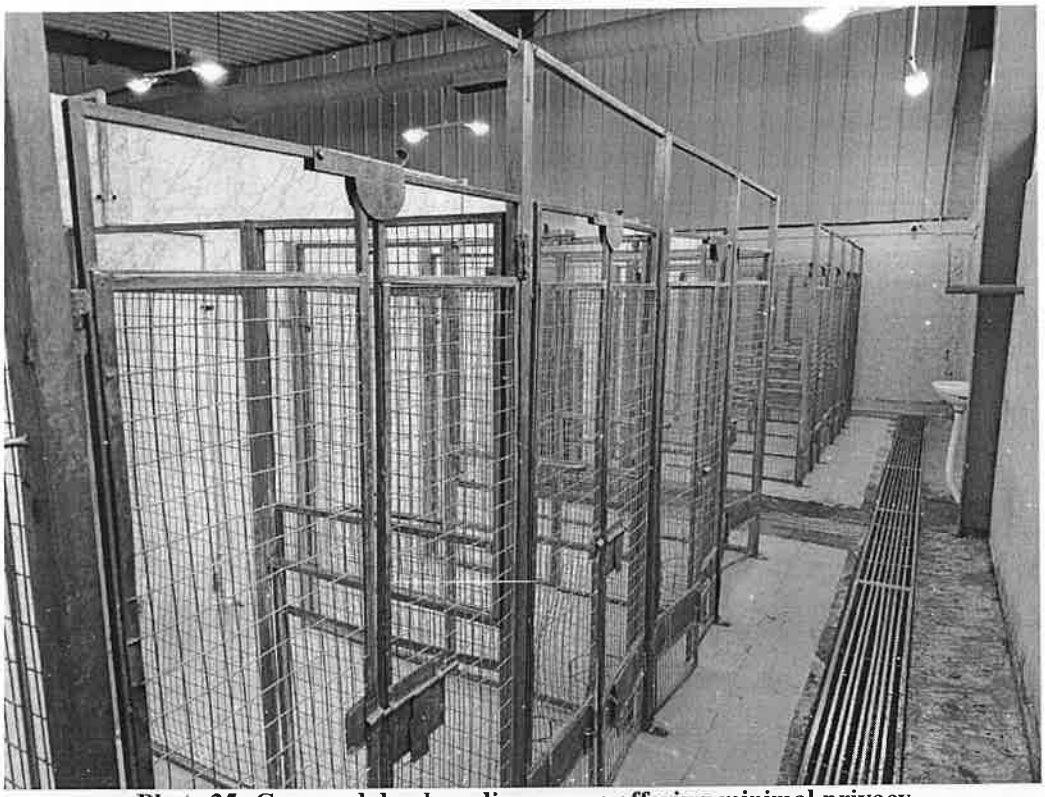


Photo 25: Cramped dog breeding rooms offering minimal privacy

Photo 26: Kennels designed for human cleaning convenience, with flooring and drainage prioritised accordingly

Photo 27: Minipigs housed on uncomfortable flooring with no enrichment

Photo 28: Minipigs housed on uncomfortable flooring with poorly designed enrichment

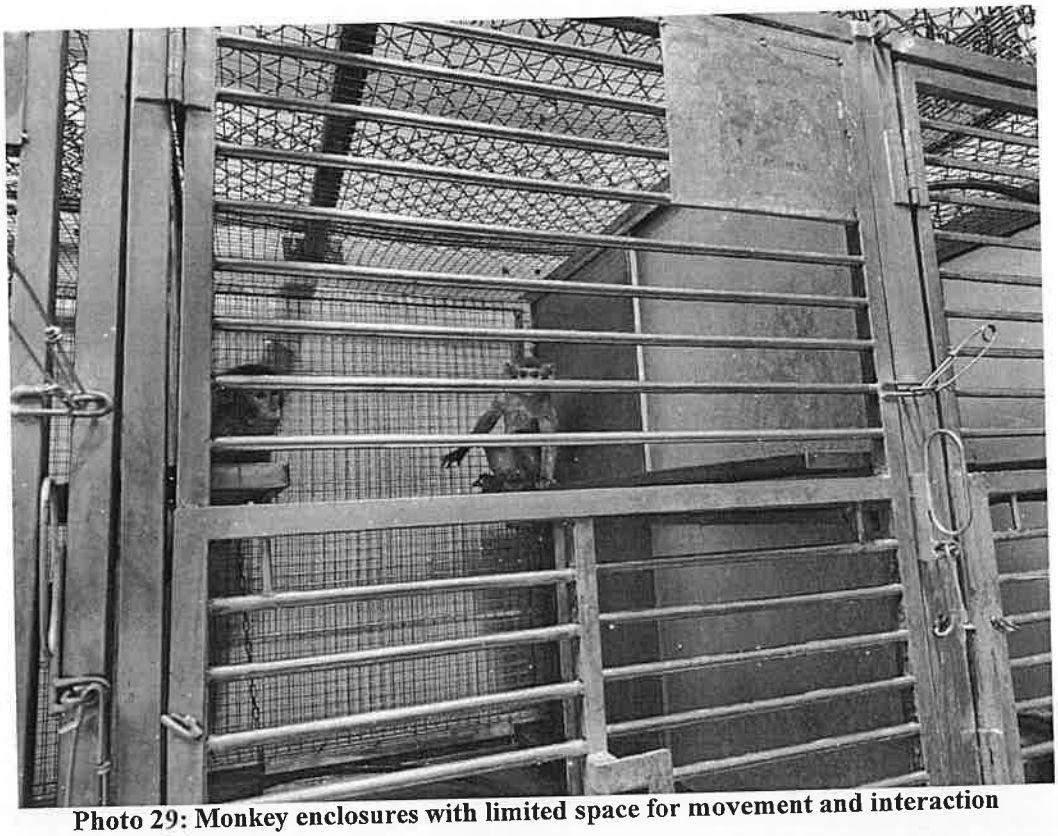


Photo 29: Monkey enclosures with limited space for movement and interaction

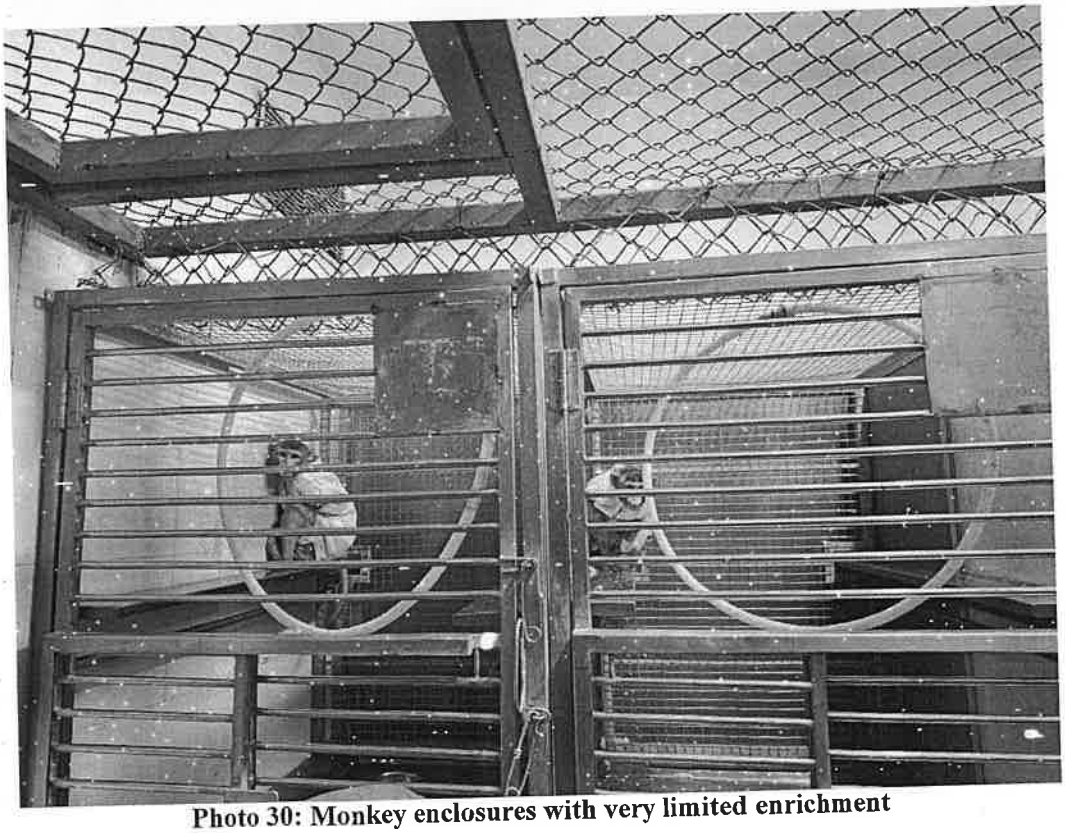


Photo 30: Monkey enclosures with very limited enrichment

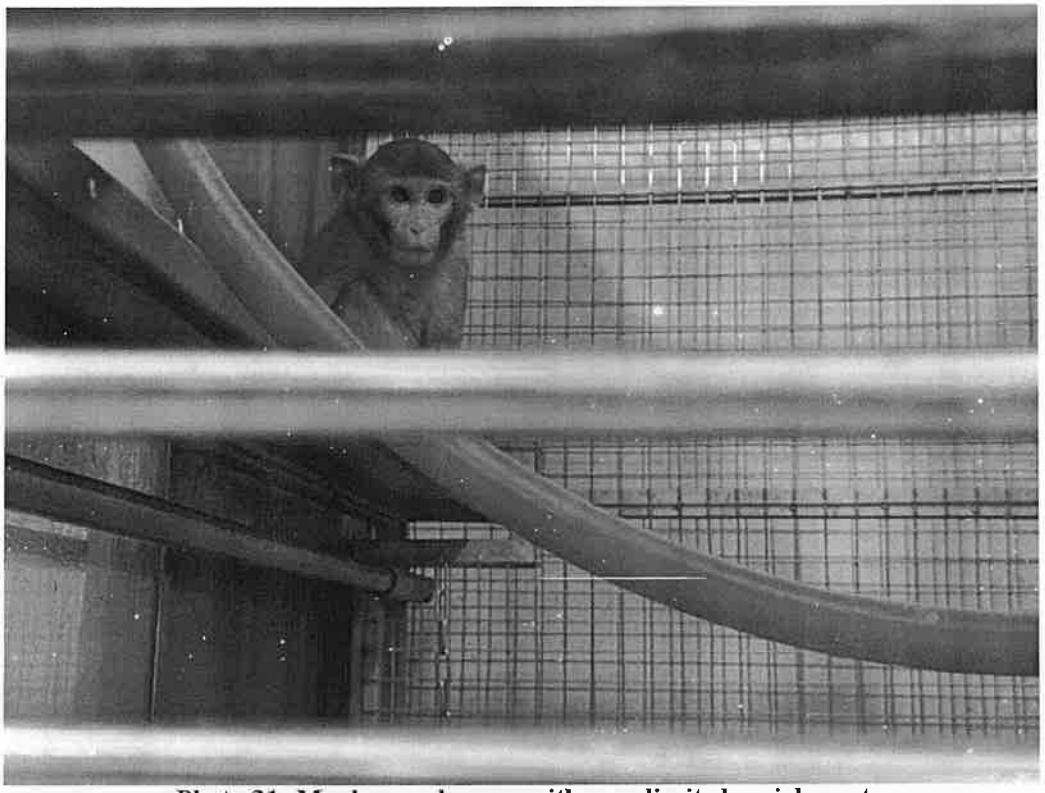


Photo 31: Monkey enclosures with very limited enrichment

Photo 32: Narrow metal platforms restricting animals' comfort while resting

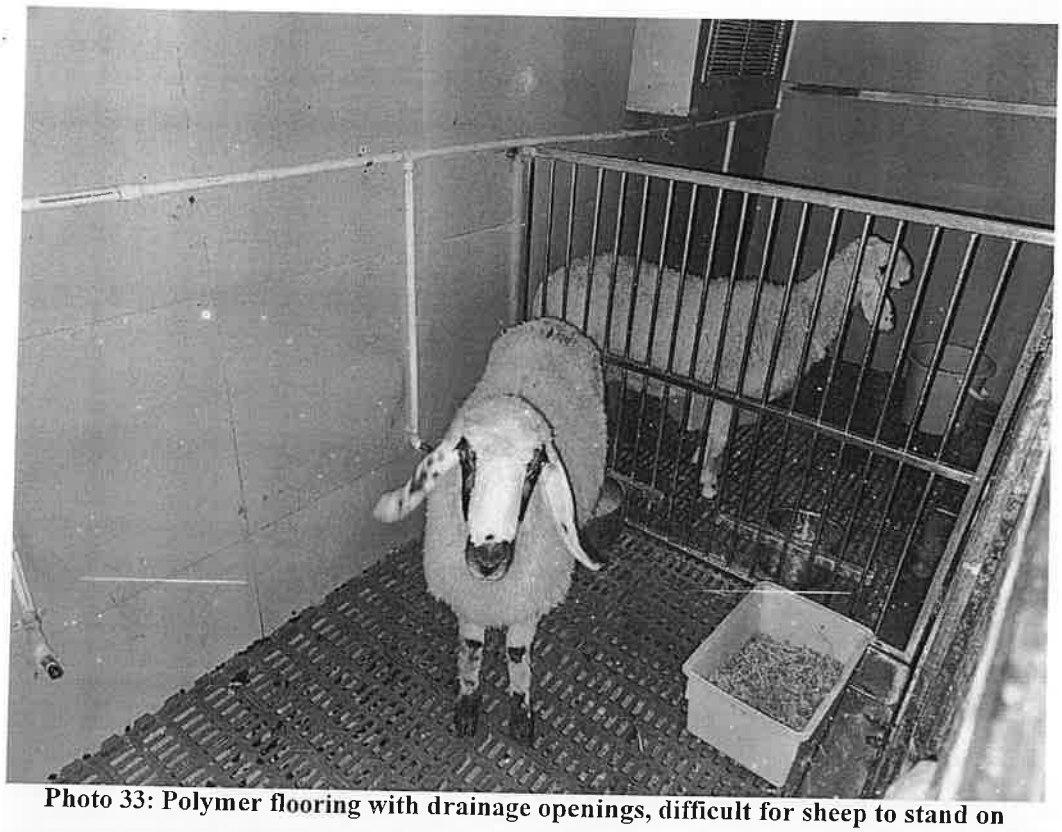


Photo 33: Polymer flooring with drainage openings, difficult for sheep to stand on

Photo 34: Restricted space and lack of socialisation opportunities

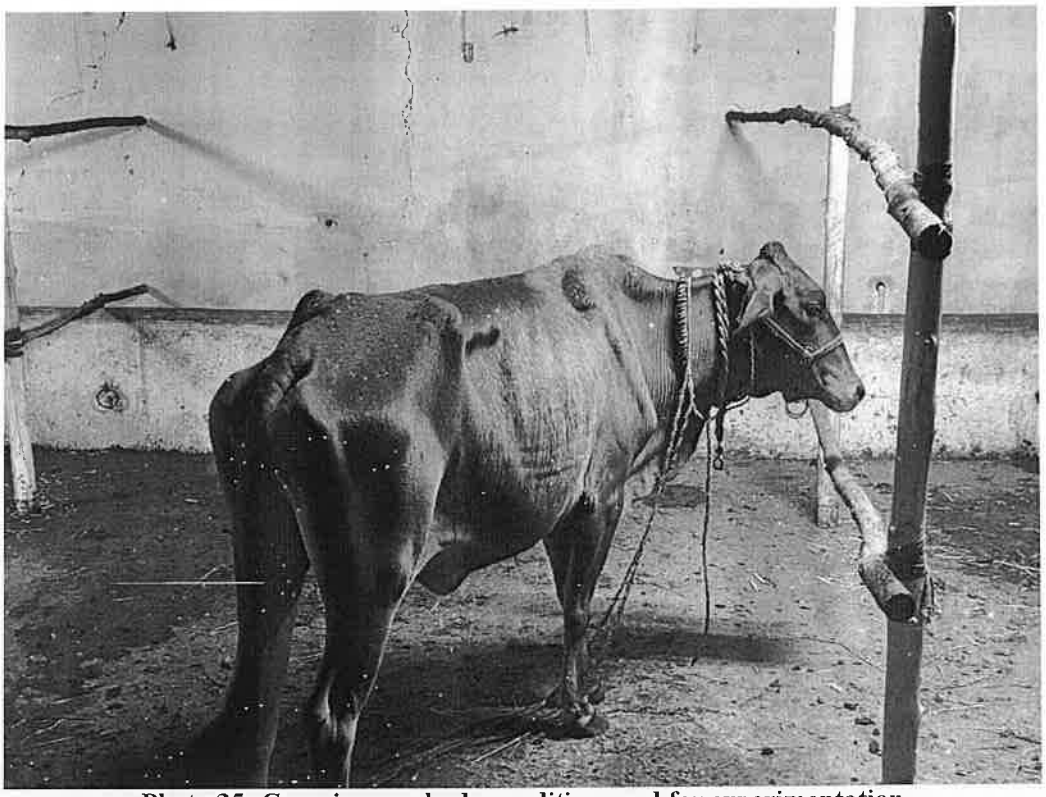


Photo 35: Cows in poor body condition used for experimentation

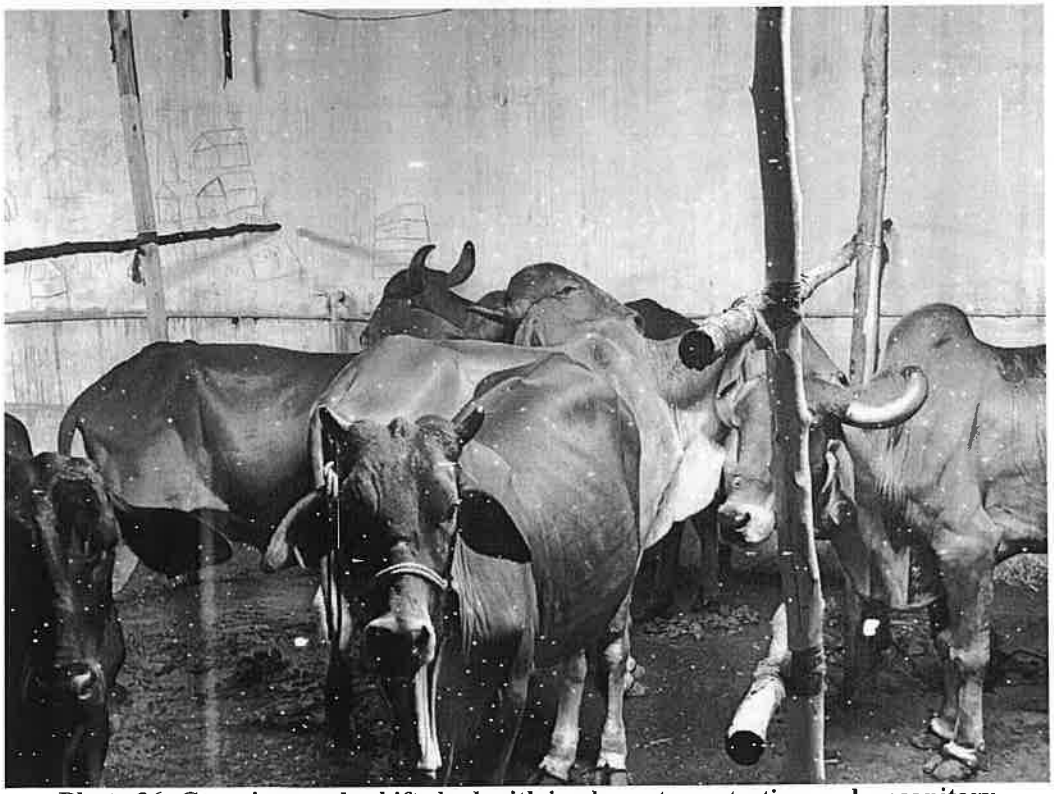


Photo 36: Cows in a makeshift shed with inadequate protection and unsanitary conditions

Medical Inventory:

Photo 37: Medical inventory lacks essentials medicines like sedatives and analgesics

Photo 38: Inadequate medical inventory with only general medicines

Photo 39: Inadequate medical inventory with only general medicines

Photo 40: Inadequate surgical inventory with only general items

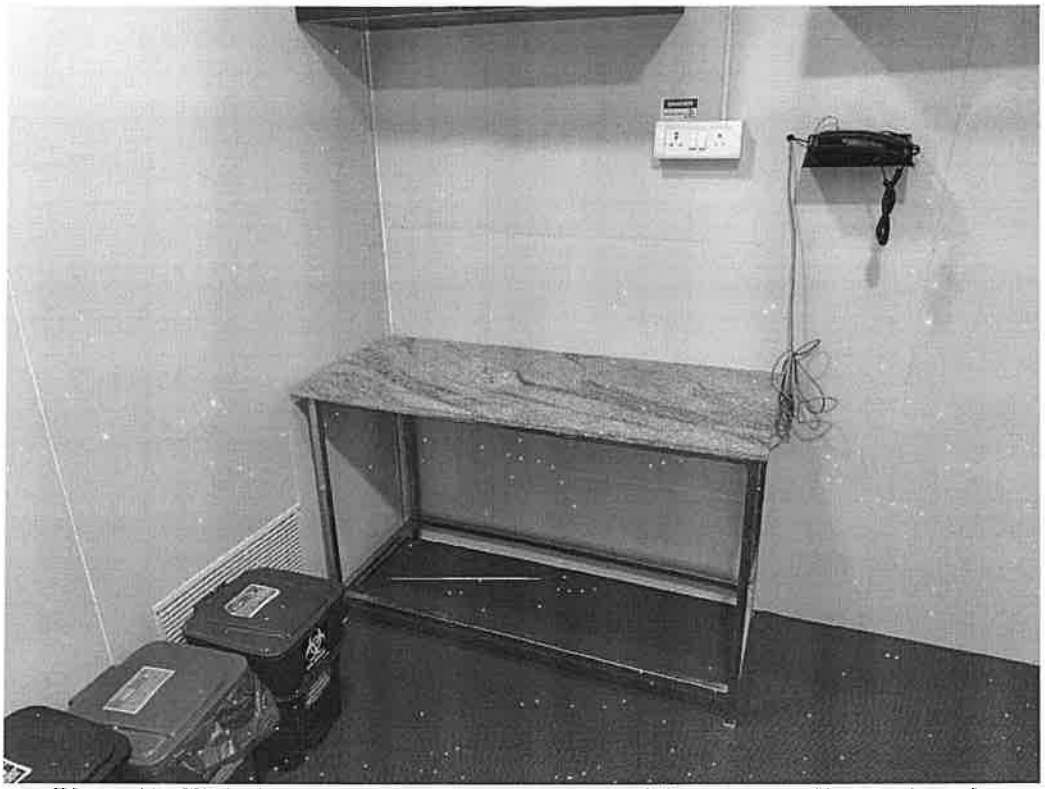


Photo 41: Clinical examination room without medicine stock or diagnostic tools

Photo 42: Clinical examination room without medicine stock or diagnostic tools

Veterinary Documentation:

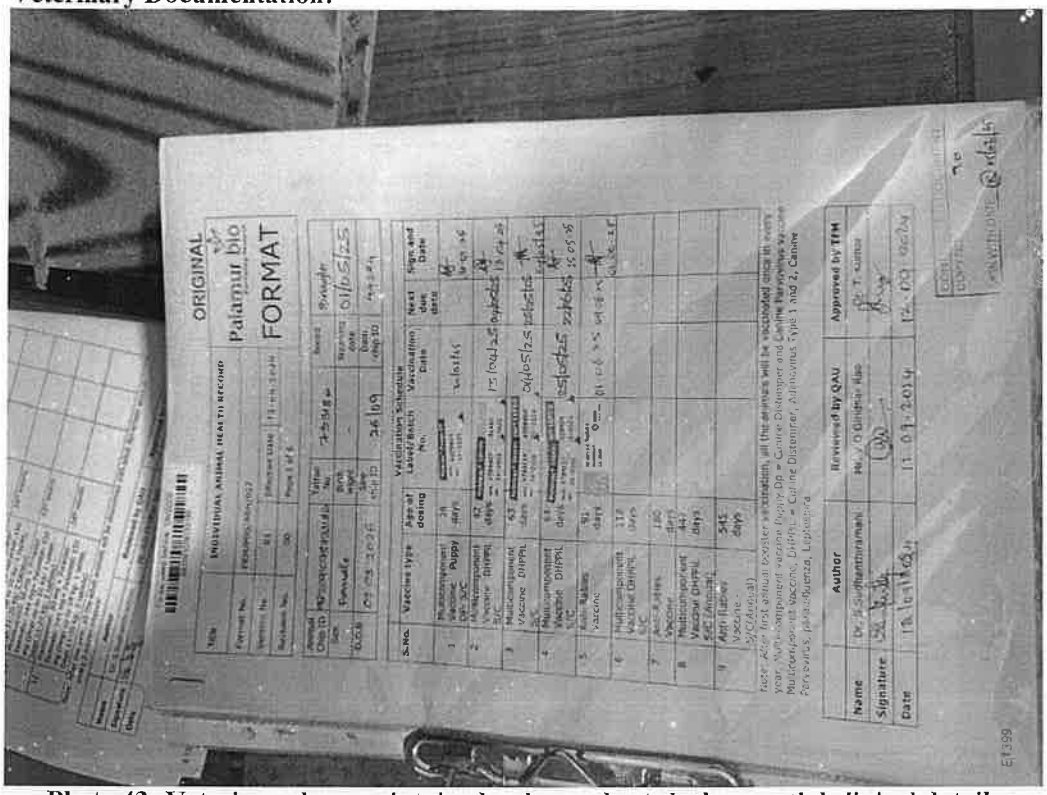


Photo 43: Veterinary logs maintained as loose sheets lack essential clinical details

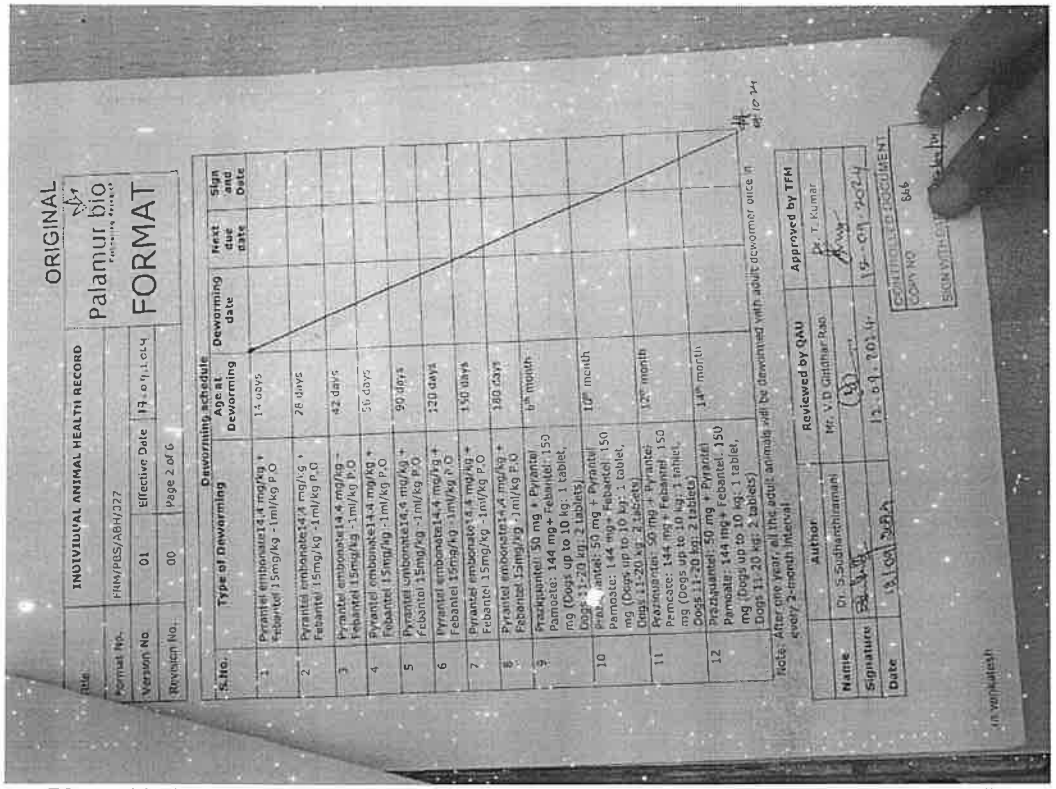


Photo 44: Veterinary logs maintained as loose sheets lack essential clinical details

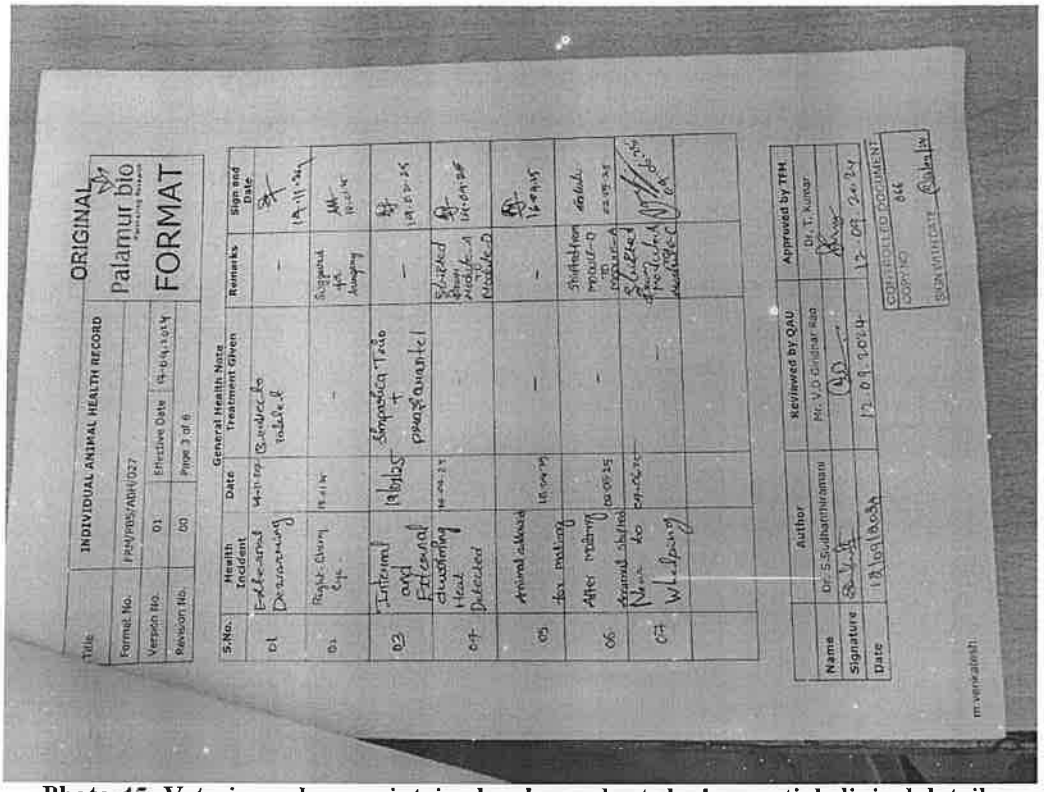


Photo 45: Veterinary logs maintained as loose sheets lack essential clinical details

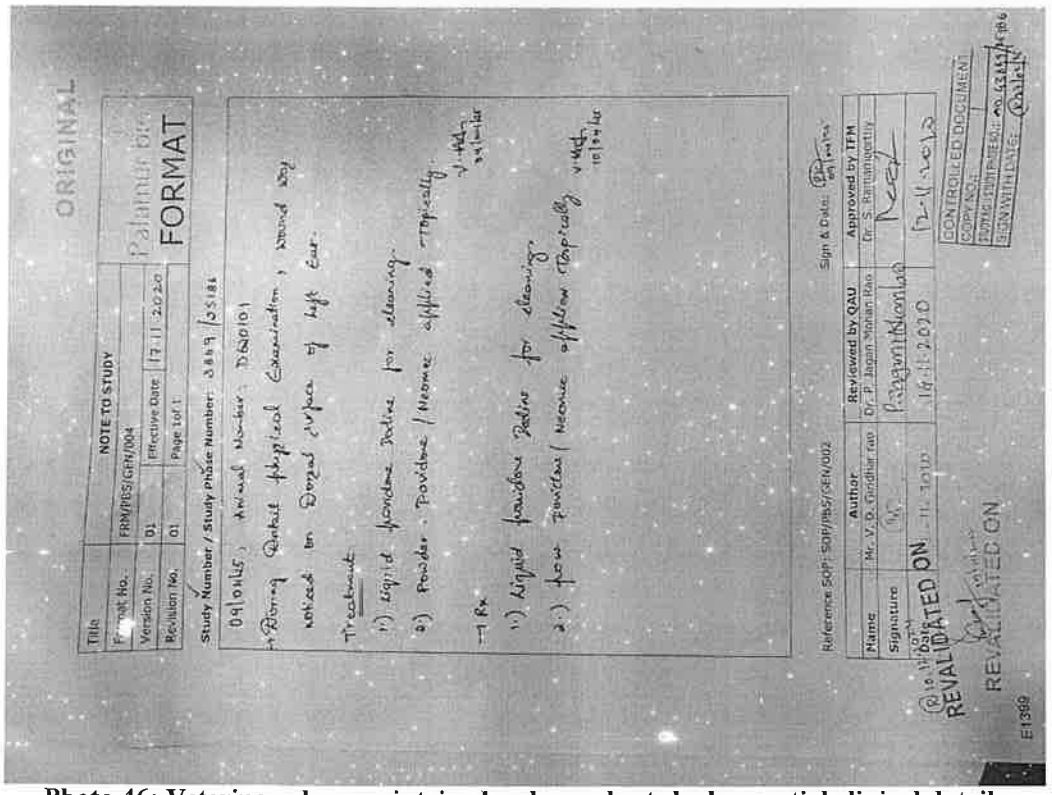


Photo 46: Veterinary logs maintained as loose sheets lack essential clinical details

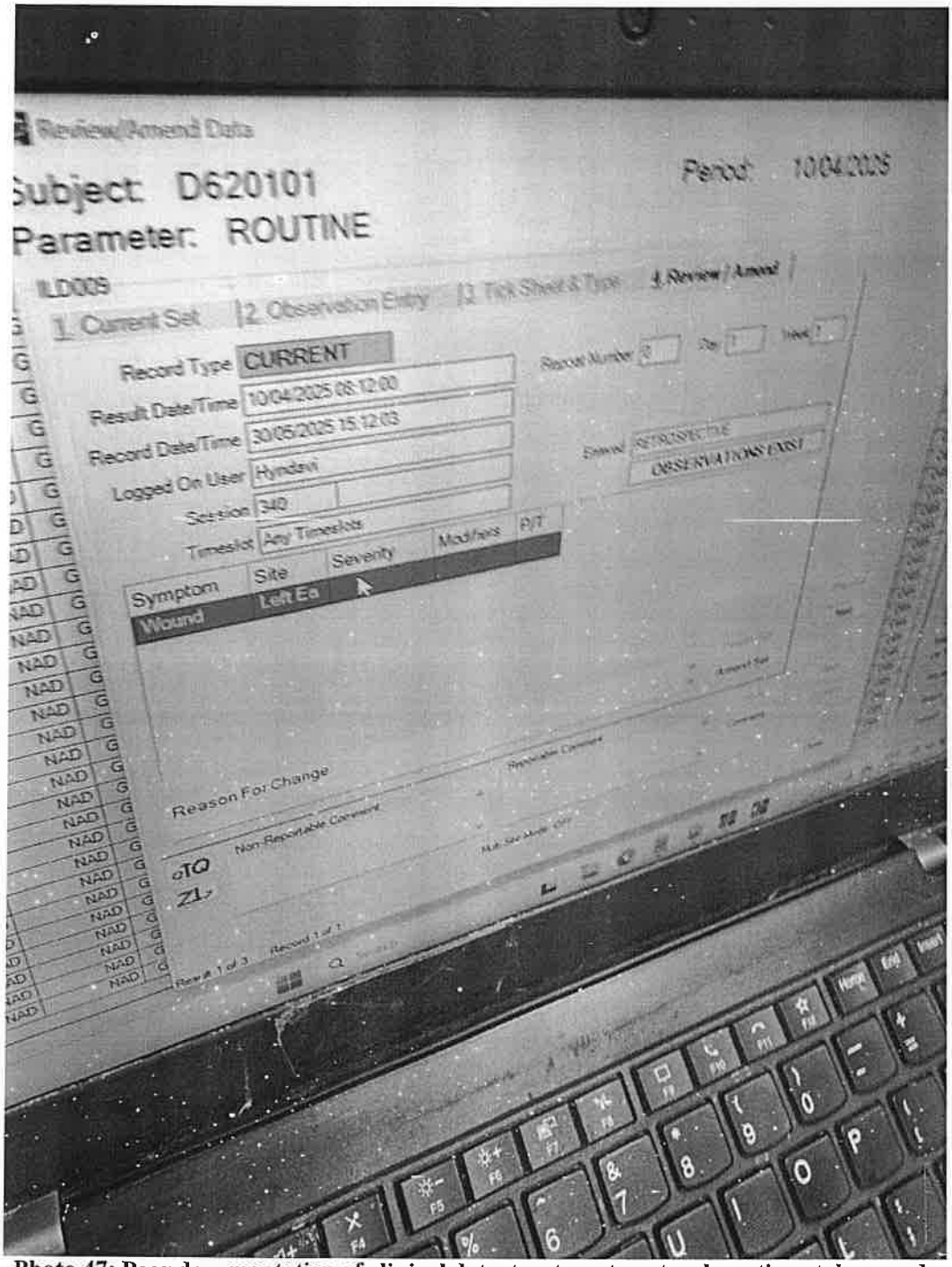


Photo 47: Poor documentation of clinical data, treatment protocols, actions taken, and outcomes

Rehabilitation:

Photo 48: Rehabilitation appears to be a makeshift arrangement, indicated by a paper label affixed to the door

Photo 49: Room and cage design indicate conversion from an experimentation room

Photo 50: Rehabilitation cages lack any form of enrichment

Photo 51: 73 dogs—62 males and 11 females—reported to be under rehabilitation

Euthanasia:

Photo 52: Only 20+ thiopentone vials stocked for 30–40 monthly euthanasia cases

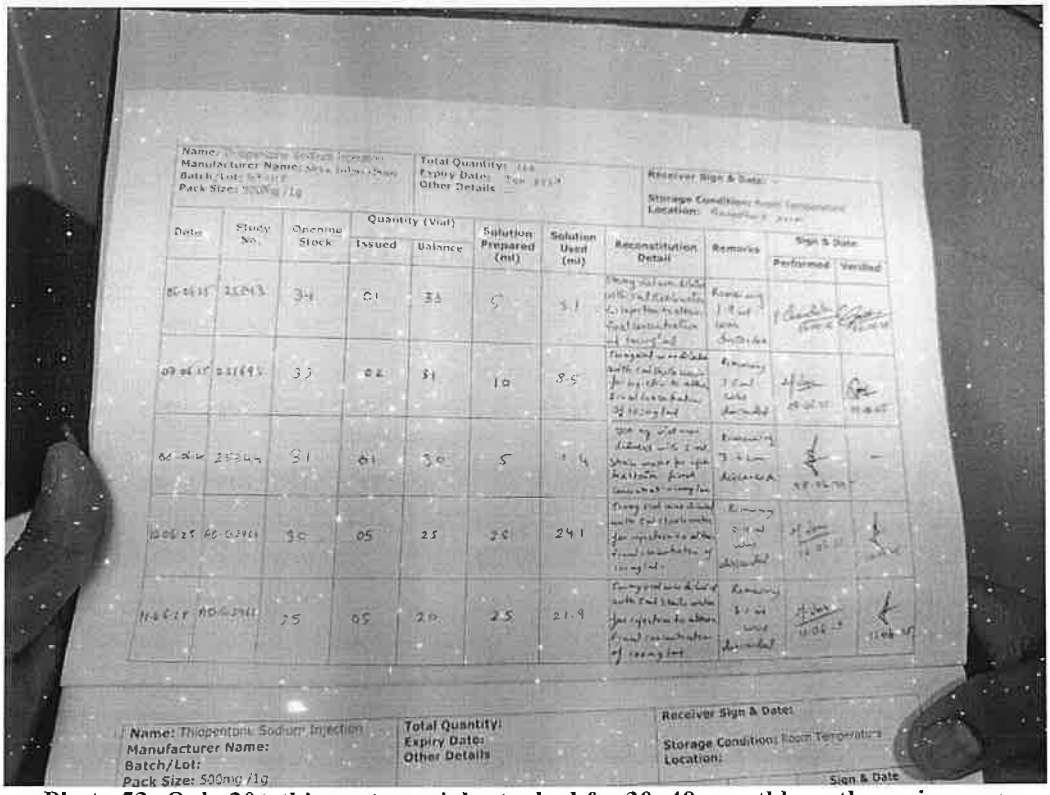


Photo 53: Only 20+ thiopentone vials stocked for 30–40 monthly euthanasia cases

Socialisation Area:

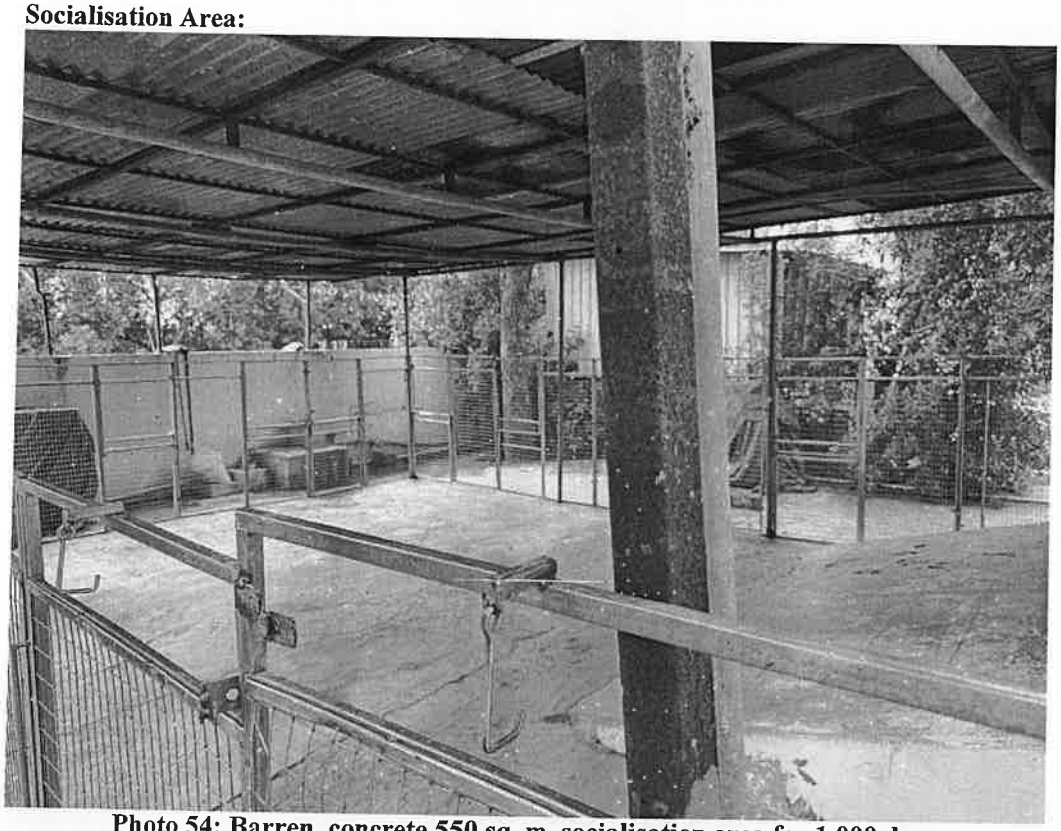


Photo 54: Barren, concrete 550 sq. m. socialisation area for 1,000 dogs

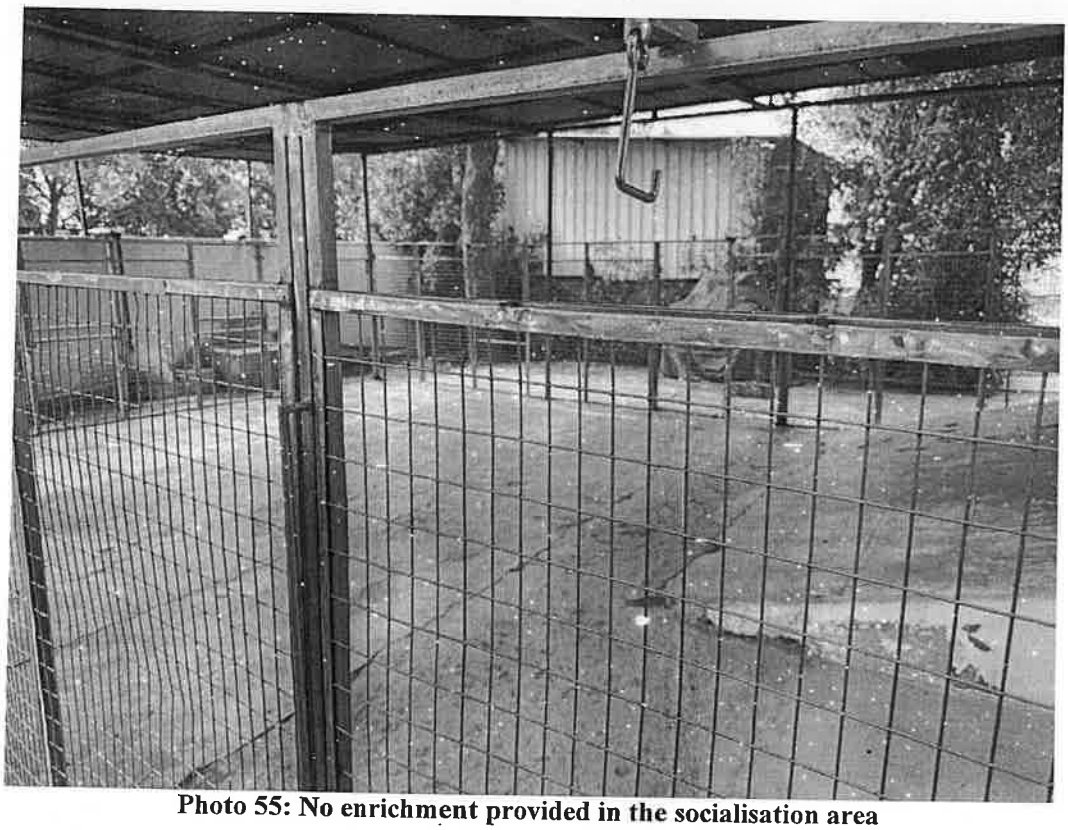


Photo 55: No enrichment provided in the socialisation area

Nutrition:

Photo 56: Animals fed commercial food with limited quantity and frequency

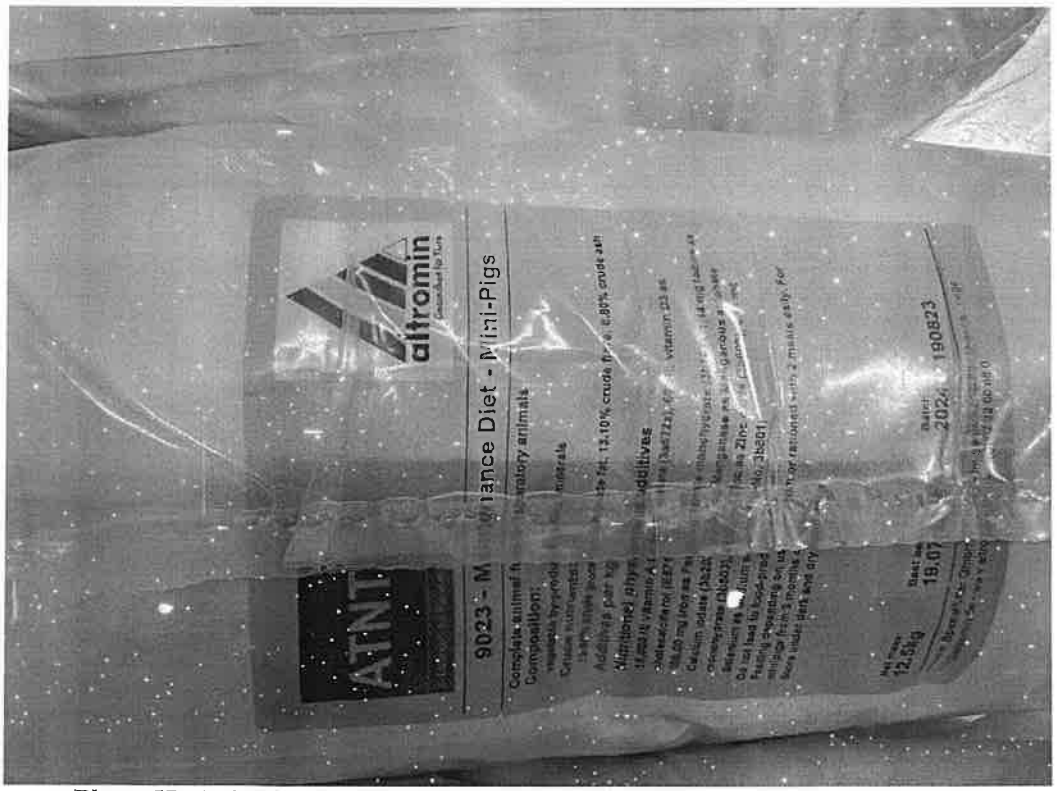


Photo 57: Animals fed commercial food with limited quantity and frequency

CCTV

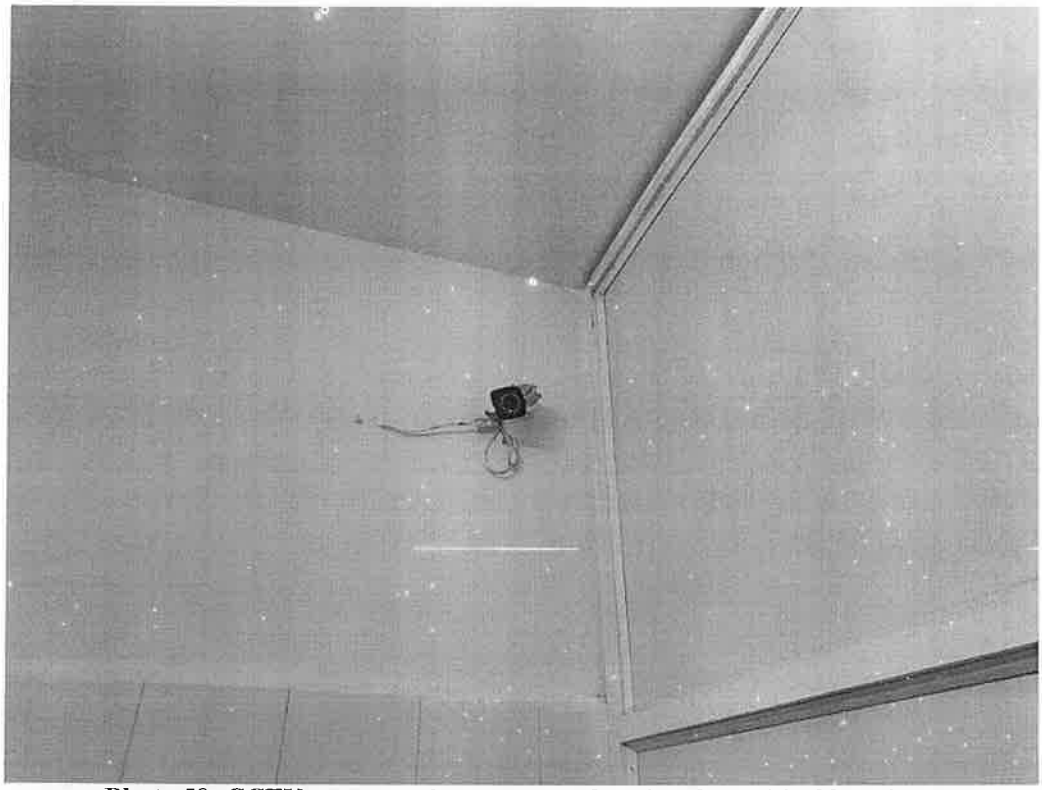


Photo 58: CCTV cameras absent or non-functional at critical locations

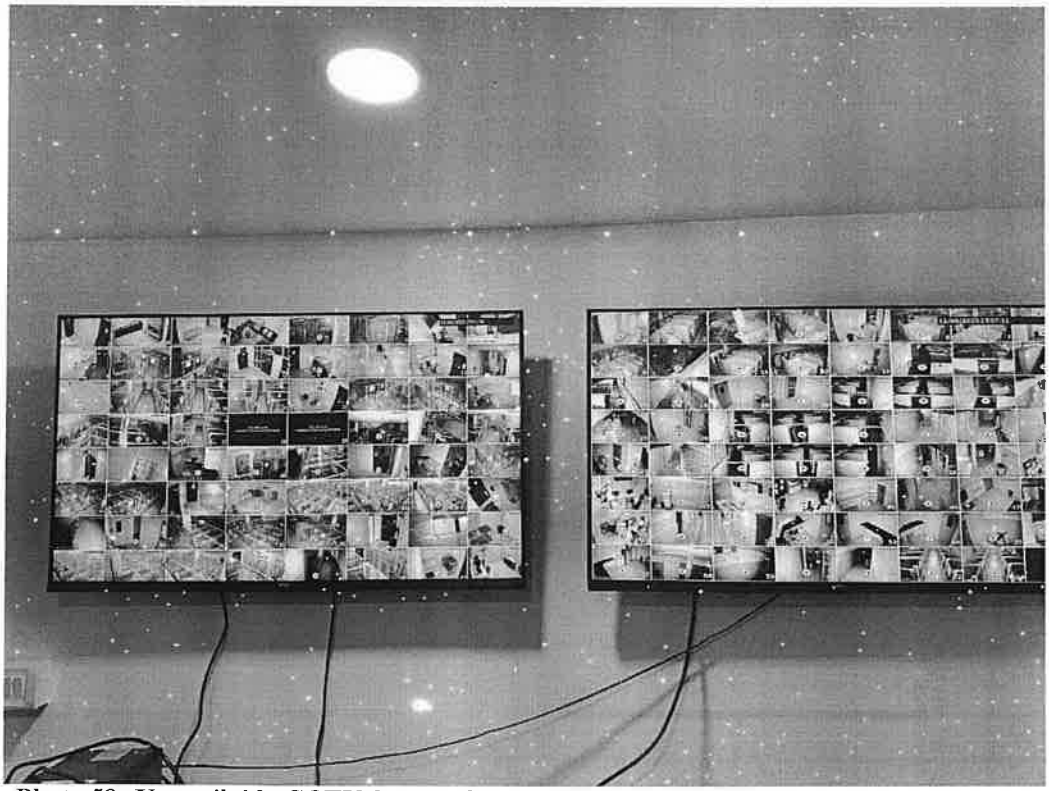
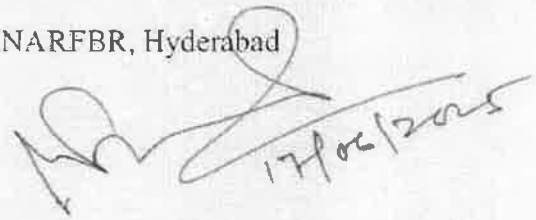


Photo 59: Unavailable CCTV footage from critical areas raises transparency concerns

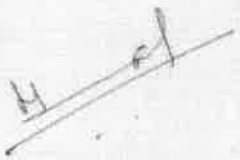
Inspection Team Signatures and Verification

We, the undersigned members of the inspection team, hereby affirm that the findings, observations, and conclusions presented in this report are accurate to the best of our knowledge, based on the on-site inspection conducted at Palamur Biosciences Pvt. Ltd, on 11 June 2025, and the information made available by PBPL representatives. This report is respectfully submitted to the Committee for the Control and Supervision of Experiments on Animals (CCSEA) for its consideration and appropriate action in accordance with the applicable rules and regulations.

1. Dr. Mukesh Kumar Gupta
   Member, CCSEA & Director, ICMR-NARFBR, Hyderabad



2. Dr. Manilal Valliyate
   Member, AWBI



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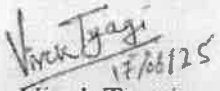
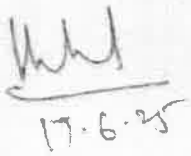
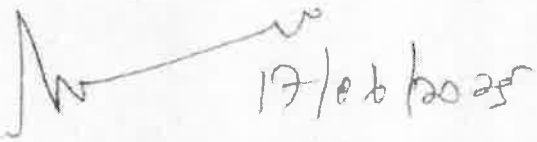


3. Dr. Vivek Tyagi
   Senior Consultant, CCSEA



4. Dr. B.D.P. Kala Kumar
   Main Nominee, IAEC



5. Shri A. Madhava Rao
   Socially Aware Nominee, IAEC

6. Ms. Alokparna Sengupta
   Managing Director, Humane World for Animals India Foundation

Annexure D:

First Complaint letter dated 10 June 2025 to CCSEA

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10 June 2025

Dr. Abhijit Mitra
Chairman, CCSEA
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Ministry of Fisheries, Animal Husbandry and Dairying,
Government of India,
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Via post and email: ahc-dadf@nic.in

Dear Dr. Mitra:

I am writing on behalf of People for the Ethical Treatment of Animals (PETA) India and our more than 2 million members and supporters. We recently received alarming video footage, photographs and testimonials from insiders who were employed at Palamur Biosciences Pvt Ltd (CCSEA Registration Number: 1312/PO/RcBiBt-S/RcBiBt-L/09/CPCSEA) (hereinafter, "the Company"), located at Karvena (Village), Bhoothpur (Mandal), Mahabubnagar-District- 509001 Telangana, documenting the reported abuse and neglect of dogs, pigs, and monkeys used at the Company from 2021 to 2023. **You can see the referenced video footage, photographs, and more details here.**

The details described below present a deeply troubling and persistent pattern of apparent noncompliance with The Prevention of Cruelty to Animals Act, 1960 (hereinafter, "the PCA Act, 1960"), The Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998 (hereinafter, "the Breeding of and Experiments on Animals Rules, 1998"), the Guidelines on the Regulation of Scientific Experiments on Animals, 2007 (hereinafter, "the CPCSEA Guidelines, 2007"). the CPCSEA Guidelines for Laboratory Animal Facility, 2015 (hereinafter, "the CPCSEA Guidelines, 2015"), and the Guidelines of CPCSEA for Reuse Rehabilitation of Large Animals 2020 (hereinafter, "the CPCSEA Guidelines, 2020").

Based on the information presented below, we request that the Committee for the Control and Supervision of Experiments on Animals (CCSEA) pursue the following immediate actions with respect to the Company due to serious and repeat apparent violations of the PCA Act, 1960, the Breeding of and Experiments on Animals Rules, 1998, the CPCSEA Guidelines, 2007, the CPCSEA Guidelines, 2015, and the CPCSEA Guidelines, 2020:

- **Permanently terminate the Company's CCSEA registration to conduct animal experiments now or in the future, and permanently close the company's breeding centre;**
- **Direct the Company to immediately cease to perform any experiment on any animal, or transfer any animal to any other laboratory or breeding centre, or acquire any new animal; and,**
- **Ensure care and protection of all surviving animals under the custody or control of the Company by rehabilitating them—at the Company's**

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expense—at loving homes and recognised sanctuaries, for which PETA India would offer to rally and work with NGOs to assist in animal placement and care.

Whistleblower Reported Facts

The aforementioned insiders reported the following details of apparent abuse and neglect of animals at the Company to PETA India:

1. Beagles:
    a. The Company kept more beagles than its facility could properly house or was licensed for—some nearly 1,500 dogs in a space designed for some only 800, forcing three to four dogs into cages meant for just two. Overcrowding was particularly pronounced in the Company's breeding facility. According to one insider, when auditors came to inspect the facility, representatives from the Company were careful to show them the research and other facilities, steering clear of the breeding facility.
    b. The Company begins breeding dogs when they are approximately 18 months of age. They are bred twice a year, and although the Company's policy is to breed the dogs for a maximum of five breeding cycles, the Company often ignores its own policy, exceeding that limit. Dogs as old as 13 years have been used for breeding. The Company's practice of breeding dogs too frequently—without allowing the mothers adequate time to rest and recover between pregnancies—placed immense physical strain on their bodies. This overbreeding significantly increased the risk of dystocia (difficult labor) and the need for cesarean (C-section) deliveries.
    c. The Company's overcrowding of dogs, coupled with a lack of socialisation, led to extreme frustration, food aggression, and frequent fights, often causing serious injuries, especially to the dogs' ears. Despite these wounds, the Company failed to provide basic care, neglecting both proper wound cleaning and pain management.
    d. The Company's animal care staff were seen handling dogs roughly, with some workers kicking the animals or carelessly closing cage doors on their legs. Workers would pick up dogs, some weighing as much as 15 kilograms, by the scruff of the neck or the skin on their backs. Although the Company offers some training, it fails to ensure workers follow proper handling methods, as captured on closed-circuit television (CCTV), where an employee slammed a cage door on a dog's leg, prompting the dog to yelp in pain.
    e. In some studies conducted by the Company, dogs were injected subcutaneously with test compounds. According to a whistleblower, animals developed abscesses, ulcers, and signs of severe pain following these injections. In several cases, the injection sites reportedly became inflamed or developed open wounds. Depending on the location of the abscess, there can be further health issues suffered by the dogs. For example, if the abscess is in the shoulder, that can inhibit the dog's ability to move. They can be in severe pain;

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they will lose their appetite and lose weight. These infections could spread, eating through the skin and damaging the underlying tissue, leaving the dogs with open, painful wounds.

f. In the Company's other studies, dogs became very sick, and in one case, a dog vomited excessive quantities of blood before dying.
g. Although the Company specifies "humane endpoints" in its protocols, that endpoint only exists on paper. Management at the Company will tell veterinarians to wait for the sponsor to give permission before euthanizing an animal who is suffering. Everything is decided by the sponsor. If the sponsor approves euthanasia or some other action, the veterinarians can pursue such actions. Otherwise, the animal is kept suffering.
h. One insider reported that some animals suffered ulcers in their mouth and intestine from the oral dosing procedure—but they were kept alive unless the sponsor approved euthanasia.
i. The Company kills dogs using thiopentone but fails to sedate them beforehand—a basic step that could reduce their fear and distress in their final moments of life.

2. <u>Minipigs:</u>
    a. The Company purchased Göttingen minipigs from a supplier in Denmark but the Company did not have a license to breed them. At one point, a minipig became pregnant, and the head veterinarian ordered the euthanasia of the eight to ten piglets born. The piglets were killed via intracardiac injection, but were not sedated first—a basic step that could have significantly reduced their fear and suffering.
    b. Despite a written policy requiring playtime and social enrichment for pigs, the Company routinely failed to provide either. Pigs would only be given access to enrichment when there were external visitors; otherwise, they remained confined to their cages and were only removed for experimental procedures.
    c. During a visit to the Company, representatives from the Danish company mentioned above in point 2.a. observed that pigs' feet were getting injured due to improper flooring.

3. <u>Monkeys:</u>
    a. The Company used rhesus macaques captured from the forest in the state of Rajasthan. The Company had permission from the Indian government to capture 12 monkeys, but it captured 14. The monkeys were approximately 1.5 years of age and weighed less than 4 kilograms. The monkeys were sedated and placed in plastic bags, with up to five monkeys in each bag.
    b. In Rajasthan, blood samples were taken from the monkeys captured by the Company and two of these monkeys tested positive for monkeypox, which is a zoonotic disease. The other monkeys tested negative for monkeypox. However, all of the monkeys were already on their way to the Company's facility in Telangana. When the monkeys arrived at the Company's facility, the two monkeys who had tested positive for monkeypox were killed, but the others were

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kept alive—even though they had been transported from Rajasthan with the positive monkeys and monkeypox can be transmitted between monkeys.[1] The 12 surviving monkeys were again tested for monkeypox one week after they arrived at the Company's facility, even though the incubation period for the virus can be longer. However, the Company needed the monkeys for a client-sponsored test and decided to go forward with using the monkeys for the test.

c. Despite the public health risks to Company employees and the community-at-large, the Company kept the matter quiet and simply killed the monkeys.

Apparent Violations of Standards Under CCSEA Purview

These incidents *prima facie* point to serious lapses in the Company's adherence to the PCA Act, 1960, the Breeding of and Experiments on Animals Rules, 1998, the CPCSEA Guidelines, 2007, the CPCSEA Guidelines, 2015, and the CPCSEA Guidelines, 2020, overseen by CCSEA, including apparent violations of the following provisions:

APPARENT VIOLATIONS	LEGAL/REGULATORY REFERENCES
Overbreeding and Housing Too Many Beagles Without Permission **The Company held up to some 1,500 dogs severely overcrowding cages (e.g., 3–4 dogs in space meant for 2).** ***(Refer to Whistleblower Reported Fact 1.a, 2.a and 3.a above.)***	• Rule 4, Rule 5, and Rule 8(a), Breeding of and Experiments on Animals Rules, 1998[2]
Overcrowding, Lack of Veterinary Care, and Rough Handling **Beagles were kept in overcrowded cages; animals suffered untreated injuries; employees reportedly kicked dogs and handled them roughly, including slamming cage doors on limbs.** ***(Refer to Whistleblower Reported Fact 1.c, 1.d above.)***	• Rule 7(b), 7(d), 7(e), and 7(f), Breeding of and Experiments on Animals Rules, 1998[3] • Paragraphs 11 and 16, CPCSEA Guidelines, 2015[3]

[1] According to the scientific literature, transmission of monkeypox can occur through direct contact with lesions, bodily fluids, or respiratory secretions; fomites, such as contaminated bedding or cages; aerosol transmission, especially in confined settings.

[2] Breeding of and Experiments on Animals (Control and Supervision) Rules, 1998, notified under the Prevention of Cruelty to Animals Act, 1960. Ministry of Environment and Forests, Government of India. Available at: https://ccsea.gov.in/WriteReadData/userfiles/file/1998.pdf

[3] CPCSEA Guidelines for Laboratory Animal Facility, 2015. Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India. Available at: https://ccsea.gov.in/WriteReadData/userfiles/file/Compendium%20of%20CPCSEA.pdf

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	• Paragraph B.i.7, of the CPCSEA Guidelines, 2020[4] • Principle 5, CPCSEA Guidelines, 2007[5]
Excessive Whelping and Breeding **Female dogs were reportedly bred twice a year and beyond the permitted five-litter limit, without rehabilitation.** ***(Refer to Whistleblower Reported Fact 1.b above.)***	• Paragraph 9, CPCSEA Guidelines, 2015[4] • Paragraph B.i.7, of the CPCSEA Guidelines, 2020[5]
Unsanitary Housing Conditions **Animals were kept in unclean enclosures and bedding; overcrowding also compromised hygiene.** ***(Refer to Whistleblower Reported Fact 1.c and 2.c above.)***	• Rule 7, Breeding of and Experiments on Animals Rules, 1998[3] • Paragraph 22, CPCSEA Guidelines, 2015[4]
Unlicensed Breeding of Mini Pigs and Housing of Rhesus Macaques **The Company allegedly bred Göttingen minipigs without CPCSEA license, and housed rhesus macaques without CCSEA or wildlife approvals.** ***(Refer to Whistleblower Reported Fact 2.a and 3.a above.)***	• Rule 3 and Rule 10, Breeding of and Experiments on Animals Rules, 1998[3]
Improper Quarantine Practices **Monkeys were reportedly captured from the wild and killed after testing positive for zoonotic diseases, without following proper quarantine protocols.** ***(Refer to Whistleblower Reported Fact above 3.a, 3.b and 3.c .)***	• Paragraph 4(b), CPCSEA Guidelines, 2015[4]

[4] Guidelines of CPCSEA for Rehabilitation/Reuse of Large Animals Post-Experimentation, 2020. Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment, Forest and Climate Change, Government of India. Available at: https://ccsea.gov.in/WriteReadData/userfiles/file/Guidelines%20of%20CPCSEA%20for%20Reuse%20Rehabilitation%20of%20Large%20Animals%202020.pdf

[5] Committee for the Control and Supervision of Experiments on Animals. (2021). *Compendium of CPCSEA*. Ministry of Fisheries, Animal Husbandry and Dairying, Government of India. Retrieved from https://ccsea.gov.in/WriteReadData/userfiles/file/Compendium%20of%20CPCSEA.pdf (p. 53)

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Improper Euthanasia by Unqualified Personnel **Dogs were killed using thiopentone without sedation; piglets born to an accidentally pregnant minipig were killed by intracardiac injection without sedation.** ***(Refer to Whistleblower Reported Fact 1.g, 1.h and 2.a above.)***	• Rule 9(d), 9(ff), and 9(cc), Breeding of and Experiments on Animals Rules, 1998[3] • Paragraphs 31 and 32, CPCSEA Guidelines, 2015[4]
Failure to Report Adverse Reactions and Falsification of Records **Deaths and zoonotic infections were allegedly concealed from Institutional Animal Ethics Committee (IAEC) and employees, risking public health and violating documentation protocols.** ***(Refer to Whistleblower Reported Fact 1.e, 1.f, 3.b and 3.c above.)***	• Rule 11, Breeding of and Experiments on Animals Rules, 1998[3] • Paragraph 27, CPCSEA Guidelines, 2015[4]
Cruelty and Grievous Harm to Animals **Beating, maiming, overcrowding, and unauthorised killing of animals constitute cruelty under Indian law.** ***(Refer to Whistleblower Reported Fact 1.c, 1.d, 1.e, 1.g, 2.a , 3.a, 3.b and 3.c above.)***	• Section 11(1)(a), (b), (c), (e), (g), (h), (k), and (l), PCA Act, 1960 • Rule 9(ff), Breeding of and Experiments on Animals Rules, 1998[3] • Section 117(2) (Voluntarily causing grievous hurt), 271 (Negligent act likely to spread infection of disease dangerous to life) and 291 (Negligent conduct with respect to animal) of the Bharatiya Nyaya Sanhita, 2023[6]

The alleged egregious conduct at the Company, described herein, represents a serious breach of both Indian law and basic ethical standards in animal welfare and compromises scientific integrity. We trust CCSEA will treat this matter with the utmost urgency and gravity it demands.

[6] Government of India. (2023). *Bharatiya Nyaya Sanhita, 2023*. Sections 117(2) (Voluntarily causing grievous hurt), 271 (Negligent act likely to spread infection of disease dangerous to life) and 291 (Negligent conduct with respect to animal). Retrieved from https://www.mha.gov.in/sites/default/files/250883_english_01042024.pdf

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Global Regulatory Shifts Away From Animal Experimentation

We also wish to draw your attention to significant global developments concerning the growing shift away from the use of animals in experimentation. In April 2025, US federal agencies announced historic plans to phase out animal testing, in alignment with PETA US' Research Modernization NOW strategic framework for modernising research toward human-relevant methods[7]:

- The US Food and Drug Administration (FDA) announced on April 10, 2025, a "groundbreaking step to advance public health by replacing animal testing in the development of monoclonal antibody therapies and other drugs with more effective, human-relevant methods", in an effort to "improve drug safety and accelerate the evaluation process, while reducing animal experimentation, lowering research and development (R&D) costs, and ultimately, drug prices".[8]
- The US National Institutes of Health (NIH) announced a major new initiative on April 29, 2025, "to expand the development and use of cutting-edge, non-animal models—such as organoids, tissue chips, computational models, and real-world data—to address long-standing translational challenges in biomedical research. This initiative reflects NIH's commitment to advancing innovative, translationally effective research while aligning with broader federal efforts to reduce reliance on animal models".[9]

Furthermore, specifically regarding experiments on dogs, the NIH announced on May 4, 2025, that it is "getting rid of all the beagle experiments on the NIH campus"[10], and the US Secretary of the Navy announced that he is terminating all US Navy testing on dogs and cats.[11,12]

I encourage CCSEA to take these groundbreaking shifts in the global animal testing landscape into consideration, as they likely will have an impact on the Company's

[7] PETA US. Research Modernization NOW. November 2024. https://www.peta.org/wpcontent/uploads/2025/01/Research-Modernization-NOW-Biomedical.pdf
[8] US FDA. FDA Announces Plan to Phase Out Animal Testing Requirement for Monoclonal Antibodies and Other Drugs. April 10, 2025. https://www.fda.gov/news-events/press-announcements/fda-announces-plan-phase-out-animal-testing-requirement-monoclonal-antibodies-and-other-drugs
[9] US NIH. NIH to prioritize human-based research technologies. April 30, 2025. https://oacu.oir.nih.gov/about-oacu/news-events/news/nih-prioritize-human-based-research-technologies
[10] NIH [@NIH]. May 4, 2025. [Watch @NIHDirector_Jay on @FoxNews with @RCamposDuffy where he discusses a new NIH initiative to expand innovative, human-based science while reducing animal use in research, including getting rid of all the beagle experiments on the NIH campus.] [Post]. X. https://x.com/NIH/status/1919070337225855335.
[11] Bedard P. May 28, 2025. Trump cheered as 'best friend of animals' after research grants nixed. Washington Examiner. Accessed May 29, 2025. https://www.washingtonexaminer.com/news/washington-secrets/3423973/trump-cheered-bestfriend-of-animals-after-research-grants-nixed/.
[12] Secretary of the Navy [@SECNAV]. May 27, 2025. [Today it gives me great pleasure to terminate all Department of the Navy's testing on cats and dogs, ending these...] [Post]. X. https://x.com/SECNAV/status/1927500765817393569?s=19

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business model that is currently predicated on using animals in experiments. As the first step, the Company should be required to release all of its surviving animals to loving homes or recognised sanctuaries, and PETA India would be happy to work with other NGOs to assist in finding them good forever homes to live out the remainder of their lives in peace.

I can be reached at **aaggarwal@petaindia.org** or +91-9958840994. We stand ready to assist you further as needed. Thank you for your attention to this important matter, and we look forward to your response.

Sincerely,

Dr Anjana Aggarwal
Scientist and Research Policy Advisor
PETA India

cc: **CCSEA Core Committee Members**

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Annexure E:

Updated list of Core committee members of CCSEA

PEOPLE FOR THE ETHICAL TREATMENT OF ANIMALS INDIA

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Appendix E: Analysis of the Committee for the Purpose of Control And Supervision of Experiments on Animals (CCSEA) Core Committee Members

S.No.	Member Name and Title at Institute	Institute Name	Relation to Animal Testing/Labs
1.	Dr. Muthukumarasamy B, IAS, Joint Secretary	Animal Welfare Board of India (AWBI)	NA
2.	Representative (name not revealed)	Animal Welfare Board of India (AWBI)	
3.	Dr. Rajendra Gulabrao Bambal, Secretary (Addl. Charge)	Veterinary Council of India	Significant amount of animal experimentation on livestock 'productivity'[1].
4.	Dr. S. Kavimani, Professor & HOD, Department of Pharmacology	Mother Theresa Post Graduate & Research, Institute of Health Sciences, Puducherry	Active animal experimenter (e.g., toxicity tests) and has published various experiments on mice[2].
5.	Dr. Aanam Vishala, Joint Drugs Controller	Central Drugs Standards Control Organization, New Delhi	Associated with CDSCO that routinely accepts, reviews, and relies on animal testing-derived data for decision-making.
6.	Chairman or his representative (name not revealed)	National Medical Commission (NMC)	Current regulations of NMC continue to include animal experimentation as an integral part of the curriculum for certain postgraduate medical courses, specifically in Physiology and Pharmacology.
7.	Prof. Rana Pratap Singh	Jawaharlal Nehru University (JNU), School of Life	JNU's Centre for Laboratory Animal Research (CLAR) conducts CCSEA-

[1] Bambal, Rajendra Gulabrao. "Scientific Contributions." *ResearchGate*. ResearchGate, n.d., www.researchgate.net/scientific-contributions/Rajendra-Gulabrao-Bambal-2084502230. Accessed 10 Nov. 2025.
[2] Kavimani, S. "S. Kavimani | Professor (Full) | M.Pharm., PhD." *ResearchGate*, ResearchGate, n.d., www.researchgate.net/profile/S-Kavimani. Accessed 10 Nov. 2025.

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		Sciences, New Delhi	registered animal experimentation for life sciences, likely involving rodents for genetic and biomedical experiments.
8.	Dr Ruchi Singh, Scientist G & Head	Indian Council for Medical Research (ICMR)	ICMR's Discovery Research Division oversees animal testing projects[3], and ICMR institutes like National Animal Resource Facility for Biomedical Research (NARFBR) are engaged in animal experimentation.
9.	Dr. Karthikeyan Vasudevan, Chief Scientist	Laboratory for the Conservation of Endangered Species (LaCONES), Centre for Cellular and Molecular Biology (CCMB), Hyderabad, Telangana	LaCONES routinely conducts reproductive / biotechnology intervention experiments on animals, including wildlife species and rodents.
10.	Dr. Raghavendra Bhatta, Deputy Director General (AS)	Indian Council of Agricultural Research (ICAR), New Delhi	ICAR's mandate explicitly includes conducting, funding, and promoting experiments on animals.
11.	Dr Nagendra R. Hegde, Scientist-H	National Institute of Animal Biotechnology (NIAB), Hyderabad	NIAB's portfolio demonstrates extensive, long-term, and systematic dependence on animal experimentation and animal disease models.[4]

[3] Development of mice models for amyloidosis: a rare disease. Rare Diseases Registry & Data Bank (RDRDB), Indian Council of Medical Research (ICMR) [Internet]. File no. 33/15/2019-TF/Rare/BMS; 2020 Jan 6–2023 Jul 5 [cited 2026 Jan 15]. Available from: https://rdrdb.icmr.org.in/view_record1/19/

Entities:
- PETA US
- PETA Asia
- PETA France
- PETA Australia
- PETA Germany
- PETA Switzerland
- PETA Netherlands
- PETA Foundation (UK)

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12	Dr. Asmita Gajbhiye, Professor and Dean	School of Engineering and Technology, Dr. Harisingh Gour University, Sagar, Madhya Pradesh	Dr. Harisingh Gour University, Sagar, Madhya Pradesh conducts animal-based teaching and experiments across its Zoology, Pharmacy, Biotechnology, and Life Sciences departments, making it an active user of animals in laboratories.
13.	Prof. Dr. Arvind Dasharath Ingle, Scientific Officer 'H' and Officer-in-Charge	Laboratory Animal Facility & Histopathology, Tata Memorial Centre, Advanced Centre for Treatment Research & Education in Cancer (ACTREC), Mumbai, Maharashtra	Prof. Dr. Arvind Dasharath Ingle uses animals in various experiments at ACTREC[5].
14.	Dr. Subeer S. Majumdar	Gujarat Biotechnology University, Gandhinagar	Operates a CCSEA-registered animal facility for biotechnology experiments, focusing on transgenic and genetic models[6].
15.	Dr. Ramachandra S.G.	Indian Institute of Science (IISc), Central Animal Facility, Bangalore	IISc's Central Animal Facility is CCSEA-registered, and uses animals for basic and applied experiments across disciplines like

[4] Hegde NR. Dr. Nagendra R. Hegde [Internet]. National Institute of Animal Biotechnology; [cited 2026 Jan 4]. Available from: https://www.niab.org.in/PeopleResearchNagendraHegde.aspx

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[6] Majumdar, Subeer S. "Dr Subeer S. Majumdar." *NII Former Faculty*, National Institute of Immunology. https://gbu.edu.in/faculties/dr-subeer-s-majumdar/ Accessed 10 Nov. 2025.

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			neuroscience and pharmacology[7].
16.	Dr. Suresh Pothani (retired)	ICMR-NARFBR, Hyderabad	Previously directed NARFBR, where he supervised animal breeding and preclinical testing, with direct involvement in experimentation on animals[8].
17.	Dr. R. Gopinath, Member	All India Institute of Medical Sciences (AIIMS), New Delhi	AIIMS's Central Medical and Imaging Experimentation (CMIE) Animal Testing Centre uses animals including rats, mice, rabbits, guinea pigs, and hamsters for pharmacology and physiology experiments[9].
18.	Dr. Pradeep Bhatu Patil, Member	ICMR-National Institute of Nutrition (NIN), Hyderabad	NIN's CCSEA-registered animal facility breeds, supplies, and uses animals for nutrition and dietary experiments, making it directly involved in extensive animal experimentation. [10]
19.	Dr. SK Dutta, Joint Commissioner	Department of Animal Husbandry and Dairying (DAHD)	

[7] "Centre for Animal Facility." *CAF IISc*, Indian Institute of Science, https://caf.iisc.ac.in/ . Accessed 10 Nov. 2025
[8] Pothani, Suresh. "Scientific Contributions." *ResearchGate*, ResearchGate. https://www.researchgate.net/scientific-contributions/Suresh-Pothani-2108342037 . Accessed 10 Nov. 2025
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Annexure F:

PETA India's Research Modernisation Deal

The Research Modernisation Deal

2020

PETA People for the Ethical Treatment of Animals (PETA) India

People for the Ethical Treatment of Animals (PETA) India is an animal rights organization dedicated to establishing and protecting the rights of all animals. Please visit www.petaindia.com for more information.

Version 1.1 • last updated 19 May 2020

Executive Summary

Astonishing advances in research technology are already revolutionising biomedical research and regulatory testing, and even more progress is expected in the coming years.

The transition away from research relying on the use of animals to model human disease or as tools to predict human responses to drugs or other substances and towards human biology–based methods is changing policy and practice around the globe. Research funders are becoming increasingly aware that failing animal methods used to establish both efficacy and toxicology risk are holding back the development of potential cures. In the existing animal research paradigm, novel drugs take 10 to 15 years to reach the market at a cost of over $2 billion, and over 95 per cent of them fail when they reach clinical trials. These failure rates cannot be supported economically or ethically, and efforts to transform the research environment are urgently needed.

Consider the following key points:

- Systematic reviews published in peer-reviewed journals document limitations in translating results from studies using animals to treatments for humans for numerous disease areas. Fewer than 10 per cent of highly promising basic science discoveries enter routine clinical use within 20 years.
- Between 50 and 89 per cent of preclinical research is not reproducible, with animal experimentation implicated as a serious problem area.
- Major scientific breakthroughs in disease areas such as diabetes and breast cancer have relied on studies of human disease in patients; they would not have been possible using animal research.

Along with growing evidence that experiments on animals do not faithfully translate to treatments for humans – as well as the development and implementation of technology that supplants animal use in laboratories – our society has also witnessed growing moral concern regarding the practice of using animals in experiments.

Public, private, and charitable funding bodies must cut budgets for experiments using animals and redirect funds to non-animal methods. In order to end the use of animals in experiments, we recommend the development of a strategy that includes the following critical steps:

1. **Immediately eliminate animal use in areas for which animals have already been shown to be poor and unreliable predictors for humans and have impeded progress.**
2. **Conduct critical scientific reviews to identify the areas in which the use of animals can be ended immediately**
3. **Implement an ethical harm-benefit analysis system.**
4. **Harmonise and promote international acceptance of non-animal testing methods for regulatory toxicity testing requirements.**
5. **Redirect funds from animal studies to the development of non-animal methods.**

Table of Contents

I. Introduction

The Indian Department of Science and Technology's (DST) gross expenditure on research and development (R&D) has consistently increased over the years and more than quadrupled from Rs 24,117.24 crores in 2004–2005 to Rs 1,04,864.03 crores in 2016–2017.[1] A percentage of this funding is used for animal experimentation, even though an increasing number of studies show – and more and more scientists acknowledge – that the results of experiments on animals are often not reproducible or translatable to humans. The failure of animal testing is also evident in the fact that more than two decades of drug discovery in India have not led to a single new chemical entity that has entered national and global markets from India, although a few molecules have been out-licensed to pharmaceutical companies globally.[2]

The US National Institutes of Health (NIH), the world's largest funder of biomedical research, reports that "failure rates [for novel drugs] occur in about 95 percent of human studies", even though these drugs showed success in preclinical experiments using animals.[3] A 2015 investigation concluded that between 50 and 89 per cent of all preclinical research, a large part of which involves animal testing, could not be reproduced. At the most conservative US estimate, the abundant failure to reproduce preclinical research results in approximate annual spending of $28 billion on misleading experimentation.[4]

Acceptance of non-animal techniques in one region or country is an open door to international harmonisation and the wider statutory elimination of animal experiments. Over the past two decades in particular, significant progress has been seen in the development, validation, implementation, and regulatory acceptance of non-animal technology for the assessment of human health endpoints such as skin irritation and corrosion, serious eye damage, skin sensitisation, skin absorption, and phototoxicity. We've also seen an end to notoriously cruel international test guidelines such as the Organisation for Economic Co-operation and Development (OECD) Test No 401, also known as the Lethal Dose 50 (LD_{50}) test. Opportunities exist to increase and harmonise the use of validated non-animal test methods for regulatory assessment, and by taking them, we can achieve better protection of human health and the environment within the appropriate legal framework.

In order to work towards this goal, we present in this report a roadmap for replacing the use of animals in experimentation. We identify a number of strategic priorities and append further information regarding areas of both regulatory (government-required) and non-regulatory research where there are opportunities for the immediate and near-future replacement of animal use. We have also included information outlining areas in which further development, validation, and implementation of non-animal methods are required.

II. Limited Predictive Value of Research Using Animals

A great deal of scholarly research in the last 15 years shows that animal studies are flawed and divert both monetary and intellectual resources from methodologies better suited to curing human disease. There are many factors at play in the failure of animal experimentation to predict human outcomes reliably.[5] Critically, intrinsic biological and genetic differences between species contribute significantly to problems in extrapolating results from non-human animals to humans, even in the best controlled and best-executed study designs.

A stunning 2014 analysis published in *The BMJ* found that – contrary to public perception – studies using animals have not furthered knowledge in the field of human health or led to the development of treatments for conditions affecting humans.[6] The authors note:

> If research conducted on animals continues to be unable to reasonably predict what can be expected in humans, the public's continuing endorsement and funding of preclinical animal research seems misplaced.

The difficulties in applying data derived from animals to human patients are compounded by the confinement and unnatural conditions of laboratory life, which thwart animals' ability to engage in natural behaviour.[7] This deprivation contributes to their stress and alters their physiology and neurobiology, causing them to exhibit various psychopathologies.[8,9,10] Importantly, the fact that animals in laboratories have altered physiology and neurobiology means that they will not be good "models" for their counterparts in the wild. A mouse in a laboratory will not respond to a drug in the same way that a mouse in a field would. One then has to ask, how does this biologically distinct mouse reliably represent the biology of human beings?

The opposite is also true: therapies that have not worked well in animals have sat useless on the shelf while patients have gone without life-saving treatment. For example, penicillin was first tested in rabbits in 1929, but as it had no apparent effect in this species, it was ignored for more than a decade – costing countless human lives. The first human clinical trials weren't conducted until the 1940s.[11, 12] Researchers later remarked on the good fortune that it was not first tested in guinea pigs, for whom the antibiotic is lethal. Had experimenters seen this result, penicillin may have never been tried in humans.[13,14]

III. The Need for a Paradigm Shift

Research involving animals is impeding the development of treatments and cures for human ailments. Many within the scientific community have begun to advocate for change.

In support of using an evidence-based approach to accelerating the delivery of useful drugs to the patients who need them, 15 Vanderbilt University researchers published a 2017 article calling for the elimination of experiments using animals where there is clear evidence that animal "models" are not useful or predictive of human disease.[15] As another example, *The Turkish Journal of Gastroenterology* officially banned the publication of studies involving experiments on animals from its pages. Journal editor Dr Hakan Şentürk wrote that the new policy represents "growing concern about the lack of applicability of animal research to humans."[16]

Significantly, a move away from animal-based research will allow for substantial growth in the science and technology sectors and for a faster return on investment in drug research and development.[17] An evolution of research funding priorities toward human-relevant methods will get treatments to the patients who need them more safely and likely in less time.[18,19]

IV. Opportunities for Economic Advancement

By mandating a move away from animal experimentation and towards advanced scientific methods, India has the opportunity to expand job growth rapidly in science and technology and reduce health-care costs for the entire population. The country is among the top 12 biotechnology hot spots in the world and ranks third in the Asia-Pacific region, mainly because of its more than 600 core biotechnology companies and 2,600 biotech start-ups.

India also has the second-highest number of US Food and Drug Administration (FDA)–approved manufacturing plants outside the US and is currently home to more than 523 FDA-approved drug-manufacturing facilities. The Indian biotechnology industry that was valued at $64 billion in 2019 is expected to reach $150 billion by 2024–2025.[20,21]

As Meigs and colleagues report in their review, "Animal Testing and Its Alternatives – the Most Important Omics Is Economics", "an economy of alternative approaches has developed that is outperforming traditional animal testing".[22] Using the traditional process, bringing a new drug to market may cost up to $2 billion and take as long as 15 years.[23] The high cost of R&D may be transferred to patients who

are compelled to pay increasingly unmanageable prices for prescription drugs.[24] With the use of human-relevant technology in place of expensive, time-consuming, and inaccurate animal experiments, the cost of drug discovery has the potential to decrease dramatically.

The market for human-based *in vitro* technology for biomedical research and testing is growing rapidly. According to a report by Grand View Research, Inc, "The global in-vitro toxicology testing market is expected to reach USD 44.7 billion by 2022 growing at an estimated CAGR [compound annual growth rate] of 10.5% from 2015 to 2022 …. This expected rise in demand can be ascribed to novel and promising technologies in analytical laboratories."[25] For example, in the US, the Boston-based start-up Emulate, Inc, raised $36 million in financing to expand its organ-on-a-chip technology, which is being used by AstraZeneca, Roche, Merck, Johnson & Johnson, and others to predict the safety and efficacy of drug candidates more accurately.[26,27] New technology will streamline drug development, making the process safer, cheaper, and more effective.

V. Regulatory Opportunities for Humane Toxicity Assessment

The past quarter-century has seen a revolution in the way in which chemicals are tested – non-animal tests are rapidly replacing animal tests. This is the result of our better understanding of biological processes and the emergence of new technology, which has allowed for the development of testing methods that can look directly at cellular mechanisms rather than at the crude, inscrutable results that come from using animals. It is also the result of public pressure and, as explained below, dissatisfaction among scientists with the results from animal tests. Concurrently, there is growing recognition among regulators and the regulated community that animal tests do not adequately protect either human health or the environment and that "the current approach is time-consuming and costly, resulting in an overburdened system that leaves many chemicals untested, despite potential human exposure to them".[28]

In 2007, the US National Academies of Sciences, Engineering, and Medicine published a landmark report titled "Toxicity Testing in the 21st Century: A Vision and a Strategy". The report states that advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on *in vitro* methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin. The proposed changes will generate better data on the potential risks humans face from environmental chemicals, building a stronger scientific foundation that can improve regulatory decisions to mitigate those risks and reducing the time, money, and number of animals used for testing.[29]

VI. Public Opinion and Animal Sentience

Public opposition to animal research is a major factor driving policy change. A 2009 YouGov survey conducted in six EU countries found overwhelming opposition to animal experiments – 84 per cent of respondents were in favour of prohibiting all experiments in which animals would be subjected to severe pain and suffering.[30] Public support for investment in non-animal methods is also high –74 per cent of respondents to a UK survey backed increased efforts to develop alternatives to animal use.[31]

Given the growing recognition of animal sentience, public opposition to animal experimentation is not surprising. In 2012, a prominent international group of neuroscientists issued *The Cambridge Declaration on Consciousness*, which definitively stated that "humans are not unique in possessing the neurological substrates that generate consciousness" and that, like humans, "[n]on-human animals have the … capacity to exhibit intentional behaviours".[32]

More than 150 academics, intellectuals, and writers have also backed a report by the Oxford Centre for Animal Ethics that condemns experiments on animals as both morally and scientifically indefensible. "The deliberate and routine abuse of innocent, sentient animals involving harm, pain, suffering, stressful confinement, manipulation, trade, and death should be unthinkable. Yet animal experimentation is just that: the 'normalisation of the unthinkable'," write the report's authors. They conclude that experimenting on animals contradicts what we now know about animals' ability to experience not only pain but also shock, fear, foreboding, trauma, anxiety, stress, distress, anticipation, and terror.[33]

VII. Leadership

There is movement internationally that reflects the growing consensus in the scientific community that using animals in basic biomedical research or for regulatory assessment requirements is neither ethical nor efficacious.

In 2016, the Dutch government announced its plan to phase out toxicology tests for chemicals, food ingredients, pesticides, veterinary medicines, and vaccines by 2025. This was after the Dutch National Committee for the Protection of Animals Used for Scientific Purposes stressed the need for a paradigm shift away from procedures on animals. Its report on the Netherlands' transition to non-animal research included objectives for the country to become an international leader in the field of innovation without animals in applied and

translational research.[34] This led to the initiation, in 2017, of the Transition Programme for Innovation without the use of animals (TPI), which aims to bring together regulators, scientists, funding bodies, and industry and offer them a platform for identifying and developing innovative activities within their fields that will increase the pace of the transition towards animal-free innovation. The TPI is supervised by the Minister of Agriculture, Nature and Food Quality. In 2019, the US Environmental Protection Agency announced that it would eliminate all funding of and requests for tests on mammals by 2035, reduce testing by 30% by 2025, provide funding for non-animal test method development, and hold an annual conference to discuss the advancement of non-animal testing.[35]

In order to advance animal-free testing, the Indian Council of Medical Research (ICMR) published a paper titled "Need for Alternatives to Animals in Experimentation: An Indian Perspective", which encourages the use of a 21st century toolbox of human-relevant, non-animal techniques to make India self-reliant in the development of non-animal technologies. The paper sheds light on the current status of non-animal testing in the country, identifies gaps, and presents a roadmap for addressing the remaining gaps.[36] ICMR also plans to establish a Centre of Excellence in Human Pathway–Based Biomedicine and Risk Assessment in Hyderabad for the advancement of human-specific approaches in medical research and product safety testing.

The current COVID-19 pandemic presents a unique opportunity to set new and improved standards for testing the safety and efficacy of new therapeutics. Currently, regulatory agencies and governing bodies in India[37] and around the world are demonstrating that regulations requiring extensive animal testing before human clinical trials are unnecessary barriers to introducing lifesaving drugs. For example, the Indian Central Drugs Standard Control Organisation (CDSCO) has approved open randomised clinical trials to study the efficacy and safety of convalescent plasma therapy.[38] Moreover, to encourage the development of a vaccine or treatment for COVID-19, CDSCO has made the allowance that "[d]ata requirement for animal toxicity study, clinical study, stability study etc. may be abbreviated, deferred, or waived on case to case basis".[39]

VIII. Plan for Action: Recommendations to Modernise Scientific Research and Assessment

There is a need and an abundance of opportunities for India to shift the regulatory testing and biomedical research paradigm towards innovative animal-free techniques and become a world leader in the application of such methods. The country has the necessary academic and industrial strength to develop and employ new non-animal forms of technology, which could position it as a global powerhouse in this area. Implementation of the following strategies will generate rapid, economical, and reproducible data; improve regulatory decision-making; reduce the rate of clinical trial failures; and reduce the ethical burden of using animals in experiments.

1. Immediately eliminate animal use in areas for which animals have already been shown to be poor and unreliable predictors for humans and have impeded progress.

Multiple reviews have documented the overwhelming failure of animal use to benefit human health in specific areas, including neurodegenerative diseases, neuropsychiatric disorders, cardiovascular disease and stroke, cancer, diabetes and obesity, inflammation and immune responses, HIV/AIDS research, addiction studies, trauma research, and medical training. As such, animal experiments in these research areas should be ended as soon as possible and replaced with more effective and efficient non-animal research methods. Please find appended further elaboration and recommendations on these areas.

2. Conduct critical scientific reviews to identify the areas in which the use of animals can be ended immediately

For those areas of investigation where there is still some question as to whether the use of animals is beneficial, a thorough systematic review should be conducted to determine the efficacy of using animals. Systematic reviews, which critically analyse multiple research studies, are the first step in assessing the effectiveness of animal research. Some countries, such as the Netherlands, require that systematic reviews be conducted before animal studies can receive funding. Furthermore, Article 58 of Directive 2010/63/EU mandates that the European Commission conduct periodic reviews concerning the use of animals in scientific procedures, thus providing a clear mechanism for advancing the replacement of animals in scientific procedures.

It is recommended that the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) meticulously maintain and publish information on the number of animals used in experiments and the results of these experiments. Moreover, CPCSEA and funding agencies such as the DST, ICMR, and Department of Biotechnology (DBT) should conduct critical scientific analyses of animal research and funding proposals by determining their scientific relevance and reject those in areas where non-animal methods exist.

3. Implement an ethical harm-benefit analysis system.

Directive 2010/63/EU on the protection of animals used for scientific purposes requires that applications to conduct research using animals be evaluated to ensure full use of available alternative techniques and test methods as well as consideration of whether the expected outcome of the research can justify the level of pain, distress, and suffering likely to be experienced by animals.[40] While these project evaluations are generally conducted through government bodies, they at least provide a means by which ethical evaluations can take place.

Likewise, in order to improve the robustness of the regulatory system, the UK government's Animals in Science Committee has recommended that the prospective harm-benefit analysis should be improved and that societal concerns about animal research should be explored and addressed. Furthermore, the committee recommended that methods to avoid those procedures predicted to cause severe pain, distress, and lasting harm should be explored – the ultimate goal being the elimination of these types of procedures in their entirety.

Section 17(1) of The Prevention of Cruelty to Animals Act, 1960, requires "that animals are not subjected to unnecessary pain or suffering before, during or after the performance of experiments on them".[41] However, the current system does not adequately determine an animal's suffering in experiments, and therefore, this assessment is not possible.

4. Harmonise and promote international acceptance of non-animal testing methods for regulatory toxicity testing requirements.

The regulatory acceptance of non-animal techniques in one region or country is an open door to international harmonisation and the wider statutory elimination of animal testing methods. As a signatory to the OECD Mutual Acceptance of Data, India must accept the data generated by other OECD member countries in order to avoid duplicating the generation of toxicity data. The ICMR paper noted, "[Human-derived, three-dimensional] models are rapidly evolving and replacing animal studies either to reduce or minimize animal usage in research. In addition, regulatory agencies in the western world are very receptive to the evolving novel, non-animal technologies to replace animal studies. Collaborative efforts between regulatory agencies, academia and industry in the western countries are simply revolutionizing the development of human-derived, non-animal technologies, not only for less animal dependence but also to improve the successful translational outcomes in humans."[42]

We advocate that national and international regulatory bodies and standards organisations liaise with industry, research agencies, and relevant NGOs worldwide to establish and promote clear paths to the validation and harmonisation of non-animal techniques for regulatory testing requirements.

5. Redirect funds from animal studies to the development of non-animal methods.

Indian researchers are involved in the advancement of animal-free tests. For example, various protocols have been standardised for efficient generation of three-dimensional spheroids using hepatic cell lines[43] and human umbilical cord mesenchymal stem cells[44] derived hepatospheroids as *in vitro* models for routine drug metabolism and hepatotoxicity testing – and three-dimensional bio-printed tissues and organs such cartilage, liver, and skin are being successfully generated.[45,46,47] *In silico* models have also been routinely employed for assessing the ecotoxicological risk of a wide

range of chemicals[48,49,50] and for identifying and designing molecules as inhibitors or vaccines against various therapeutically important drug targets.[51] A predictive, comprehensive system biology tool, eSkIN, has been developed by the Indian Council of Science and Industrial Research – Institute of Genomics and Integrative Biology (CSIR-IGIB) and Pune-based firm Persistent System to ascertain the efficacy and adverse effects of chemicals used by cosmetics and pharmaceutical companies.[52]

For these benefits to be realised, these models must be scientifically validated, rapidly scaled up for industrial use, and fully integrated into the pharmaceutical and chemical development pipelines. An ICMR paper states, "It is important that the Government encourages the creation of 'Centers of Excellence (COE)' where 'Alternatives to Animals' research in India can be compared and compete with the elite COE in western countries. … Funding for research focusing on human-based biology, rather than 'improved' animal models, should be prioritized." The authors add, "Government funding agencies should fund private sectors with these kinds of innovations in alternatives to animal technology startups to give the necessary boost for many of the concepts to grow to a prototype stage."[53]

National and international institutes must take the next step and end the funding of crude experiments that have failed to provide effective treatments and cures. With greater investment in innovative non-animal methods and bold policy initiatives, far more promising cures and treatments for humans can be developed. This will also alleviate the almost unimaginable suffering of millions of animals.

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Appendices

Please find below further detail on opportunities to replace animals in the following areas of biomedical research and training, forensic sciences, toxicity assessment, and laboratory production methods. Also included is information regarding the expertise of the scientists who work for PETA and its international affiliates.

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Glossary

3Rs	replacement, reduction, and refinement (of animal use)
AD	Alzheimer's disease
ADHD	attention deficit hyperactivity disorder
AIDS	acquired immune deficiency syndrome
AOP	adverse outcome pathway
ATLS	Advanced Trauma Life Support
BCOP	bovine corneal opacity and permeability
CTA	cell transformation assay
DPRA	direct peptide reactivity assay
ECHA	European Chemicals Agency
EDQM	European Directorate for the Quality of Medicines & HealthCare
EDSP	Endocrine Disruptor Screening Program
EMA	European Medicines Agency
EPA	Environmental Protection Agency
EURL ECVAM	European Union Reference Laboratory for Alternatives to Animal Testing
FBS	foetal bovine serum
GEMM	genetically engineered mouse model
GHS	Globally Harmonized System of Classification and Labelling
h-CLAT	human cell line activation test
HD	Huntington's disease
HIV	human immunodeficiency virus
hPL	human platelet lysate
IATA	integrated approach to testing and assessment
ICCVAM	Interagency Coordinating Committee on the Validation of Alternative Methods
IET	Institution of Engineering and Technology
IFV	Influenza virus
ISO	International Organization for Standardization
JaCVAM	Japanese Center for the Validation of Alternative Methods
LAL	Limulus amoebocyte lysate test
MAT	monocyte activation test
MND	motor neurone disease
NICEATM	NTP Interagency Center for the Evaluation of Alternative Toxicological Methods
NIH	National Institutes of Health
NOS	nitric oxide synthase
NRU	neutral red uptake
NTP	National Toxicology Program
OECD	Organisation for Economic Co-operation and Development
PD	Parkinson's disease
PDAC	pancreatic ductal adenocarcinoma
Ph Eur	European Pharmacopoeia
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RhCE	reconstructed human cornea-like epithelium
RHE	reconstructed human epidermis
RPT	rabbit pyrogen test
SA	structural alert
SCCS	Scientific Committee on Consumer Safety
SCI	spinal cord injury
SCHEER	European Commission Scientific Committee on Health, Environmental and Emerging Risks
SIV	simian immunodeficiency virus
STAIR	Stroke Therapy Academic Industry Roundtable
STE	short time exposure
T2DM	type 2 diabetes mellitus
TER	transcutaneous electrical resistance
TZD	thiazolidinedione
WoE	weight of evidence

Basic and Applied Biomedical Research

Detailed below are opportunities to end the non-regulatory use of animals immediately in a number of specific areas of biomedical research.

Cancer

Recommendation: End the use of animals immediately

Oncology drugs have the lowest "likelihood of approval" among all disease categories. A survey of 4,451 drugs made by 835 companies between 2003 and 2011 found that only 6.7 per cent of cancer drugs were approved after entering the first phase of clinical trials, even though they were all successful in preclinical testing. A 2018 analysis of data collected between 2000 and 2015 shows that the success rate for oncology drugs dropped to 3.4 per cent,[1] suggesting that the problem is getting worse. The authors admit that the "current animal models (e.g., xenograft tumor models in mice) can be poor predictors of clinical outcomes in humans".[2] Even though study design and other logistical issues can be problematic, cancer physicians at McMaster University in Ontario state the following:

> [M]ost futilities in fact originate from molecular mechanisms of the drug(s) tested… Crucial genetic, molecular, immunologic and cellular differences between humans and mice prevent animal models from serving as effective means to seek for a cancer cure.[3]

Following an analysis of 1,110 mouse xenograft tumour models, which involve the transplantation of human tumour cells into mice, scientists and physicians from Harvard University, Massachusetts Institute of Technology, the Dana-Farber Cancer Institute, and other respected institutions reached a conclusion that challenged the ability of xenograft models to predict patients' response to therapy. They found that transplanting human cancer cells into these mice altered the genetic composition of those cells in ways that would be unlikely to happen in humans. That, in turn, altered the responses that the cells had to chemotherapy drugs,[4] invalidating one of the foundational animal models for human cancer research.

There are numerous examples of the ways in which rodent models have misled cancer researchers. For brevity, we will present three cases. Scientists now know that endogenous bile acids, if dysregulated, can induce DNA damage and several forms of cancer, such as colon cancer, in humans. However, previous experiments on rats show that bile acids are not carcinogenic on their own. The profiles of bile acids, metabolism of bile acids (by the liver and gut microbiome), and colon epithelial cell accumulated turnover rate (adjusted by age) are all different between rodents and humans, contributing to the discrepancy.[5]

Another example of the disconnect between human cancer and rodent cancer research is the formerly proposed link between soya and breast cancer. It is now recognised that isoflavones in soya may be protective against several types of cancer, such as breast and prostate cancers,[6] particularly if people are exposed to it early in life.[7] However, it was observed that genistein, a major isoflavone in soya, induces oestrogen-sensitive tumours in some animals used in studies, including rodents. The inconsistency was later explained to be due to differences in phase II metabolism of genistein in rodents, whose level of unconjugated, and hence active, genistein is about 20 to 150 times higher than that of humans (depending on the strain). Additionally, rodent models had low endogenous oestrogen levels and different metabolic profiles compared to humans, and high experimental levels of isoflavones were used in those initial studies.[8]

Rodents are not suitable for radiation-induced carcinogenesis research, including for thyroid cancer. The nuclear architecture and spatial positioning of genes involved in radiation-induced injury are drastically different between rodent and human thyroid cells.[9] Similarly, rodents are not suitable for research into pancreatic ductal adenocarcinoma (PDAC). As some scientists have pointed out, "Although it may seem obvious that there are important differences between men and mice, this is often overlooked by those modeling human disease. … The potential for species differences to be relevant is greatest in models that use nonhuman PDACs, such as genetically engineered mouse models (GEMMs) and syngeneic xenografts."[10]

Given the many shortcomings described above as well as the astonishingly low translational success rate of cancer research, despite the popularity of using rodents in such research, it is clear that they are not good models for any type of human cancer experimentation. Therefore, it is wise to move away from rodent models and focus on human-relevant methods.

The prestigious Institution of Engineering and Technology (IET) global Harvey Engineering Research Prize was recently awarded to Portuguese scientist Rui L Reis for his work using tissue engineering to create reliable 3-dimensional (3-D) engineered functional cancer disease models. According to IET, his innovative research will "help to predict the efficacy of novel cancer drugs and potential therapies, avoiding a range of unnecessary animal tests, and preclinical and clinical trials of doomed to fail new drugs".[11]

Other recent, human-relevant cancer research includes the development of a human blood vessel-on-a-chip to aid in the advancement of new cancer therapies that may inhibit new blood vessel formation to slow tumour growth,[12] the study of patient-derived human brain organoids to develop personalised therapies for deadly glioblastomas,[13] the use of a tumour microenvironment-on-a-chip to create precision medicine tailored to individual patients and specific cancer types,[14] and the application of 3-D printing to producing precise replicas of tumours using patients' own cells in the bioink.[15] In addition, by sequencing DNA and RNA in human skin cells, researchers at the University of California–San Francisco have analysed which signalling pathways are disrupted in the evolution of melanoma.[16]

Former National Cancer Institute Director Dr Richard Klausner stated the following:

> The history of cancer research has been a history of curing cancer in the mouse. We have cured mice of cancer for decades – and it simply didn't work in humans.[17]

Cancer is a highly variable, individualised disease that will require individualised treatment to overcome.[18] Scientists using non-animal methods for cancer research are faced with a smaller translational hurdle, since they are able to use patients' own cancer cells and because all human-relevant methods are grounded in human – instead of rodent – biology.

Cardiovascular Disease

Recommendation: End the use of animals immediately

Cardiotoxicity is a primary reason that drugs fail in clinical trials. Experts point out the "lack of concordance between the effects of compounds in animals (or animal-derived tissues) and those in humans",[19] that "substantial differences in drug responsiveness between species can limit the effectiveness of predicting clinical outcome from animal toxicity testing",[20] and the many known species-related differences in cardiac contractile function and calcium handling.[21] In a co-authored review, scientists from Stanford University, the US Food and Drug Administration, and the biopharmaceutical company AbbVie refer to testing cardiotoxicity in animal models as a "black box" approach.[22]

The properties of calcium-handling proteins and their composition differ in the hearts of rats, mice, rabbits, dogs, and humans, and rodents and humans do not have the same profiles or functions of contractile proteins.[23] This makes the profile of ventricular repolarisation and susceptibility of arrhythmia different, leading to varied drug responses. A meta-analysis evaluating 11 measured functional parameters of the heart, comparing rodents with humans, concluded that only one (systolic pressure) was within an acceptable range for comparison between the two species.[24] Rodents are also resistant to atherosclerosis, a major cause of many cardiovascular diseases, owing to their lack of cholesteryl ester transfer protein.[25]

For heart failure research, "insights gleaned from animal-based research efforts have shown poor translation in terms of deciphering human heart failure and developing effective therapies", and "lack of concordance between animal models and human disease state has been acknowledged as a major contributing factor [to this translational failure]".[26] It is clear that human-relevant *in vitro* and *in silico* methods are much more suitable for cardiotoxicity testing and cardiovascular research in general.

The global stem cell biotechnology company Novoheart is using a platform called MyHeart™ composed of engineered human cardiac tissues, which has been able to "detect the devastating arrhythmogenic hazards of certain 'anti-arrhythmic' drugs that had previously caused fatalities in human patients despite passing through the flawed process of animal testing for FDA approval".[27] Scientists in Singapore and New York are using organ-on-a-chip models of blood vessels and beating heart tissue, respectively, to model human atherosclerosis and test human reactions to various drug compounds.[28,29] Worcester Polytechnic Institute's Marsha Rolle, a tissue engineer, has created functional blood vessels from human cells to "replicate what happens when [human blood vessels are] diseased".[30] In a news release, she noted that the 10-year average for developing new medications is "exacerbated by the fact that animal testing, which is the way most new drugs are tested, is not always an accurate indicator of how human blood vessels will respond to the same drugs".[31]

Other recent advancements in human tissue engineering for cardiovascular research include the ability of scientists to control the electrical pace of lab-grown heart cells using light,[32] the use of plant-derived cellulose framework as scaffolding to build networks of human veins,[33] and the development of an *in vitro* 3-D model of human early heart development that "could serve as an embryotoxicity screening assay in drug discovery, regulation, and prescription for healthy fetal development".[34] This 3-D "organogenesis-in-a-dish" model could provide a way to determine drug safety in pregnant women.

Computer modelling is also rapidly advancing human cardiovascular research. Recently, Clemson University Assistant Professor Ethan Kung was given a prestigious National Science Foundation grant for his work "aimed at reducing human and animal testing and addressing concerns that the skyrocketing cost of developing new devices and surgeries is unsustainable". His research merges numerical computer models with experimental data to create modern cardiovascular biochemical models.[35] University of Oxford researchers have demonstrated that *in silico* methods are more accurate than animal models at predicting the cardiotoxicity of certain drugs.[36]

Diabetes

Recommendation: End the use of animals immediately

From 1984 to 2014, more than 50 papers were published per month describing experiments on rodent models of type 2 diabetes mellitus (T2DM).[37] Considering these numbers, we now know a great deal about diabetes, or metabolic disturbances that look like diabetes, in rodents, but "many details of human T2DM pathogenesis remain unclear, and means of preventing disease progression remain elusive".[38] Rodent studies were used to identify thiazolidinedione (TZD) drugs as possible therapeutics for humans with T2DM or insulin dysfunction.

Unfortunately, the studies did not predict that TZDs would increase the risk of cardiovascular death in these patients by 64 per cent; in fact, they provided contradictory evidence.[39]

T2DM is a disease of glucose misregulation that leads to broad physiological effects. Rodents differ from humans on every tier of glucose regulation, from the level of nucleic acids to differences in proteins, pathways, cells, tissues, and organs. The two species also differ in terms of disease progression at the organism level and, dramatically, in environmental exposure and autonomy of lifestyle.[40,41] "Because mice rely principally on the liver for glucose homeostasis, while humans rely on skeletal muscle where transport mechanisms and biochemical pathways differ, mice may not be expected to be analogous to [T2DM] patients in regards to mechanisms of glucose metabolism or its dysfunction."[42] Despite these clear discrepancies, diabetes research in animals continues while more relevant, human-based methods are often ignored.

Many genetic models of T2DM are based on leptin or leptin-receptor deficiency, even though neither of these represent an important contributor to T2DM in humans.[43] Mice who have been genetically modified to lack select insulin-signalling genes are also poor models. For example, mice with a complete deletion of the insulin receptor die within a few days of birth, while humans with this rare condition can survive until age 2.[44] Overall, observed phenotypes in these and similar animal models of diabetes are only "secondary to genetic mutations that do not reflect disease etiology in humans".[45]

Human-relevant alternatives to the use of animals in diabetes research include human imaging, *in vitro* technology using human heterologous cell lines, human induced pluripotent stem cells, organotypic 3-D cell culture, the use of human organs *ex vivo*, post-mortem human tissue, non-invasive human imaging, epidemiological and human genetic studies – including nutrigenomics and nutrigenetics – as well as *in silico* modelling.[46,47] For example, scientists at Glasgow Caledonian University recently used human cells from a tissue bank to generate wound-healing models for diabetic patients, who have difficulty with wound healing and controlling skin infections.[48] Additionally, the US Food and Drug Administration has approved a closed-loop insulin pump developed using *in silico* modelling as a substitute for animal testing, providing just one example of how "[r]ealistic computer simulation is capable of providing invaluable information about the safety and the limitations of closed-loop control algorithms, guiding clinical studies, and out-ruling ineffective control scenarios in a cost-effective manner".[49]

In their recent publication, Ali, Chandrasekera, and Pippin discuss a wealth of relevant methods for studying diabetes, stressing the need to focus on human biology for human diabetes research:

> As we continue to uncover major species differences in factors affecting glucose biology – such as cell division, stimulus-secretion coupling and autocrine–paracrine interactions … it is now becoming unquestionable that **new information should be derived solely from human primary cells, tissues and organs**, obtained from nonpatient controls and patients in the various progressive stages of T2DM. … If the ultimate goal of the diabetes research community is to understand disease mechanisms that will lead to better T2DM prevention and therapeutic outcomes for patients, then the best way to achieve that goal is by prioritising human-centred research [emphasis added].[50]

HIV/AIDS

Recommendation: End the use of animals immediately

The failures of animal experiments to translate into useful human application of HIV/AIDS vaccines were recognised more than 20 years ago when, in 1995, the US National Institutes of Health (NIH) instituted a moratorium on the breeding of chimpanzees, the most commonly used animal in HIV/AIDS research at the time, acknowledging the failure of studies using the species to produce clinically useful data in this field. Following NIH's acknowledgement that chimpanzees aren't human-relevant surrogates for this research, experimenters began to use other non-human primate species, notably macaques.

Because macaques are unreceptive to HIV, experimenters who wanted to use them shifted their focus to studying simian immunodeficiency virus (SIV), even though it is known that SIV isn't related to the most widespread HIV virus, HIV-1, but rather is a relative of the rarer and less pathogenic HIV-2.[51] The genetic homology between HIV and SIV is only 55 per cent, and SIV is less genetically diverse than HIV.[52,53] Owing to differences in surface proteins and other molecular markers, antibodies that neutralise SIV have no effect on HIV, and vice versa,[54] making them useless in HIV research. Importantly, the dose of SIV administered to non-human primates in experiments is much higher than the typical amount of HIV-1 to which a human is exposed during sexual transmission.[55] AIDS researcher Mark Girard has stressed, "Extrapolating from vaccine protection results in non-human primate [SIV/SHIV] studies to efficacy in man may be misleading."[56]

Immune system and genetic variances between humans and non-human primates weaken non-human primate HIV/AIDS research. Here are some examples:

- Non-human primates have more leukocyte antigen genes and therefore wider variety in antigen recognition than do humans.[57]
- Non-human primate T cells contain molecules called siglecs, which act as "brakes" on the immune system, preventing hyper-responsiveness. The absence of siglecs in human T cells dramatically affects how humans respond to infection and treatment.[58]
- The primate TRIM5α gene codes for a restriction factor that affects responsiveness to retroviruses such as SIV, giving some non-human primates greater resistance to infection, a function mostly lost in human TRIM5α.[59]
- Even in chimpanzees, humans' closest non-human relatives, transcript expression in the liver differs by 40 per cent,[60] a species difference that becomes more pronounced following the varying translation of these transcripts into proteins.

For these reasons and more, HIV/AIDS vaccine research involving non-human primates has been called "one of the most notable failures in animal experimentation translation".[61]

Because of broad failures in non-human primate HIV/AIDS research, experimenters have recently shifted some focus to a species even more genetically removed from humans: the mouse. The "humanised" mouse model for HIV/AIDS research is a mouse who has been partially repopulated by human immune cells, allowing the animals to be infected with HIV-1. However, humanised mice are limited in their longevity with the disease and retain murine major histocompatibility complex antigens, "complicating immune response interpretations".[62] Not surprisingly, the use of "humanised" mice has also failed to generate useful results for clinical HIV/AIDS treatment.

Considering the differences between an animal laboratory environment and human society, it is clear that animal experiments will never capture the complexity of this human disease. Animals used in experiments are kept in mostly pathogen-free conditions, and cofactors that may be present in human patients, such as other

microbial infections, are absent, significantly altering the acquisition and course of the virus.[63] Additionally, researchers at Emory University in Atlanta state, "HIV persistence is a very complex virological and immunological phenomenon, with infection of several cell types in a wide array of anatomic tissues that are all regulated differently,"[64] and recognise that human *in vitro* models are needed to replicate this human disease and develop treatment. Thinking progressively about non-animal methods, UK scientists have said, "Existing animal models predicting clinical translations are simplistic, highly reductionist and, therefore, not fit for purpose," and that clinical attrition data "focusses the attention back on to early target selection/lead generation, but it also questions the suitability of current animal models with respect to congruency with and extrapolation of findings for human hosts".[65]

Scientists admit that even after costly and unreliable animal experiments, human data is still needed to determine whether a drug is fit for the clinical setting. Rao and Alving of the US Military HIV Research Program state that "human clinical trials still appear to be the only reliable way to determine whether an HIV vaccine candidate will have activity or efficacy in humans".[66] In a comprehensive review of preclinical and clinical data, Jarrod Bailey reported that of 85 candidate vaccines that were tested in 197 clinical trials, zero were successful; some drugs even increased the risk of HIV infections compared to the placebo.[67] A current search of ClinicalTrials.gov will return more than 700 AIDS vaccine trials, and still, none has been successful.

Recently, scientists from Australia, France, Italy, and the UK have been studying the immune cells of individuals called "HIV controllers", who can become infected with HIV but are able to control the virus's spread without any intervening therapy.[68] The hope is that immune cells from HIV controllers can be transferred to HIV-infected patients to help them fight the virus. This promising research is human-specific and requires human-specific testing methods. As Nobel laureate Sydney Brenner declared, "We don't have to look for model organisms anymore because we are the model organism."[69] Similarly, in 2007, the associate editor of *The BMJ* stated, "When it comes to testing HIV vaccines, only humans will do."[70]

Inflammation and Immunology

Recommendation: End the use of animals (particularly mice) immediately

Because of the development of tools allowing for manipulation of the mouse genome, the mouse is the most commonly used research subject worldwide. However, it should be no surprise that with this rampant use comes substantial evidence that mice are not the same as humans and that there are certain fields, in particular, in which the dramatic differences in physiology between the two species disqualify the use of mice as research subjects. One of the most noted fields in this category is immunology.

In 2004, a compelling review was published in *The Journal of Immunology* outlining the many differences between mouse and human immune systems, including in the anatomy of lymphoid tissue, ratios of white blood cell types, antimicrobial peptide profiles, cytokine profiles and functions, mechanisms for crosstalk between the adaptive and innate immune systems, antibody subtypes, development and regulation of lymphocytes, and activation of clotting factors.[71] Since then, several other analyses have been published detailing the many differences between human and mouse immunology.

A 2014 study found fundamental differences between the species in the innate immune response, stating, "[W]hile in human blood mechanisms of immune resistance are highly prevailed, tolerance mechanisms dominate for the defense against pathogenic microorganisms in mouse blood."[72] Logically, these differences make sense: we humans "do not live with our heads a half-inch off the ground",[73] and we have considerably longer lifespans and a larger body size than do mice.[74,75] As concisely stated by Leist and Hartung, "[H]umans are definitely no 70-kg mice."[76] Despite the glaring contrast, mice continue to be used for immunological research.

The use of mice as a model of influenza virus (IFV) infection has been heavily criticised: "There are … a number of drawbacks of the [mouse] model that make it unsuitable for addressing certain virological questions and can render data obtained in mice difficult to translate to the human situation."[77] Viral infection is species-specific, and mice cannot naturally catch human IFV. To bypass this problem, experimenters have altered both the strain of mice and the viruses used. The BALB/c mouse, for example, is an inbred strain and is highly susceptible to viral infection because of the lack of MX1 gene, which codes for Mx1 protein that can selectively inhibit IFV replication.[78] The lethal dose of a deadly IFV strain (H5N1) is about 100 times lower in BALB/c mice compared to their cousins in the wild.[79] BALB/c mice do not possess genetic heterogeneity nor proper immune function for virology research.

The viruses used in animal studies are often adapted through serial passage in target hosts (mice, in this case) for easy infection.[80] This is because human IFV receptors (α2,6-linked sialic acids) are not abundant in the upper airways of mice, who have a different receptor (α2,3-linked sialic acids).[81] Through serial passage, the virus can adapt to the new host and become distinct from the kind that affects humans predominantly.

There are many more differences between mice and humans in terms of IFV disease progression. For example, mice get hypothermia rather than fever following infection.[82] They do not cough or sneeze.[83] Moreover, the virus does not transmit between mice.[84] Additionally, we now know that gut microbiota are intimately linked to the immune system,[85] and studies have demonstrated drastic differences between the microbiomes of humans and mice. For example, 85 per cent of bacterial species in mice don't exist in humans.[86] The aforementioned evidence supports the inapplicability of mouse immunity to human immunity.

Considering the obvious failure of mice as surrogates in the study of human immune systems, investment in human-relevant *in vitro* and *in silico* models is needed. Advances in data collection and computer analyses have allowed for the development of human-relevant multiscale models that "can consistently integrate immunological data generated at several scales, and can be used to describe and optimize therapeutic treatments of complex immune diseases".[87]

Vanderbilt University researchers have used a dual-chamber blood-brain barrier microfluidic device called the NeuroVascular Unit to study the human blood-brain barrier's response to neuroinflammation.[88] German scientists developed a computer model that gives them the capability to assess, for the first time, the electrophysiological consequence of the acidosis in human immune cells accompanying most forms of inflammation.[89] Additionally, a University of Tennessee mathematician, along with surgical and immunological specialists at the University of Pittsburgh, used a mechanistic mathematical model to characterise human immune responses during organ transplantation.[90]

A review summarising the progress of immune-competent human skin disease models recognises the failures of animal studies to translate into effective treatments for diseases such as fibrosis, psoriasis, cancer, contact allergy, and autoimmune diseases, due, in part, to the immunological nature of these conditions. The authors go on to describe how co-culture, 3-D organotype systems and organ-on-a-chip technology will "enable human models of well-controlled complexity, yielding detailed, reliable data; thus providing a fitting solution for the drug development process".[91]

Nerve Regeneration

Recommendation: End the use of animals immediately

Many neuroprotective agents have been developed that are successful in treating spinal cord injury (SCI) in animal models, but clinical trials have been disappointing. Neurologist Aysha Akhtar has described three major reasons for this failure: "differences in injury type between laboratory-induced SCI and clinical SCI, difficulties

in interpreting functional outcome in animals, and inter-species and interstrain differences in pathophysiology of SCI".[92] In their systematic review of the use of animal models to study nerve regeneration in tissue-engineered scaffolds, Angius and colleagues noted, "The large majority of biomaterials used in animal models have not progressed for approval to be tested in clinical trials in spite of the almost uniform benefit described in the experimental papers."[93] The authors lamented the low quality of described animal experiments, in that necessary detail and rationale had been omitted, making it difficult to compare data.

For example, methylprednisolone, a routinely used treatment for acute SCI, has generated inconsistent results in animal models. A systematic review examining 62 studies of the drug on a wide variety of species, from rodents to monkeys, found that 34 per cent of the studies reported beneficial results, 58 per cent no effect, and 8 per cent mixed findings.[94] The results were inconsistent both among and within species, even within strains. Furthermore, the variability in results remained even when many of the study design and procedure variables were controlled. The authors pointed out numerous intrinsic differences between, and limitations of, each species/model and suggested that as a result of these immutable inter- and intra-species differences, no human-relevant animal model can be developed. They concluded that the "research emphasis should be on the development and use of validated human-based methods".[95]

Among species, rats are particularly unsuitable for nerve repair or regeneration research. Experts have pointed out three major problems with rat models in this field:

> (1) The majority of nerve regeneration data is now being generated in the rat, which is likely to skew treatment outcomes and lead to inappropriate evaluation of risks and benefits. (2) The rat is a particularly poor model for the repair of human critical gap defects due to both its small size and its species-specific neurobiological regenerative profile. (3) Translation from rat to human has proven unreliable for nerve regeneration, as for many other applications.[96]

More specifically, the inconsistencies between animal models and the clinical situation include the following:

> (1) healthy animals versus sick patients; (2) short versus long gap lengths (the clinical need for *large* gap repairs, while 90% of in vivo studies are in rats and rabbits where gap lengths are usually ≤3 cm); (3) animal models that almost always employ *mixed sensory-motor* autografts for repairing mixed defects, versus clinical repairs that almost always involve *sensory* autografts (usually sural nerve) for repairing mixed defects; (4) protected anatomical sites in animal models, versus repairs that must often cross articulating joints in humans; and (5) inbred, highly homogeneous animal strains and ages, versus diverse patient populations and ages: It is well recognized that animal models fail to mimic the human condition in terms of the *uniformity* of animal subjects used.[97]

University of Florida biomedical engineers Mobini and colleagues add, "We are incapable of truly mimicking human neural injures in animal models because of the extensive anatomical, functional, molecular, immunological, and pathological differences between humans and frequently studied animals."[98] Human-relevant methods such as human stem cells and clinical research can bypass these limitations and should be the focus.

Human-relevant methods for studying nerve injury and regeneration have been reviewed by a number of research groups and include human organoids, microfluidics, engineered human tissue scaffold moulds, bioprinting, and other *in vitro* uses of humans cells. *Ex vivo* models, such as those that use 3-D engineered scaffolds, bioreactors, neurospheres, and organoids, allow for more controlled studies on specific parameters

than do animal experiments.[99] Bioprinting can use bioinks containing human cells and materials to construct heterogeneous tissue models in a single step and with great consistency,[100] an aspect of nerve regeneration research that has been particularly lacking in animal models.[101]

Shrirao and colleagues at Rutgers University recommend microfluidic devices, which are "adaptable for modeling a wide range of injuries" and provide advantages over traditional *in vivo* and *in vitro* experiments by "allowing researchers to (1) examine the effect of injury on specific neural components, (2) fluidically isolate neuronal regions to examine specific effects on subcellular components, and (3) reproducibly create a variety of injuries to model TBI and SCI".[102] Mobini and colleagues note that microfluidics, or lab-on-a-chip devices, offer advantages in precision, scalability, and cost-effectiveness when compared to traditional cell culture or animal experiments and that these are currently on the market and available for neural regenerative medicine research.[103]

Neurodegenerative Diseases

Recommendation: End the use of animals immediately

There is sufficient literature documenting the failings of various animal models of neurodegenerative diseases, including Alzheimer's (AD), Parkinson's (PD), Huntington's (HD), and motor neurone disease (MND), to write a lengthy appendix for each disease. However, since many of the same limitations of animal models prohibit translation across these conditions, they will be discussed briefly as a whole. For one, all these diseases are human-specific, meaning that none of them occurs naturally in other animals. No animal model has been developed that recapitulates all aspects of a particular neurodegenerative disease.[104] For AD research, the clinical failure rate for new drugs is 99.6 per cent.[105] This includes the recent failure of AstraZeneca and Eli Lilley's lanabecestat, which was hailed as extremely promising, due to futility.[106] There have been no new discoveries that slow the progression of AD for 12 years.[107]

In a bioinformatic analysis comparing transcriptional signatures of human AD, PD, HD, and MND with mouse models of these diseases, Stanford scientists made the following findings:

> [M]ost available mouse models of neurodegenerative disease fail to recapitulate the salient transcriptional alterations of human neurodegeneration and … even the best available models show significant and reproducible differences compared to human neurodegeneration. Although the reasons for the poor transcriptional performance of mouse models varied, the unifying theme was the failure of mouse models to exhibit the variety and severity of diverse defects observed in human neurodegeneration.[108]

These molecular discrepancies underscore the artificial ways in which such models are created. Physical and chemical lesioning and systemic administration of toxins are often used. These are acute stressors, not long-term degenerative processes, and as such, they initiate events in animal models that are not present in human patients. The acute and immediate nature of particular disease models, such as the 6-OHDA and MPTP models of PD and the 3-NP model of HD, fail to capture the progressive nature of the disorders that they aim to mimic. In addition, it is commonplace for scientists to use young animals, both rodents and primates, to "model" diseases associated with ageing,[109] further reducing the likelihood that their observations will be of use to humans.

Genetically modified mouse models of neurodegenerative disease exhibit an inconsistent range of pathological and behavioural phenotypes, in part because of the transgenes used, inconsistencies in transgene insertion and expression, and mouse background strains.[110] The most commonly used genetic mouse model of MND, the

SOD1 model, is based on a gene that accounts for only 3 per cent of MND cases in the human population.[111] Literature reviews have concluded that findings from this model have not translated into any effective human therapy for MND, that "a biased estimation of treatment efficacy in animals may lead to unnecessary (and possibly harmful) clinical trials in humans",[112] and that "animal models are not an ideal system for studying [amyotrophic lateral sclerosis (MND)] or for developing drug therapies".[113] In PD, even non-human primate studies do not "constitute a valid scientific modality for the complete understanding of PD and for the development of future neuromodulation therapeutic strategies".[114]

As in much of biomedical research, animal subjects suffer greatly when they are used to mimic neurodegenerative disease. In an analysis of published studies on animal models of HD, 51 studies referenced experiments "in which animals were expected to develop motor deficits so severe that they would have difficulty eating and drinking normally";[115] however, only three out of 51 reported making adaptations to the animals' housing to facilitate food and water intake. The authors of this analysis concluded that experimenters are not following the 3Rs principle (replacement, reduction, and refinement of animal use) and, in their failure to do so, are compromising not only animal welfare but also the relevance of their studies to HD.[116]

As animal studies fall short, scientists and policymakers are realising that research strategies should be more human-relevant. Following a review of AD research, an interdisciplinary panel recommended that funding be allocated away from animal studies and towards more promising techniques involving patient-derived induced pluripotent stem cell models, "omic" technology (genomics, proteomics, etc.), *in silico* models, neuroimaging, and epidemiological studies.[117] For advancements in human blood-brain barrier research, which will greatly benefit scientific progress in developing treatments for human neurodegenerative disease, please see the section on **Stroke**.

The following are highlights in cutting-edge, human-relevant AD research:

- Scientists at the University of Texas Southwestern Medical Center have discovered a "Big Bang" of AD, identifying the genesis of tau pathology in the disease, not by experimenting on animals but by extracting proteins from human brains and isolating single molecules.[118]
- Thanks to developments in human brain imaging, scientists at the University of Cambridge were able to trace tau protein in human brains.[119] Chemists there also used mathematical modelling to understand the role of cholesterol in the aggregation of amyloid proteins.[120]
- Patient-derived stem cells were used by Hungarian and Danish scientists to compare neurons from the brains of patients with sporadic AD to those with the familial form of the disease, discovering key similarities and differences between the two pathologies and concluding that stem cell technology is suitable for modelling both forms of the disease.[121]
- At the Karolinska Institute in Sweden, researchers identified a molecular fingerprint for dementia present in the synapses of brains collected post mortem from patients and subject to proteomic analyses.[122]

Biological engineering is also transforming MND research. A team of researchers in the Hickman Hybrid Systems Lab at the University of Central Florida have developed a human neuromuscular junction-on-a-chip, the first of its kind, which can be used for toxicity testing of drugs designed to treat neuromuscular diseases, such as MND and spinal muscular atrophy.[123] When the researchers tested three known drugs on this model, the results matched live human data. Scientists at Harvard University and Lawrence Livermore National Library are also using brain-on-a-chip technology to study how neurons communicate and how exposure to certain chemicals may affect the human brain over time.[124,125]

For many years, animal experimenters have tormented monkeys, mice, dogs, and other animals in an effort to create drugs to treat these devastating diseases; however, since other animals don't get these human diseases, experimenters have manipulated their genomes in order to force certain symptoms. The results, after decades

of tests, include more than 100 failed drugs, an untold number of animal deaths, and the continued suffering of human victims of the disease. For these patients, a switch to human-relevant methods is long overdue.

Neuropsychiatric Disorders

Recommendation: End the use of animals immediately

Animal models of neuropsychiatric disorders such as depression, schizophrenia, bipolar disorder, anxiety, and attention deficit spectrum disorders lack two critical aspects of model validity: (1) construct validity, meaning that the mechanistic underpinnings creating the observed symptoms in animals are different from those that lead to the disorder in humans, and (2) face validity, meaning that animals lack the ability to "recapitulate important anatomical, biochemical, neuropathological, or behavioural features of a human disease."[126] No single animal model is able to replicate all aspects of a particular condition, and features of human behaviour representing hallmarks of these disorders cannot be produced or properly assessed in animals.

Human depression, for example, is characterised, in part, by a generalised feeling of sadness, hopelessness, and despair. In an effort to measure "despair" in rodents, the most commonly used behavioural test is the forced swim test, in which a rat or mouse is placed in a container of water with no way to escape and no place to rest out of the water. Naturally, the rodent will spend some time swimming and trying to find a way out of the stressful situation but will eventually become immobile and float. The time spent swimming may be extended by giving the animal some forms of human antidepressant drugs, a finding that led some scientists to assert that less time spent immobile was a sign that animals were less "depressed" and that more time spent immobile meant they were more "depressed," as if they had "given up" and were in despair.

However, as Molendijk and de Kloet discuss, immobility in the forced swim test is simply animals' adaptation to their situation and should not be used to determine their mood.[127] Individual animals who are quicker to float also save their energy and are less likely to sink, meaning that those who pick up on this sooner and spend less time struggling are simply learning this adaptive behaviour more readily. Furthermore, the immobility response occurs after treatment with drugs that do not have antidepressant effects at all, such as caffeine and other miscellaneous drugs,[128,129] and is sometimes not observed after treatment with drugs that do.[130] Time spent swimming versus floating is also influenced by an animal's strain as well as experimental variances, such as water depth and temperature.[131,132,133] Nevertheless, thousands of published papers ignore these warnings and use the forced swim test to make erroneous conclusions about an animal's mood.[134]

Experiments on neuropsychiatric conditions in animals are of poor quality. In a survey of 121 animal studies claiming to investigate attention deficit hyperactivity disorder (ADHD), only five were found to be in any way relevant to the hypotheses of the human medical papers in which they were cited. The authors of the survey concluded that "animal research has contributed very little to contemporary understanding of ADHD".[135] A similar failure of animal studies to translate into a clinical setting has been noted with bipolar depression research,[136] and animal studies have been cited as the primary source of attrition (failure of drugs) in neurobehavioral clinical trials.[137] Significant differences in physiology between humans and other animals likely account for a large percentage of failed translation. For example, the gene encoding tyrosine hydroxylase, the enzyme involved in the formation of dopamine, was found to be regulated in an entirely different manner in humans from the way it is in mice.[138] Misregulation of tyrosine hydroxylase has been implicated in several psychiatric illnesses, such as bipolar disorder and schizophrenia.

To quote Dutch animal behaviourists van der Staay, Arndt, and Nordquist, "If evidence accumulates that the intended goal/purpose cannot be reached, then one should consider abandoning further development of the model."[139] This group also points out that in all cases, "benefits must outweigh the ethical costs of the animals. These costs include pain and suffering, distress and death".[140] Funds should be allocated to more relevant,

human-based experimental models, such as computational modelling using already well-defined biomarkers[141] and the use of patient-specific stem cells for personalised medicine, which "affords the ability to generate neuronal cell-based models that recapitulate key aspects of human disease"[142] and can be used in drug discovery. Complex diseases like schizophrenia are ideal disorders "to model through stem cell approaches due to … heterogeneous, complex genetics that are hard to recapitulate in animal models".[143]

Recent developments in the field of human neuropsychiatric research include the following examples:

- A research group at the University of Michigan used induced pluripotent stem cells from bipolar and nonbipolar individuals to grow patient-specific neurons and glial cells. They found that cells from bipolar people were genetically and behaviourally distinct from those from non-bipolar people and that they responded differently to a commonly used therapeutic. The group is now further characterising these cells and testing other treatments.[144]
- German neuroscientists are using virtual reality to simulate anxiety-causing events in humans.[145]
- In Australia, researchers performed gene expression studies in post-mortem human brains, and their analyses indicated that schizophrenia may be related to the developmental complexity of the human brain.[146]
- Scientists at the Albert Einstein College of Medicine used neurons derived from human induced pluripotent stem cells, along with the gene-editing tool CRISPR-Cas9, to identify misregulated genes following the knock-out of a gene implicated in autism and other disorders.[147]
- A team at the Salk Institute for Biological Studies used a human cellular model of bipolar disorder to pinpoint key features of the disease, such as hyperexcitability of bipolar neurons and differences in responsiveness to lithium.[148]
- At the University of São Paulo, induced pluripotent stem cells were derived from samples collected from three patients with autism spectrum disorder. By generating mixed cell cultures, researchers were able to study the interplay between neurons and astrocytes and pinpoint interleukin-6 as a potential mediator of autism-specific neural defects.[149]

In addition to the lack of applicability of animal neuropsychiatric models to the human condition, animals used in this research suffer immensely. To induce "depression", experimenters subject them to uncontrollable pain through electric shocks or chronic stressors such as restraining them for extended periods of time, starving them or denying them water, tilting their cages, forcing them to live in wet bedding, shaking them, or disrupting their circadian rhythms. Animals are often made to live in complete isolation from members of their species, bullied and physically assaulted by other animals, deprived of parental care, and subjected to genetic or surgical manipulations in an effort to induce a depressed or altered mental state. Owing to the psychological distress inherent in animals provoked to display neuropsychiatric disease tendencies and the inapplicability of the results to humans, we recommend that the use of animals in such studies be ended immediately.

Sepsis

Recommendation: End the use of animals immediately

Sepsis is estimated to affect more than 30 million people worldwide each year. Although the incident rate varies by country, the incidence of severe sepsis to the point of organ dysfunction in the European Union has been estimated at 90.4 cases per 100,000 population, as opposed to 58 per 100,000 for breast cancer.[150] Mice are the animals most commonly used in sepsis research – not because they make good models of human sepsis but because they're cheap, plentiful, small, and docile.[151] The difficulty in reliably translating results from mice to humans is believed to be the primary cause of the failure of practically all human trials of sepsis therapies.

In 2013, *Proceedings of the National Academy of Sciences of the United States of America* (*PNAS*) published a landmark study that had been 10 years in the making and involved the collaboration of 39 researchers from institutions across North America, including Stanford University and Harvard Medical School. Dr Junhee Seok and his colleagues compared data obtained from hundreds of human clinical patients with results from experiments on animals to demonstrate that when it comes to serious inflammatory conditions such as sepsis, burns, and trauma, humans and mice are not similar in their genetic responses.[152]

NIH Director Dr Francis Collins authored an article about these results, lamenting the time and resources spent developing 150 drugs that had successfully treated sepsis in mice but failed in human clinical trials. He called this disaster "a heartbreaking loss of decades of research and billions of dollars".[153] The *PNAS* paper reveals that in humans, many of the same genes are involved in recovery from sepsis, burns, and trauma but that it was "close to random" which mouse genes might match these profiles. Collins explains it as follows:

> Mice, however, apparently use distinct sets of genes to tackle trauma, burns, and bacterial toxins – when the authors compared the activity of the human sepsis-trauma-burn genes with that of the equivalent mouse genes, there was very little overlap. No wonder drugs designed for the mice failed in humans: they were, in fact, treating different conditions![154]

Even before this landmark study, the criticism of mouse models had been documented in more than 20 peer-reviewed scientific papers. The mice used in sepsis experiments are young, inbred, and of the same age and weight, and they live in mostly germ-free settings; in contrast, it is mostly infant and elderly humans, who live in a variety of unsterilised, unpredictable environments, who develop sepsis.[155,156] When experimenters induce the condition in mice, the onset of symptoms occurs within hours to days, whereas it takes place within days to weeks in humans. Mice are not typically provided with the supportive therapy that human patients receive, such as fluids, vasopressors, and ventilators.[157] Unlike humans, mice are rarely given pain relief,[158] another difference that undermines data of already questionable value, as pain affects other physiological processes.

The "gold standard" method of inducing sepsis in mice is through caecal ligation and puncture. However, mice's responses to this procedure vary depending on age, sex, strain, laboratory, the size of needle used, and the size of the incision, which makes results incomparable between laboratories.[159] In addition, the procedure causes the formation of an abscess, whose effects may disguise or be disguised by the effects of the sepsis itself.[160] This means that an intervention that appears to be beneficial for sepsis may actually be beneficial only because of its effects on the abscess.

Rats, dogs, cats, pigs, sheep, rabbits, horses, and non-human primates, including baboons and macaques, have also been used in sepsis experimentation. None of these species reproduces all the physiologic features of human sepsis. The pulmonary artery pressure responses of pigs and sheep differ from those of humans, so this aspect of sepsis cannot be compared between these species.[161] Furthermore, baboons and mice are less sensitive to a species of bacteria commonly used to induce sepsis in experimental settings.[162]

Fortunately, researchers do not have to use animals to study and find treatments for sepsis in humans. In 2015, an expert working group consisting of veterinarians, animal technologists, and scientists issued a report on the implementation of the 3Rs in sepsis research.[163] The group noted several methods that could be used instead of animal models, such as *in vitro* cell culture models for studying sepsis mechanisms, systems and computation biology for laying out the inflammatory processes occurring during sepsis, 3-D cell culture models for exploring human disease progression and infectious disease mechanisms, synthetic human models to recreate human disease–related cell types and tissues, and human genomic information to discover how sepsis affects individuals differently and which groups may be more at risk. The authors state that genomic information "will complement or even replace the need for mouse models in disease discovery and drug development".[164]

The following are examples of recent developments in human-relevant sepsis research:

- Scientists at Emory University and the Georgia Institute of Technology have engineered a microfluidic vascularised bleeding model that allows them to test the effects of therapies on clot and plug formation in human blood.[165]
- Because the clinical trajectory of sepsis can be drastically different for every individual, University of Chicago researchers propose that human genetic algorithms "can serve as a guide on the path towards true 'precision control' of sepsis".[166]
- Physicians from Cincinnati Children's Hospital support using microfluidic devices to study sepsis in infants, whose cells could be captured from a very small amount of blood.[167]
- Researchers from the Harvard TH Chan School of Public Health, Brigham and Women's Hospital, and the University of Sheffield compared public datasets of the blood transcriptome profiles of adults and children with sepsis, populations that have different mortality rates from the disease. This led them to identify 10 candidate drugs that had never been linked to sepsis before.[168,169]
- By analysing blood from patients with sepsis, a German group identified a specific microRNA as an independent risk factor for mortality and a biomarker for discriminating between sepsis and infection.[170]

In fact, there may have already been a breakthrough in sepsis research. Physicians have recently had impressive results by treating sepsis patients with an intravenous vitamin C combination.[171] One patient whose chance of dying from sepsis was nearly 100 per cent was well enough to leave the intensive care unit within seven days of receiving this treatment.[172] An estimated 10 to 20 per cent of intensive care specialists around the world have already started using this therapy, and studies involving 13 hospitals are underway to confirm its efficacy.[173] Importantly, these successes have been achieved using only human patients, not mice or other animals, and many patients were helped tremendously in the process.

Stroke

Recommendation: End the use of animals immediately

According to researchers at the Institute for Stroke and Dementia Research in Munich, "More than 1000 neuroprotective compounds have been tested in rodent models with the aim to improve stroke outcome. … Indeed, many agents reduced brain damage (in most cases measured as decreased infarct volume) in rodent models of experimental stroke. Out of these candidates approximately 50 neuroprotective agents were tested in more than 100 clinical stroke trials, but none has improved outcome in clinical stroke patients."[174]

Many factors contribute to this failure, such as flaws in experimental designs, publication bias, disease-management inconsistencies between animal models and clinical populations, and physiological differences between species. Experts in the field admit that "animal models of stroke mimic at best less than 25 percent of all strokes".[175] The Stroke Therapy Academic Industry Roundtable (STAIR) published its first recommendations in 1999, but the success rate of clinical trials has not improved. One drug, NXY-059, which fulfilled the STAIR criteria, failed in clinical trials.[176] This illustrates the need to shift away from animal models and focus on human-centred methods.

In a 2017 review,[177] Clemens Sommer, MD, of the University Medical Center at Johannes Gutenberg University Mainz, details the following aspects of animal experimentation that limit the translatability of animal-based stroke research to the clinical setting:

- Most animals studied in stroke research have lissencephalic, or smooth, brains, unlike the gyrencephalic brains of humans.
- The expression of certain signalling molecules differs between rodents and humans in three types of brain cell – neurons, astrocytes, and microglia – both at baseline and in response to oxygen deprivation.
- In humans, ischaemic damage to the white matter of the brain is important in the prognosis of stroke, but white matter content in humans is much higher than in other animals. "While in humans the percentage of white matter accounts for 60%, it decreases to about 35% in dogs, 20% in rabbits, 15% in rats and is as low as 10% in mice,"[178] meaning that a major factor in stroke outcomes for humans cannot be accurately compared in animal models.
- Blood vessels in the brain have a different anatomy in humans compared to other animals; even strains of rodents differ in their vascular framework. These "functional differences may have deeper implications concerning the pathophysiology of the ischemic cascade".[179]
- In humans, the gene for the neurotransmitter nitric oxide synthase 2 (NOS2) is regulated differently than it is in mice. NOS is important, since nitric oxide may be an essential gas-signalling molecule during stroke.[180]
- As discussed elsewhere in this report, immune system differences between humans and other species are drastic. Sommer describes this as follows:

[T]he percentage of neutrophils in mice and rats is about 10–20% compared to 50–70% in humans, while the opposite situation is seen for lymphocytes, which comprise about 50–100% in rodents compared to 20–40% in humans, respectively. Moreover, there is only a minimal intersection of whole-genome mRNA and microRNA expression in leukocytes from rodents versus humans at both baseline and after stroke, raising the question whether rodents are acceptable models at all for the human immune system after stroke.[181]

- The RNA profile of a mouse brain is more similar to that of other tissues in a mouse's body, such as the lungs, liver, and heart, than it is to that of a human brain.[182]
- Ischaemic stroke typically occurs in heterogeneous elderly patients with comorbid conditions, whereas animal stroke experiments are predominantly carried out in young, healthy, male, inbred animals.

Kaya and colleagues made the following observation:

> In animal studies, prolonged survival and neurological improvement rates are not documented realistically. Histopathological findings and treatment effects are rarely adequate to reveal the mechanisms in behavioral and functional improvement. There is great difference between animal experiments and clinical practice in terms of outcome evaluation. The cerebral infarct area is used in animal experiments while neurological function and quality of life are more important in humans.[183]

On the other hand, human-based models of stroke do not suffer from these deficiencies. Instead, they allow for high-throughput analyses and are "increasingly important" for "testing novel potentially neuroprotective pharmaceuticals".[184] Scientists from the Department of Molecular and Cellular Physiology at Louisiana State University have written that a "key benefit of *in vitro* systems is the opportunity to work with human cells, as such Werth *et al*., utilized the brain slice method in human cortical slices to provide the first direct evidence of glutamate receptor involvement in ischemic injury in the human brain".[185]

Thanks to technological advances, including accurate 3-D representations of multiple neuronal cell types and structures of the human brain, researchers are able to overcome some of the previously limiting factors of human *in vitro* brain research. As part of a $70 million NIH programme, an interdisciplinary team of researchers at Vanderbilt University have engineered a blood-brain barrier-on-a-chip, which they are using to study human brain inflammation induced by various compounds.[186] Similarly, the Seattle-based biotechnology company Nortis was recently awarded a federal grant to develop its predictive preclinical living model of the blood-brain

barrier as an alternative "to traditional pharmaceutical drug development testing on laboratory animals", which will "reduce costs and minimize clinical trial failures".[187] Disruption of the blood-brain barrier following a stroke[188] is a critical factor to consider in attempting to move a potential therapeutic compound from a patient's bloodstream to the brain. Scientists at the University of California–Irvine opine that "[blood-brain barrier]-on-a-chip models offer tremendous potential for recreating microvasculature in the laboratory that will allow controlled study of the mechanics of [blood-brain barrier] permeability and immune infiltration as they relate to the process of stroke",[189] particularly those that employ human cells, such as human induced pluripotent stem cells, which "can be used to create clinically relevant models for [central nervous system] disease".[190]

A report authored by 42 scientists following a US National Institute of Neurological Disorders and Stroke workshop on translational stroke research concluded, "With increased availability of human cell lines/tissues, organoids, and inducible pluripotent stem cell technologies and high-throughput assays, *in vitro* strategies, in combination with data from animal models, may hold increasing prominence in future drug development strategies."[191] Animal models will never be able to recapitulate the nature of human stroke nor the human-specific inflammatory response that follows. Considering that in the US, someone suffers a stroke every 40 seconds and that someone dies of one every four minutes,[192] we cannot afford to spend our limited resources on substandard animal-based research.

Substance Abuse

Recommendation: End the use of animals immediately

Fundamental aspects of non-human animals make them inappropriate for the study of human addiction. First, the use of and addiction to drugs of abuse in humans is a vastly complex experience, one that has been impossible to mimic using animals in a laboratory setting.[193] It has been argued that attempts to model human disorders such as addiction in non-human animals, especially rodents, are "overambitious" and that the "'validity' of such models is often limited to superficial similarities, referred to as 'face validity' that reflect quite different underlying phenomena and biological processes from the clinical situation."[194]

Second, the pharmacokinetic actions of drugs are different among species. For example, "the rate of metabolism of MDMA [street name: Ecstasy, E, or Molly] and its major metabolites is slower in humans than rats or monkeys, potentially allowing endogenous neuroprotective mechanisms to function in a species specific manner".[195] Pharmacokinetic differences between humans and "model" animals likely explain why the neurotoxicity seen in rodents after MDMA administration has not been observed in the clinical setting.[196] Since MDMA is being explored because of not only its illegal use as a recreational drug but also its potential use as a therapeutic, accurate knowledge regarding its safety in humans is paramount.

Third, serious flaws in experimental design of addiction experiments greatly skew interpretation of their results. In the human experience with drugs, the user chooses to consume the addictive substance. They choose it over other substances or activities that they may find rewarding. Animals in laboratories are typically not given this option. When they are, the vast majority of them will choose an alternative reward, such as sugar, over the drug of abuse.[197] This holds true for primates as well as mice and rats.[198] Even in animals with very heavy previous drug use, only about 10 per cent would continue to give themselves a drug when they had the option to make another rewarding choice.[199] In a review on the "validation crisis" in animal models of drug addiction, French neuroscientist and addiction researcher Serge Ahmed asserts that the lack of choice offered to animals in these experiments elicits "serious doubt" about "the interpretation of drug use in experimental animals".[200]

The non-human animal has been called a "most reluctant collaborator" in studying alcohol addiction and noted to have a "determined sobriety" that the experimenter must fight against in order to overcome "their consistent failure to replicate the volitional consumption of ethanol to the point of physical dependency".[201] National Institute of Mental Health researchers reason that "it is difficult to argue that [drug self-administration by rodents] truly models compulsion, when the alternative to self-administration is solitude in a shoebox cage".[202]

Despite the prevalence of addiction research conducted on animals, "drugs that effectively curb opioid or psychostimulant addiction by promoting abstinence and preventing relapse have yet to be developed" and "very little clinical development is currently ongoing".[203] The data from animal studies was promising in certain drug classes, but these have either failed to be effective in human trials or not been tolerated well by humans, a negative outcome that was not predicted by animal trials.[204]

Non-invasive human research methods can provide us with answers to the questions that non-human animals, in their distaste for drugs of abuse, are fundamentally unable to answer. Rutgers University Robert Wood Johnson Medical School researchers recently authored a review article describing how the use of human induced pluripotent stem cells can provide a "unique opportunity to model neuropsychiatric disorders like [alcohol use disorders] in a manner that … maintains fidelity with complex human genetic contexts. Patient-specific neuronal cells derived from [induced pluripotent stem] cells can then be used for drug discovery and precision medicine".[205]

Human-relevant, non-animal research on alcohol use disorder is being carried out by scientists at the University of Connecticut, who recently used stem cells donated by alcoholic and non-alcoholic subjects to study the effects of alcohol on a specific receptor in the brain that is targeted by alcohol. Their results were at odds with some of the findings from animal experiments.[206] At Rutgers, scientists used patient-derived cells to generate neural cell types specific to individuals in which they could study alcohol's effects on various aspects of cell physiology. Their results demonstrated a role for neuronal inflammation in the pathophysiology of alcohol use disorder.[207] Others are using human induced pluripotent stem cells to study the effects of alcohol on the human liver.[208]

In addition, the funds used to support ineffective and wasteful animal substance-abuse studies could instead be used to aid effective and directly human-relevant drug prevention, rehabilitation, and mental health-care programmes.

Trauma

Recommendation: End the use of animals immediately

After rodents, pigs are the species most commonly used in trauma experimentation. However, notable species-specific differences between pigs and humans render results from this research unintelligible. For example, pigs' coagulation activity differs from that of humans, making it difficult to achieve a state of coagulopathy, or the inability to clot, in pigs. In instances of human trauma, coagulopathy represents part of the "lethal triad" for patients and is a great concern for researchers and physicians.[209] In addition, there are differences in the administration of mechanical ventilation and drugs such as vasopressin and heparin in research.[210,211] Importantly, as with mice and humans, immune responses are different between pigs and humans.

Trauma is extremely heterogeneous: patients differ in age, gender, ethnicity, medical history, alcohol and drug use, and the presence of other injuries, making the production of an appropriate animal model difficult,[212] if not impossible. In studies of traumatic brain injury, all promising therapeutics identified in animals have failed

in human clinical trials.[213] There is a significant amount of discussion regarding the limitations of animal models of trauma and haemorrhagic shock, which is summarised in this excerpt from a review by Combes:

> Scientific problems with the animal models include the use of crude, uncontrolled and non-standardised methods for traumatisation, an inability to model all key trauma mechanisms, and complex modulating effects of general anaesthesia on target organ physiology. Such effects depend on the anaesthetic and influence the cardiovascular system, respiration, breathing, cerebral haemodynamics, neuroprotection, and the integrity of the blood-brain barrier. Some anaesthetics also bind to the NMDA brain receptor with possible differential consequences in control and anaesthetised animals. There is also some evidence for gender-specific effects. Despite the fact that these issues are widely known, there is little published information on their potential, at best, to complicate data interpretation and, at worst, to invalidate animal models. There is also a paucity of detail on the anaesthesiology used in studies, and this can hinder correct data evaluation.[214]

Fortunately, it has been shown that computer simulation can accurately replicate real-life trauma and predict patient outcomes.[215] For example, scientists at the University of Pittsburgh used a computer model to examine the relationship between spinal cord injury and pressure ulcers in human patients and found that a certain treatment was effective at reducing inflammation and tissue damage.[216] This Pittsburgh group also used data-driven and mechanistic modelling to discover that the inflammatory response of patients who survive traumatic brain injury is different from that of individuals who do not survive, information that "may point to both novel mechanistic insights and clinically translational applications".[217]

In addition to the already-mentioned human-relevant methods that can be used to study molecular aspects of the side effects of and comorbidities related to trauma, clinical research remains invaluable in this field and informs mathematical and computer modelling. German researchers conducted a study of 35,232 patients over the course of 12 years and revealed a reduction in intubation rates, ventilation, and systemic complications such as sepsis.[218] A study conducted at the US Army Institute of Surgical Research used data from more than 250 human experiments to model mechanistically the physiology that underlies blood loss and shock in humans suffering from haemorrhage. The authors describe the study as follows:

> Unlike an animal model, we introduce the utilization of lower body negative pressure as a noninvasive model that allows for the study of progressive reductions in central blood volume similar to those reported during actual hemorrhage in conscious humans to the onset of hemodynamic decompensation (i.e. early phase of decompensatory shock), and is repeatable in the same subject. Understanding the fundamental underlying physiology of human hemorrhage helps to test paradigms of critical care medicine, and identify and develop novel clinical practices and technologies for advanced diagnostics and therapeutics in patients with life-threatening blood loss.[219]

As a result of the heterogeneity of the causes and outcomes of trauma, and because of physiological and immunological differences among species, only human-relevant research methods are suitable for informing human trauma research.

Training and Forensic Enquiries

Detailed below are opportunities to end the use of animals immediately in forensic research and biomedical education.

Forensic Sciences

Recommendation: End the use of animals immediately

Forensic science is a unique research area and deserves serious ethical scrutiny, as its goal is to understand crime-related issues, rather than improving human health or life conditions, and the experimental methods are often horrific and conducted without anaesthesia. Italian scientists Cattaneo and colleagues explain that there is a "moral obligation to pursue and respect this [responsibility to take care of other animal species], especially where mankind's actual survival is not at risk".[220]

The use of animals in forensic research was heavily criticised as early as 1992, when Bernard Knight asserted that "painful, sometimes mutilating experiments on conscious animals" in order to obtain "tenuous potential benefit to some medico-legal problem" cannot be condoned, particularly when one considers that such works "are not regularly used in routine forensic practice" and just "gather dust in university libraries".[221] He also observed that "a vast amount of published material using animal experimentation seems to have little practical relevance, other than to expand the curriculum vitae and the career prospects of the researcher".[222]

In 2015, Cattaneo and colleagues published a meta-analysis and review examining 404 forensic science articles and found that 69.1 per cent "concerned studies involving animals sacrificed exclusively for the sake of the experiment" and that "killing still frequently includes painful methods such as blunt trauma, electrocution, mechanical asphyxia, hypothermia, and even exsanguination; of all these animals, apparently only 60.8% were anesthetized".[223] In 2018, another meta-analysis was conducted by South African researchers Calvin Gerald Mole and Marise Heyns, who examined 204 original forensic science studies, using 5,050 animals, which were conducted between 2012 and 2018.[224] In these, animals, including rats, pigs, mice, rabbits, sheep, and cows, were drowned, electrocuted, cut, beaten, and made to ingest acid, among other cruel procedures. Mole and Heyns conclude that not enough is being done in forensic science research to uphold basic ethical principles of research and to adhere to the 3Rs.

Cruelty aside, Cattaneo and colleagues stress, "[T]he history of forensic sciences has provided us with much evidence of the inapplicability of data obtained from studies performed on animal models,"[225] given the anatomical, physiological, and genetic differences between species. Mole and Heyns suggest that "much of the reported animal tissue use in the traumatic research articles in the current study could be minimized using human tissue obtained at medico-legal autopsy" and that "[m]edico-legal autopsies may be an underutilized resource for scientific research specimens".[226]

In addition, there are a plethora of alternative methods, such as manikins, simulators, artificial materials, and *in vitro* technology, and "applying alternative methods rather than using animals has provided, in the forensic field, important and reproducible results".[227] Taken together, the ethical problems and scientific and practical issues associated with animal experimentation as well as the abundant and readily available alternative methods signify that forensic research is a prime area for animal use to end immediately.

Medical Training

Recommendation: End the use of animals immediately

Animals have traditionally been used in biomedical education to teach human physiology and pharmaceutical principles, study human anatomical form and function, and practise human surgical procedures. Yet the following recent developments have contributed to a paradigm shift in this field: improvements in human-patient simulation and computer-assisted learning technology that teaches biomedical education as well as or better than animal dissection and experimentation,[228] rising public opposition to animal use in laboratories,[229] increasing animal laboratory cost burdens,[230] and a renewed focus by the medical community on improving patient safety and reducing clinical errors through simulation-based training.[231]

Medical experts have recommended a transition from an animal-based pedagogy to "a robust curriculum composed of didactics, task trainers, virtual reality, cadavers, computer software, high-fidelity patient simulators, and supervised clinical work".[232] Unlike animal-based laboratories, these non-animal training methods accurately model human anatomy, physiology, and pharmaceutical intervention and can effectively prepare students for the workplace. Further benefits include allowing students to repeat medical procedures until proficiency is achieved, improving provider confidence and transference of learned skills to clinical practice, and allowing educators to receive real-time objective performance feedback.[233]

Microsurgery Training

There now exists an array of low- and high-fidelity non-animal methods that researchers have developed for the effective teaching of a wide variety of basic and advanced microsurgical skills to novice and expert physicians and that have been endorsed as replacements for live-animal use. These include task trainers and perfused human cadavers that can teach procedures such as anastomoses, resection of artificial tumours, bypasses, and aneurysm creation, dissection, and clipping.

For example, a study from the University of Toronto comparing the microsurgical anastomosis skills of surgical residents trained on live rats versus those trained on a silicone model found that, following identical initial training on inanimate models, the latter group was as proficient at performing single-layer, microsurgical anastomoses as those trained on live animals. The authors concluded, "[T]raining with low-fidelity bench models is as effective as training with high-fidelity, live animal models for the acquisition of technical skill among surgical trainees."[234]

A systematic review of microsurgical training methods supported these findings:

> It would appear from the best available evidence that simulated microsurgery training on low fidelity models can be as effective as on high fidelity models. … In the UK and elsewhere, the mainstay of microsurgical simulated training has historically been exposure to an in vivo rat microsurgery course, but generally this at a far too early stage in training where the bridge with clinical hands-on exposure to relevant cases cannot be made, and without repetition.[235]

Trauma Training

A study published by a US Air Force team compared the self-efficacy reported by military trainees taught emergency procedures on human simulators versus those taught using live animals and found equivalent results in both groups, concluding that "the belief in the superiority of animal training may just be a bias" and that "if the goal for trainers is to produce individuals with high self-efficacy, artificial simulation is an adequate modality compared with the historical standard of live animal models".[236] The lead author published a separate letter in the same medical journal stating, "We have entered into an age where artificial simulator models are

at least equivalent to, if not superior to, animal models. … [T]he military should make the move away from all animal simulation when effective equivalent artificial simulators exist for a specific task. For emergency procedures, this day has arrived."[237]

Non-animal methods are used exclusively instead of animals for military trauma training by nearly 80 per cent of NATO member states,[238] and the US Coast Guard has become the first branch of the US Armed Forces to end the use of animals for this practice.[239] These developments confirm that animal use for trauma training is neither necessary nor justified.

Efforts to replace animals with human simulators in military trauma training have gained many prominent supporters, including, recently, The New York Times Editorial Board[240] as well as numerous medical and veterans organisations representing more than 255,000 physicians and doctors-in-training, which have former US Surgeons General among their leadership.[241]

In the civilian sector, the American College of Surgeons has affirmed that human simulators can replace the use of animals in Advanced Trauma Life Support (ATLS) training, and national ATLS programmes in numerous countries have made this transition and ended animal use for this purpose.[242]

Given the non-animal training methods already available, we recommend that the use of animals for military and civilian trauma training and microsurgery training be ended immediately.

Toxicity Assessment

Detailed below are opportunities to end or significantly reduce the use of animals for the toxicity assessment of substances in the context of regulatory toxicity requirements. Also described are areas in which greater support is required to develop innovative methods that are relevant for the assessment of human health endpoints.

Please note that where tests are required for regulatory purposes, the OECD website (www.OECD.org) should be consulted for the most recent versions of test guidelines and guidance documents.

Exposure-Based Assessment

Recommendation: Immediately promote the use of exposure-based waiving as an opportunity to reduce the use of animals dramatically

Exposure-based waiving will reduce animal testing by shifting the focus of regulatory decision-making from a hazard-based to an exposure-based approach. This strategy employs "fit-for-concern" assessments rather than simple "box-ticking" by exploring safety based on real concerns and avoiding characterising hazards not relevant to human safety. The pesticide industry is actively seeking ways to promote exposure-based waiving for the assessment of their products.

Further work and collaboration by all involved stakeholders will be necessary to determine whether exposure-based waiving can be accepted and approved by regulatory authorities and the public.

Skin Irritation/Corrosion

Recommendation: Immediately eliminate the use of animals for skin irritation/corrosion testing

Skin irritation and corrosion tests for chemicals are required or recommended by multiple regulatory agencies. In these tests, rabbits are shaved, test substances are applied to their exposed skin, and they are observed for up to 14 days to assess the degree of skin damage. The tests can cause permanent skin damage, ulcers, bleeding, bloody scabs, and scarring. There is no requirement that animals be provided with pain-relieving drugs during this prolonged process.

Despite years of use, animal-based skin irritation studies have never been properly validated. Evidence exists that they are highly variable, of limited reliability, and generally poor predictors of human skin reactions. For example, a comparison of data from rabbit tests and four-hour human skin patch tests for 65 substances found that 45 per cent of classifications of chemical irritation potential based on animal tests were incorrect.[243]

The Organisation for Economic Co-operation and Development (OECD) has developed an integrated approach to testing and assessment (IATA) for skin irritation using *in vitro* skin irritation and corrosion methods that avoids or minimises animal use.[244]

- **OECD Test No 439: *In Vitro* Skin Irritation: Reconstructed Human Epidermis (RHE) Test Method:** May be used for the hazard identification of irritant chemicals (substances and mixtures), in accordance with the UN Globally Harmonized System of Classification and Labelling (GHS), as category 2, category 3, or non-classified chemicals. May be used as a stand-alone test or in a tiered testing strategy.
- **OECD Test No 430: *In Vitro* Skin Corrosion: Transcutaneous Electrical Resistance (TER) Test Method:** May be used for the identification of non-corrosive and corrosive test chemicals in accordance with the GHS.
- **OECD Test No 431: *In Vitro* Skin Corrosion: RHE Test Method:** May be used for the identification of corrosive chemical substances and mixtures. May also distinguish between severe and less severe skin corrosives.
- **OECD Test No 435: *In Vitro* Membrane Barrier Test Method for Skin Corrosion:** Allows for the subcategorisation of corrosive chemicals into the three GHS subcategories of corrosivity.

Recently, OECD TG 439 was validated for use in assessing the ability of medical device extracts to cause skin irritation, and the ISO 10993 guidance is currently being updated to include this test.[245246] A number of the above methods are currently undergoing evaluation in a joint effort by the US Environmental Protection Agency (EPA), industry, and the US NTP Interagency Centre for the Evaluation of Alternative Toxicological Methods (NICEATM) for use with pesticide products. This evaluation consists of side-by-side comparison and analysis of existing *in vitro* and *in vivo* data generated by pesticide companies for their products. Depending on the outcome of these efforts, additional work may be needed to validate the use of these methods with certain classes of chemicals that were not covered during OECD validation efforts.

Additionally, there are opportunities available to waive these tests based on criteria described in the OECD guidance document on considerations for the waiving or bridging of mammalian acute toxicity tests.[247]

Eye Irritation/Corrosion

Recommendation: Immediately eliminate the use of animals for eye irritation/corrosion testing

To assess eye irritation and corrosion using the Draize eye irritancy test, a chemical substance is applied to rabbits' eyes and the degree of damage is monitored over a 14-day period. Rabbits may endure eye swelling, discharge, ulceration, haemorrhaging, cloudiness, or blindness. The Draize test was developed 75 years ago, and advanced replacements have since been developed and validated. Furthermore, an analysis of 491 chemicals with at least two rabbit eye tests showed that there was a 73 per cent (for category 1), 32.9 per cent (for category 2A), 15.5 per cent (for category 2B), and 93.9 per cent (for no category) probability of obtaining the same GHS classification more than once.[248] Importantly, these results showed that there was a 10.4 per cent chance that a chemical once identified as category 1 would later be identified as no category. The majority of category 2A and 2B chemicals were classified differently in repeat testing: 59.4 per cent of category 2A chemicals and 80.2 per cent of category 2B chemicals were classified as no category in a second test.

While no single *in vitro* test can predict the full range of serious eye damage/irritation categories, it is possible to categorise a test substance using only one method. A top-down approach is used when chemicals are expected, based on existing information, to have a high irritancy potential or induce serious eye damage. Conversely, a bottom-up approach may be used when chemicals are expected, based on existing information, not to cause sufficient eye irritation to require a classification. An OECD guidance document on an IATA of serious eye damage and irritation was published in 2017.[249]

- **OECD Test No 491: Short Time Exposure (STE) *In Vitro* Test Method.** May be used to identify chemicals causing serious eye damage (GHS category 1) or not requiring classification (GHS no category). May also allow the classification of irritants as minimal, moderate, or severe.
- **OECD Test No 492: Reconstructed human Cornea-like Epithelium (RhCE) Test Method (EpiOcular™, MatTek).** May be used to identify chemicals not classified for eye irritation or causing serious eye damage (GHS no category).
- **OECD Test No 460: Fluorescein Leakage Test Method.** May be used to identify chemicals causing serious eye damage (GHS category 1) or not requiring classification (GHS no category). Recommended as an initial step within a top-down approach to identifying ocular corrosives or severe irritants.
- **OECD Test No 437: Bovine Corneal Opacity and Permeability (BCOP) Test Method.** May be used to identify chemicals causing serious eye damage (GHS category 1) or not requiring classification. Validated by the Interagency Coordinating Committee on the Validation of Alternative Methods (ICCVAM), the European Union Reference Laboratory for Alternatives to Animal Testing (EURL ECVAM), and the Japanese Center for the Validation of Alternative Methods (JaCVAM).
- **OECD Test No 438: Isolated Chicken Eye Test Method.** May be used to identify chemicals causing serious eye damage (GHS category 1) or not requiring classification. Validated by ICCVAM, EURL ECVAM, and JaCVAM. Recommended as the first step within a top-down or bottom-up testing strategy.

These methods are generally validated for use with cosmetics and industrial chemicals that fall under the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) regulation, and there may be limitations for some methods with certain types of chemicals (e.g. surfactants, solids, etc.). None of the current OECD-approved assays is recommended for directly determining category 2 eye irritants in a regulatory setting, but category 2 can be inferred if a substance is demonstrated not to be category 1 (severe eye damage) or no category. There is a vital need for validation of a non-animal method that can directly predict category 2 (irritant) substances for use in a regulatory setting.

The EPA currently accepts the use of *in vitro* methods for the determination of eye irritation and corrosion when classifying antimicrobial cleaning products and other pesticide products on a case-by-case basis, and it has published a guidance document describing the testing framework that industry can use for this endpoint.[250] Also, the agency, in collaboration with the PETA International Science Consortium Ltd. (the Science Consortium), NICEATM, and industry members, is currently engaged in evaluating these methods for use with agrochemical formulations through a side-by-side comparison of *in vitro* and *in vivo* data. This project is expected to be completed in 2020.

India, as per the modifications in the Drugs and Cosmetics (Amendment) Act, 2017 accepts the OECD-validated *in vitro* methods for eye irritation for all the products under its mandate.

Additionally, there are opportunities available to waive these tests based on criteria described in the OECD guidance document on considerations for waiving or bridging of mammalian acute toxicity tests.[251]

Skin Sensitisation

Recommendation: Immediately eliminate the use of animals for skin sensitisation testing

The assessment of skin sensitisation involves measuring the likelihood that a substance will cause an allergic reaction if applied to the skin. In animals, such assessments have previously been based on applying a test substance to the shaved skin of guinea pigs or to the ears of mice, who are later killed. Fortunately, for industrial chemicals and drugs, the regulatory requirement to test for skin sensitisation can be fully replaced with a combination of *in vitro* and *in chemico* assays that each address a different key event in the adverse

outcome pathway (AOP) for this endpoint.[252] The methods distinguish between sensitisers and non-sensitisers and are recommended to be used in an IATA.

- **OECD Test No 442C: In Chemico Skin Sensitisation: Direct Peptide Reactivity Assay (DPRA).** The DPRA addresses the molecular initiating event of the skin sensitisation AOP.
- **OECD Test No 442D: In Vitro Skin Sensitisation Assays Addressing the AOP Key Event on Keratinocyte Activation.** This test guideline addresses the second key event of the skin sensitisation AOP.
- **OECD Test No 442E: In Vitro Skin Sensitisation Assays Addressing the Key Event on Activation of Dendritic Cells on the Adverse Outcome Pathway for Skin Sensitisation.** This method addresses the third key event of the skin sensitisation AOP.

A recent study showed that non-animal approaches to predicting skin sensitisation are as good as or better than the mouse test when compared to human data.[253,254] While none of the methods is endorsed for potency determination, several approaches – for instance, the human cell line activation test (h-CLAT) – show promise in this regard.[255] Further efforts are underway to explore this potential.

The OECD has published a guidance document on the reporting of defined approaches to be used within IATA for skin sensitisation.[256] In general, the methods can be used to test cosmetics and industrial chemicals. The EPA accepts the use of non-animal approaches to testing single chemicals and is conducting a validation study with a goal of expanding this policy to formulations in the near-term future.[257] Likewise, the UK accepts *in vitro* methods for addressing the potential of pesticides to cause skin sensitisation for plant-protection products.[258] Additionally, there is an effort underway to validate non-animal skin sensitisation methods to replace the ISO 10993–required guinea pig skin sensitisation test for assessing medical device biocompatibility.[259] There are opportunities to waive these tests based on criteria described in the OECD guidance document on considerations for waiving or bridging of mammalian acute toxicity tests.[260]

Pyrogenicity

Recommendation: Immediately eliminate the use of animals for pyrogenicity assessment

Before drugs and medical devices can be marketed, regulators require testing to demonstrate that they are not contaminated with substances that trigger a fever response. These substances, collectively termed pyrogens, are chemically and structurally diverse but incite fever in humans through a common mechanism: peripheral blood monocytes and macrophages detect pyrogens and release pro-inflammatory cytokines that induce a rise in body temperature.

The rabbit pyrogen test (RPT) requires that rabbits be injected with a test substance and subsequently restrained for three hours, during which changes in their body temperature are monitored rectally. In Europe alone, more than 100,000 rabbits are used each year in the RPT,[261] even though it has never been formally validated for its relevance to humans and its results can vary depending on the animal's stress level. There are also differences in pyrogen sensitivity among species, and the test is incompatible with certain drug classes.[262]

The Limulus amoebocyte lysate test (LAL), also called the bacterial endotoxins test, detects only bacterial endotoxins and no other pyrogens. It requires the use of haemolymph from captured horseshoe crabs. After the biomedical bleeding process, up to 30 per cent of the crabs die. Those who live are less likely to survive in the wild.[263] A synthetic version of the LAL, in which the haemolymph is replaced by a recombinant reagent (the recombinant factor C assay), is available, but sensitivity is still limited to bacterial endotoxins.

Since 2010, the monocyte activation test (MAT) has been validated and included in the *European Pharmacopoeia* (*Ph Eur*) as a test for assessing pyrogen contamination.[264] It mimics the innate human fever

response *in vitro*, exposing human whole blood or isolated human monocytes to test articles followed by tests to detect pro-inflammatory cytokines released during exposure, and it is compatible with drugs and medical devices.[265] It avoids the aforementioned problems with the RPT and LAL tests, and case studies document instances in which the MAT detected pyrogen contamination in products that had passed the RPT and LAL but caused fever in human patients.[266]

Regulators in the EU, India, and the US accept the MAT, and the pharmacopoeias used in these regions all allow its use following product-specific validation. Nevertheless, animal tests are still used, despite their well-documented limitations.[267] To eliminate the use of animals in pyrogen tests, regulatory authorities and standards organisations must make increased effort to integrate and harmonise a preference for the MAT in international testing requirements and to encourage drug and device manufacturers to use and submit data from the MAT in their product dossiers. In September 2018, participants at a workshop organised by the PETA International Science Consortium and NICEATM discussed non-animal approaches to medical device pyrogen testing. Publication of the resulting report is forthcoming.[268]

Following a survey of pyrogen test users, the European Directorate for the Quality of Medicines & HealthCare (EDQM) revised the *Ph Eur* general chapter on the MAT to improve the method's usability and to emphasise that it is considered a replacement for animal-based pyrogen tests.[269,270] This endorsement is repeated in statements from the European Medicines Agency.[271] The International Organization for Standardization (ISO) is revising its guidance to allow use of the MAT when evaluating medical device pyrogen contamination, but the revision process has moved slowly.[272] In the 8th edition of *Indian Pharmacopoeia*, the Indian Pharmacopeia Commission revised the pyrogen testing general chapter, introduced the monograph on MAT, and replaced the RPT with the LAL.[273] Drug and device manufacturers report discomfort with regulatory ambiguity about the applicability of the MAT as a stand-alone pyrogen test, and the RPT and LAL will continue to be used until this is resolved.

Tobacco and E-Cigarette Testing

Recommendation: Immediately eliminate the use of animals for developing and testing tobacco and e-cigarette products

Around the world, animals are used to test existing tobacco products and for the development of new ones, such as e-cigarettes. In such tests, rats may be squeezed into narrow tubes, immobilised, and forced to inhale toxic substances for up to six hours each day for several years.

The European Commission Scientific Committee on Health, Environmental and Emerging Risks (SCHEER) appropriately states that, in light of the European Union (EU) policy banning animal studies for chemicals to be used in voluntary products such as cosmetics, animal studies are not endorsed to assess the safety of tobacco additives.[274] In addition, Belgium, Estonia, Germany, Slovakia, and the United Kingdom already prohibit animal tests for tobacco products because of ethical concerns.[275,276,277,278,279]

The hazard assessment of tobacco products increasingly employs innovative non-animal methods, including the exposure of cell and tissue cultures to whole cigarette smoke or e-cigarette vapour at the air–liquid interface, cell transformation assays (CTAs), and genomic analyses.[280,281,282,283] These techniques have been used to investigate cytotoxicity, genotoxicity, inflammation, and gene expression. They are more relevant to actual human exposure than are animal tests that have historically under-predicted the hazards of tobacco.

Genotoxicity

Recommendation: In light of existing non-animal methods and WoE approaches, the use of animals in genotoxicity testing can be dramatically reduced

Currently, the assessment of genotoxicity typically follows a step-wise approach, beginning with a core battery of *in vitro* tests that may be followed up by *in vivo* studies if the *in vitro* results are positive. The major endpoints that must be evaluated are gene mutation, structural chromosomal aberrations, and numerical chromosomal aberrations. In its "Strategy to Avoid and Reduce Animal Use in Genotoxicity Testing", EURL ECVAM recommends the Ames test to identify gene mutations, combined with the *in vitro* micronucleus test to identify both structural and numerical chromosomal aberrations.[284] If a substance produces negative results in both tests, it can be categorised as having no genotoxic potential and no further testing is indicated. If a substance produces positive results in either test, certain regulatory applications currently specify *in vivo* tests as the next step. This is because while *in vitro* tests are highly sensitive, producing false negative results at a low rate, they are less specific, producing false positive results at a higher rate. The number of false positive results can be reduced by using p53-competent human cells, evaluating cytotoxicity based on cell proliferation, and testing at reduced maximum concentrations.[285] These considerations have been incorporated into recent revisions of OECD test guidelines.

- **OECD Test No 490: *In Vitro* Mammalian Cell Gene Mutation Tests Using the Thymidine Kinase Gene.** Two distinct assays can be used to detect gene mutations induced by chemical substances.
- **OECD Test No 487: *In Vitro* Micronucleus Test.** This test can be used to detect micronuclei in the cytoplasm of interphase cells that have undergone cell division during or after exposure to the test substance.
- **OECD Test No 471: Bacterial Reverse Mutation Test.** This test uses amino acid–requiring *Salmonella typhimurium* and *Escherichia coli* to detect point mutations by base substitutions or frameshifts.
- **OECD Test No 473: *In Vitro* Mammalian Chromosomal Aberration Test.** This test identifies chemical substances that cause structural chromosomal aberrations in cultured mammalian somatic cells.
- **OECD Test No 476: *In Vitro* Mammalian Cell Gene Mutation Test Using *Hrpt* and *xrpt* Genes.** These tests can detect gene mutations induced by chemicals.

To undertake a better assessment of the genotoxic potential of substances that produce positive results in the core battery, additional *in vitro* tests can be used in place of *in vivo* tests. In its "Notes of Guidance for the Testing of Cosmetic Ingredients and Their Safety Evaluation", the European Commission's Scientific Committee on Consumer Safety (SCCS) recommends using a micronucleus test on 3-dimensional (3-D) reconstructed human skin or a comet assay either in mammalian cells or on 3-D reconstructed human skin.[286] However, negative results produced in these alternative tests do not necessarily rule out genotoxic potential. In such cases, expert judgement as well as mechanistic investigations may be helpful in evaluating the WoE. For example, *in vitro* toxicogenomics-based tests can provide information on the mode of action of potential genotoxicants by identifying global gene expression changes.

Validation studies of the micronucleus test and comet assay on 3-D reconstructed human skin are currently being conducted and thus providing further opportunities for phasing out the use of animals for genotoxicity testing.[287]

Acute Systemic Toxicity

Recommendation: In light of existing non-animal methods and weight-of-evidence (WoE) approaches, the use of animals for acute systemic toxicity testing can be dramatically reduced

To determine the danger of acute exposure to a product or chemical, a substance is administered to animals in extremely high doses through force-feeding (oral), skin contact (dermal), and/or forced inhalation. In this test, the dose at which half the animals would be killed – called the lethal dose 50 (LD_{50}), or lethal concentration 50 (LC_{50}) for inhalation testing – is calculated. Animals may endure severe abdominal pain, diarrhoea, convulsions, seizures, paralysis, or bleeding from the nose, mouth, or genitals before they ultimately die or are killed. The LD_{50} and its adaptations have never been scientifically validated, and their accuracy in predicting chemical effects in humans remains questioned. One analysis of the variability of the acute oral toxicity animal test showed that there is 78 or 74 per cent accuracy in obtaining the same EPA or GHS classification, respectively, if the same chemical is tested more than once.[288]

Regulatory authorities may issue waivers for acute toxicity testing in animals if certain criteria are met. The OECD has published guidance for waiving or bridging acute toxicity testing,[289] and the EPA has published similar guidance for pesticides and pesticide products.[290] This includes the use of existing data for read-across and the consideration of the physicochemical properties of the test substance.

Acute Oral Toxicity

NICEATM and ICCVAM organised a project to develop predictive models for acute oral systemic toxicity.[291] The outcome was consensus quantitative structure-activity relationship (QSAR) models for the prediction of acute oral toxicity to meet various regulatory needs, which were presented at an April 2018 workshop.[292] The models are being optimised and will be posted on the NICEATM and EPA websites.

EURL ECVAM's strategy to replace, reduce, and refine the use of animals in the assessment of acute mammalian systemic toxicity focuses on the *in vitro* 3T3 neutral red uptake (NRU) cytotoxicity assay, which can be used in a WoE approach to support the identification of non-classified substances.[293] *In vitro* tests such as the 3T3 NRU and normal human keratinocyte assays that measure basal cytotoxicity can also be useful in determining starting doses in animal tests. EURL ECVAM is currently working to improve confidence in the 3T3 NRU through the use of QSARs and by accounting for target organ information and the lack of metabolism in 3T3 cells.[294,295,296] In addition, it has proposed an approach to identifying non-classified substances using information from 28-day repeated dose toxicity studies, thereby avoiding acute systemic toxicity testing.[297]

In its "Guidance on Information Requirements and Chemical Safety Assessment", the European Chemicals Agency (ECHA) advises that an *in vivo* acute oral toxicity study can potentially be avoided if a registrant has relevant data, which are used in a WoE approach.[298] In cases in which the WoE adaptation leads to the assumption of low/no expected acute oral toxicity (>2000 mg/kg bw/d), the registrant can avoid unnecessary animal testing pursuant to REACH Articles 13(1) and 25(1).[299]

Acute Dermal Toxicity

Testing by the dermal route of exposure can be waived if data on oral toxicity are available. The EPA and NICEATM analysed the relative contributions of data from acute oral and dermal toxicity tests to pesticide hazard classification and labelling. Finding that the dermal data provided little to no added value in regulatory decision-making, the EPA published guidance allowing registrants to submit waiver requests.[300] In addition, dermal studies can be waived for substances that are non-classified by the oral route and not absorbed dermally. The European Commission recently amended REACH Annex VIII so that substances that are non-classified by the oral route do not require dermal data.

Acute Inhalation Toxicity

Testing by the inhalation route of exposure can be waived if substances demonstrate low volatility and are not aerosolised or otherwise made respirable under conditions of use. In addition, promising research efforts are underway to develop non-animal methods for acute inhalation toxicity.[301,302] A recent series of webinars (www.piscltd.org.uk/inhalation-webinars) and a workshop hosted by the Science Consortium and NICEATM presented several approaches that could eventually replace animal testing for this endpoint.[303,304]

Carcinogenicity

Recommendation: In light of existing non-animal methods and WoE approaches, the use of animals in carcinogenicity testing can be dramatically reduced

The OECD carcinogenicity study (Test No 451) currently requires that testing be conducted on rats (or other species when justified) for the majority of their life (up to two years for rodents). The test requires the use of 50 animals of each sex per dose, and a minimum of three doses and control for each study, which equates to a minimum total of 400 rats or mice per chemical. However, the National Toxicology Program, the primary organisation conducting the rodent cancer bioassay in the US, has reportedly increased the size of the dose group from 50 animals to 200 animals per dose, thus using a minimum of 1,600 animals per carcinogenicity study.[305] An updated guideline has been published to combine the one-year chronic study with the carcinogenicity study as reported in OECD Test No 453, sparing a minimum of 80 rodents per chemical.

While carcinogenicity studies are still routinely conducted, the test has been under scientific scrutiny since the early 1970s for its lack of ability to predict human outcomes. Several reviews have been conducted over the past three decades to highlight the overall lack of reliability in the carcinogenicity study.[306,307,308,309,310,311,312,313,314,315,316,317,318,319] Two assumptions underlay the bioassay: (1) rodent carcinogens are human carcinogens, and (2) high-dose chemical exposure in rodents is indicative of an environmentally relevant dose.[320] Both have been proved incorrect by 50 years' worth of carcinogenicity data.

In an assessment of 202 pesticide evaluations from the EU review programme, it has been demonstrated that the mouse carcinogenicity study contributed little or nothing to either derivation of an acceptable daily intake for assessment of chronic risk to humans or hazard classification for labelling purposes.[321] In terms of pesticide approvals, the authors showed that the mouse study did not influence a single outcome. An additional study reported that data collected from 182 pharmaceutical chemicals show that little value is gained from the carcinogenicity study when compounds lack certain histopathologic risk factors, hormonal perturbation, and positive genetic toxicity results.[322] This study highlights the opportunity to use a WoE approach to determine whether the carcinogenicity study can be waived for chemicals that meet certain criteria.

In vitro CTAs recapitulate a multistage process that closely models *in vivo* carcinogenesis, and they have the potential to detect both genotoxic and non-genotoxic carcinogens. In its recommendation on the CTA based on the Bhas 42 cell line, EURL ECVAM notes that information on the transforming potential of substances generated by CTAs may be sufficient for decision-making.[323] In a validation study, the Bhas 42 CTA was tested with 98 substances, including carcinogens and non-carcinogens; for predicting carcinogenicity, its performance was equivalent or superior to conventional genotoxicity assays.[324] As the protocols were transferable and reproducible between laboratories, they are recommended for routine use. In addition, because the Bhas 42 CTA is based on a cell line rather than primary cells, no animals are required.

In its guidance document on the Bhas 42 CTA, the OECD recommends that it be used as part of a testing strategy rather than as a stand-alone assay. When combined with other information, such as genotoxicity data, structure-activity analysis, and toxicokinetic information, CTAs in general – and the Bhas 42 CTA specifically –

can contribute to the assessment of carcinogenic potential and may provide an alternative to the use of *in vivo* testing.[325]

The structural alerts (SAs) rulebase has recently been expanded with a large number of new SAs for non-genotoxic carcinogenicity and has been incorporated into the OECD QSAR Toolbox version 4.2.[326] Additionally, the EPA has published a computer system, OncoLogic™, to evaluate chemicals for carcinogenic potential,[327] and commercial options are also available, such as the Lhasa Carcinogenicity Database, MultiCASE, UL Cheminformatics, and Leadscope. Ultimately, the identification of DNA-reactive chemicals with the Ames test or genotoxic SAs can potentially be combined with the identification of non-genotoxic carcinogens using non-genotoxic SAs, leaving CTAs to model most of what is left unexplained in a WoE approach. There is an effort underway at the OECD level to generate an IATA for non-genotoxic carcinogens.[328]

Endocrine Disruption

Recommendation: In light of existing non-animal methods and WoE approaches, the use of animals in endocrine testing can be dramatically reduced

In the 1990s, the EPA's Endocrine Disruptor Screening Program (EDSP) was established to screen approximately 10,000 chemicals for their effects on the human body's hormone systems and on wildlife. The programme has the potential to use millions of animals in testing. In order to reduce the number of animals used and rapidly and effectively screen such a high volume of chemicals, the agency has turned to several non-animal methods.

Its Toxicity Forecaster (ToxCast) ranks and prioritises chemicals using more than 700 high-throughput screening assays, which cover a variety of high-level cell responses and approximately 300 signalling pathways, as well as computational toxicology approaches. Data have already been generated on thousands of chemicals of interest to the EPA.

ToxCast is being used successfully for these purposes. After a comparative study of ToxCast oestrogen pathway assay results and uterotrophic assay results,[329] the EPA announced that it will accept ToxCast data as an alternative to at least one animal test – the uterotrophic assay – that screens for effects on the oestrogen pathway.[330] The agency is working to finalise the use of ToxCast data as an alternative to the rat Hershberger assay, which screens for effects on the androgen pathway.

The thyroid pathway has more complexity than either the oestrogen or the androgen pathways. Although ToxCast is showing promising results, more research is required in this area, and use of this system to replace tests on animals is still several years away. There are complementary efforts at the international level. An OECD scoping document for *in vitro* approaches to the thyroid signalling pathway was published in 2014.[331] The OECD Molecular Screening Group's *in vitro* Thyroid Subgroup is working to bring relevant *in vitro* thyroid assays to the attention of OECD member countries and provide recommendations for their development and use. More research and development is needed to obtain non-animal approaches to screening for thyroid disruption potential in humans and wildlife populations.

Repeat Dose, Reproductive, and Developmental Toxicity

Recommendation: Immediately fund and support the development of innovative non-animal methods for assessing repeat dose, reproductive, and developmental toxicity

In repeat dose toxicity studies, animals areexposed repeatedly to substances for one to three months in order to measure the effects of multiple chemical exposures. Chemicals are usually administered to animals using an oral gavage.

Reproductive toxicity studies measure a chemical's effects on reproductive organs and fertility, while developmental toxicity studies measure a chemical's effect on developing offspring during pregnancy.

While the assessment of repeat dose toxicity is a standard requirement in human safety evaluation, no non-animal methods are currently accepted for regulatory purposes. The European Commission's Detection of Endpoints and Biomarkers of Repeated Dose Toxicity Using *In Vitro* Systems (DETECTIVE) project was one of the six research projects funded under the Safety Evaluation Ultimately Replacing Animal Testing (SEURAT-1) cluster umbrella. The aim of the project was to set up a screening pipeline of high-content, high-throughput, and "-omics" technology to identify and investigate human biomarkers in cellular models for repeat dose *in vitro* testing. In addition, the EU-ToxRisk project integrates advancements in cell biology, -omics technology, systems biology, and computational modelling to define the complex chains of events that link chemical exposure to toxic outcome. The project focuses on repeat dose systemic toxicity and developmental and reproductive toxicity.

None of the *in vivo* methods used for testing reproductive and developmental toxicity have been validated for their relevance to humans.[332] There are considerable limitations surrounding the *in vivo* methods, with a predictivity of only around 60 per cent and large interspecies variations.[333,334]

EURL ECVAM has investigated the validation of *in vitro* reproductive toxicity test methods and is leading the development of an AOP for an aspect of reproductive toxicity, i.e. PPARγ activation leading to impaired fertility.[335,336] The EU FP6 project, ReProTect, has also investigated possible strategies to cover the entire mammalian reproductive cycle, resulting in a series of published works.[337] Furthermore, the ChemScreen FP7 project has been designed to generate a rapid screening system that is relatively simple and cost-effective.[338]

The EPA's National Center for Computational Toxicology is also exploring the potential for chemicals to disrupt prenatal development through the use of its virtual embryo model, v-Embryo™, which integrates *in vitro* and *in silico* modelling approaches.[339] While the field is gradually moving towards IATA strategies in order to cover the majority of possible mechanisms, much more research is required.

Aquatic Toxicity Testing

Recommendation: In light of existing non-animal methods and WoE approaches, the use of animals in aquatic toxicity testing can be substantially reduced

Aquatic toxicity tests are conducted to measure the effects of chemicals on the environment and wildlife. In 2011, nearly 180,000 fish were used for toxicological and other safety assessments in the EU.[340] As assessment of aquatic toxicity is required in various regulatory frameworks, strategies to replace testing using aquatic animals are urgently needed.

Several non-animal alternatives to the use of live animals are available now. In 2018, two OECD test guidelines for *in vitro* intrinsic clearance using cryopreserved rainbow trout hepatocytes[341] and rainbow trout liver S9 subcellular fraction[342] and an associated guidance document[343] were adopted. Liver intrinsic clearance values can be used either for physiologically based toxicokinetic models for fish bioaccumulation or for extrapolation to an *in vivo* biotransformation rate. The latter can be used with *in silico* models for prediction of bioconcentration factors. Thus, although these test guidelines require the use of fish to obtain primary cells, they can contribute to replacing the use of fish in OECD Test No 305 on bioaccumulation in fish.[344]

To reduce the number of juvenile and adult fish used in acute aquatic toxicity testing, ECHA will accept data from the Fish Embryo Acute Toxicity Test[345] in a WoE approach[346] on a case-by-case basis.

A promising cytotoxicity assay using the RTgill-W1 cell line has been developed for the determination of acute aquatic toxicity testing.[347] This *in vitro* assay has the potential to reduce or even replace the use of fish in the acute fish toxicity test.[348] A ring trial on transferability and both intra- and inter-laboratory reproducibility of the assay organised by the Swiss Federal Institute of Aquatic Science and Technology has been completed,[349] and a Standard Operating Procedure has been adopted by the ISO.[350] A project to develop an OECD test guideline on the fish cell line acute toxicity test using the RTgill-W1 cell line assay has been included in the work plan of the OECD Test Guideline Programme in 2019. Adoption of the test guideline is planned for April 2020.

Laboratory Production Methods

Detailed below are opportunities to end the use of animal-derived products for scientific or medical purposes and to reduce significantly the use of animals for the production of drugs and vaccines.

Biologic Drugs

Recommendation: In light of existing non-animal methods and WoE approaches, the use of animals can be dramatically reduced in the production and evaluation of biologic drugs

Many vaccines and other biologic drugs are produced or tested for quality, identity, safety, and efficacy in experiments that require the use of large numbers of animals. These procedures often cause severe suffering before the animals die or are killed. New technology has enabled the production and testing of biologics without animals, but experience has shown that validation and regulatory acceptance of these methods have not guaranteed their use.[351,352,353,354] Activities intended to phase out the use of animals in this context must ensure that regulatory authorities and industry commit to (1) making the transition to non-animal biologic production platforms, (2) ensuring that available non-animal methods are consistently used in place of animal-based tests, and (3) developing non-animal replacements for quality, identity, safety, and efficacy tests for all biologics.

Production platforms are available that replace animal-derived substances with recombinant, cell-based equivalents. Antitoxins, for example, have been produced historically by hyper-immunising horses and other large mammals and isolating the resulting immunoglobulins from animals' blood. These animal-derived immunoglobulins can be replaced with recombinant human antitoxin expressed in cell culture. Several recombinant antitoxins have been licensed for marketing, and more are in development.[355] With adequate funding and support from regulators, all biologics of animal origin, including antibodies (described above), can and should be replaced in a similar fashion in order to resolve issues inherent in using antibodies derived from animals.

Non-animal quality tests are available, but no formal mechanism exists to ensure that barriers to their implementation are resolved in a timely manner.[356] In some instances, manufacturers report difficulty meeting the technical criteria for using validated non-animal methods (as with the *in vitro Leptospira* vaccine potency tests).[357] In other instances, international regulators have yet to agree on technical criteria for using non-animal methods (as with the *in vitro* rabies vaccine potency test).[358] In the absence of formal oversight of the implementation process, these barriers are left to be resolved informally through workshops and decentralised problem-solving by consortia of interested parties. For companies seeking to use validated non-animal methods, this approach is prohibitively expensive and slow. As a consequence, industry adoption of non-animal methods remains limited, despite the documented reduction in animal use when they are implemented successfully.[359] Additional barriers to the implementation of currently available alternative tests have been discussed at length in the literature for erysipelas, clostridial, and tetanus vaccines and for recombinant therapeutic hormones.[360] Accelerating and standardising processes that facilitate the use of these existing replacement methods is crucial.

Regulatory leadership will ensure international regulatory and industrial coordination on best practices to remove these barriers. Regulatory authorities must establish harmonised manufacturing consistency

requirements, as tightly controlled manufacturing consistency policies are the foundation of many animal-replacement strategies.[361,362]

Antibody Production

Recommendation: Immediately eliminate the use of animal-derived antibodies in scientific applications

Affinity reagents such as antibodies are essential tools used in research to bind to a molecule to identify it or influence its activity. Every year, tens of thousands of animals are injected with viruses, bacteria, or other foreign substances and then killed for the antibodies that their bodies produce in response. Animals used in antibody production are subjected to a number of invasive and painful procedures, including antigen injection and repeated blood or ascites collection, before being killed. In the ascites method of antibody production, animals have been reported to be unable to eat, walk, or breathe properly. A number of countries, such as Australia, Canada, Germany, the Netherlands, Switzerland, and the United Kingdom, have restricted or banned the production of antibodies via the ascites method because of animal-welfare concerns.[363]

Growing concern about the lack of quality and reproducibility of animal-derived antibodies, which often show poor specificity or fail to recognise their targets, is also evident in the literature. In a February 2015 *Nature* commentary, 109 academic and industry scientists joined Andrew Bradbury of the Los Alamos National Laboratory in the US and Andreas Plückthun, head of the Department of Biochemistry at the University of Zurich, to call for an international shift to the use of recombinant antibodies for reasons that include increased reliability and reduced lot-to-lot variability in affinity reagents.[364] Bradbury and Plückthun note that they believe that poorly characterised antibodies were in large part to blame in a study in which the scientific results of only six out of 53 landmark preclinical studies could be replicated. In addition, a May 2015 *Nature* news feature reports that antibodies may be the laboratory tool most commonly contributing to the "reproducibility crisis".[365] Furthermore, a systematic analysis of 185 commercially available hybridoma monoclonal antibodies found that one-third were not reliably monospecific, and the authors recommended replacing the use of animal-derived monoclonal antibodies with sequence-defined recombinant antibodies as a straightforward and cost-effective solution to this serious problem.[366] This issue is not limited to monoclonal antibodies. Because only 0.5 to 5 per cent of the antibodies in a polyclonal reagent bind to their intended target, and polyclonal reagents have significant batch-to-batch variation, in 2015, 111 academic and industry scientists called for polyclonal antibodies to be phased out of research completely.[367]

In addition to the lack of scientific reliability and the animal-welfare concerns, there are significant economic issues related to using animal-derived antibodies. It is estimated that $800 million is wasted annually worldwide on unreliable antibodies.[368] Thus, there are potential cost savings associated with the more reproducible research that would result from using higher-quality affinity reagents.

Non-animal affinity reagents, such as recombinant antibodies and aptamers, can be used in all applications in which traditional antibodies are used, including in basic research, regulatory testing, and clinical applications. They are commercially available and, with appropriate resources, can be developed by researchers in their own laboratories.[369,370] The numerous scientific advantages of non-animal affinity reagents over animal-derived antibodies include high affinity and specificity, shorter generation time, reduced immunogenicity, the ability to control selection conditions, and the ability to be generated against unstable, toxic, immunosuppressant, and non-immunogenic antigens.[371]

International efforts have highlighted the importance of a large-scale transition from animal-derived antibodies to animal-free affinity reagents. In December 2018, a working group of the Scientific Advisory Committee of EURL ECVAM reviewed the scientific validity and benefits of using animal-free technology to produce affinity

reagents, concluding that the use of animal-free affinity reagents would improve scientific reproducibility and that scientists should work towards the replacement of animal-derived antibodies.[372] In the U.S., experts and organizations, including NICEATM and the PETA International Science Consortium, are working to increase access to animal-free affinity reagents. In December 2019, NICEATM and the Science Consortium convened a meeting to outline a pathway to improve the quality and reproducibility of research and testing by accelerating their production and use. Steps to overcome hurdles to a comprehensive shift from animal-derived to animal-free, sequence-defined affinity reagents that were identified at the meeting are described in the article "Increasing the use of animal-free recombinant antibodies".[373] More information on sources of animal-free affinity reagents, webinars, publications, and the scientific, economic, and ethical advantages of replacing animal-derived antibodies with animal-free options is available at www.piscltd.org.uk/our-work/antibodies/.

An EU-wide ban on the *in vivo* production of monoclonal antibodies using the ascites method should be introduced, in line with the one that has been in place in the Netherlands for more than 20 years, and the EU should further move to eliminate the import of animal-derived monoclonal antibodies and the use of animals in the hybridoma method.[374] In order to expedite such a ban, we recommend that member states and research funding bodies provide grant opportunities for the generation and implementation of non-animal affinity reagents.

Foetal Bovine Serum

Recommendation: Immediately eliminate the use of foetal bovine serum in scientific applications

Foetal bovine serum (FBS) is a supplement for cell culture media that provides an undefined mixture of macromolecules that function to maintain cell viability and facilitate cell metabolism, growth, proliferation, and spreading in culture. When pregnant cows are slaughtered, a large-gauge needle is used to draw the blood from the beating heart of the foetus. Because the unborn calves are not anaesthetised at the time of blood collection, they likely experience pain. It has been estimated that 600,000 litres of FBS are produced globally each year, which translates to the use of up to 1.8 million bovine foetuses for this purpose.[375]

Additionally, a number of scientific concerns are associated with the use of FBS, including batch variation leading to reproducibility issues for *in vitro* studies using FBS, the unknown composition of the serum, and the risk of contamination by animal proteins or pathogens, which is especially problematic in the manufacture of biologics for human therapies. Dutch organisations hosted workshops in 2003 and 2009 that called for the transition from FBS to non-animal serum supplements in cell culture.[376,377] A third workshop on FBS and alternatives was held in 2016, organised by the SET Foundation and the Deutscher Tierschutzbund (German Animal Welfare Federation).[378] The workshop report recommends increased funding and continued development of serum-free culture models and the use of serum-free media when establishing new cell lines. Because a universal chemically defined serum-free culture medium is not yet available and there is high demand for different cell types, the report recommends the use of human platelet lysate (hPL) as a replacement for FBS when a serum-free medium is not available.

Animal component–free and chemically defined serum-free media are available for some cell types. For others, researchers still need to optimise the concentration of each supplement to replace FBS. For these cell types, hPL, which is obtained from donated human platelets, contains growth factors essential for cell growth and proliferation and is a superior alternative to FBS for culturing cells.

Listings of commercially available products and FBS-free media recipes published in scientific literature are available on the Science Consortium's website (www.piscltd.org.uk/fbs) and in the Fetal Calf Serum-Free

Database (https://fcs-free.org/). Expert presentations on replacing FBS in cell culture media while maintaining robust cell growth and cellular functions are also available at www.piscltd.org.uk/fbs.

Government and regulatory agencies should move expediently to restrict the production and use of FBS when non-animal media or supplements are available. They should also provide funding for the development and optimisation of non-animal, serum-free medium. For cell types in which non-animal supplement concentrations have not yet been optimised and hPL cannot be used, they should require exemptions to be obtained before FBS can be produced or used. To obtain exemptions, measures should be taken to seek non-animal alternatives, and a plan to make the transition to non-animal media or supplements should be implemented.

Scientific Advisory Capabilities of PETA and Its International Affiliates

The Dutch government consulted with PETA scientists before making its decision to phase out certain experiments using animals. PETA and its international affiliates stand ready to offer our assistance in whatever capacity might be required.

The PETA International Science Consortium Ltd. promotes and funds non-animal research methods and coordinates the scientific and regulatory expertise of its members, the international PETA affiliates. With an eye towards championing the best non-animal methods and reducing animal testing, the Science Consortium and its members are actively involved in the development, validation, global implementation, and harmonisation of non-animal test methods. Briefly, the Science Consortium is an accredited ECHA stakeholder and a member of the EURL ECVAM stakeholder forum and regularly comments on OECD test guidelines as a member of the International Council on Animal Protection in OECD Programmes (ICAPO).

The scientists who work for PETA and its international affiliates have a proven track record of productively assisting many Fortune 100 corporations as well as regulatory and government agencies. This assistance includes providing expert opinions, regulatory advice, and technical support in a broad range of fields. Given the breadth and depth of our expertise, we believe that we can make a valuable contribution to developing and implementing a strategic plan for the future of biomedical research and regulatory testing.

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